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Clonal spread of methicillin-resistant coagulase-negative staphylococci among horses, personnel and environmental sites at equine facilities

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Running head: Methicillin-resistant coagulase-negative staphylococci at equine facilities

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Abstract

A cross-sectional study was carried out to investigate species distribution and clonality of methicillin-resistant coagulase-negative staphylococci (MRCoNS) isolated from the nasal cavity of 39 horses and 32 veterinary or caretaker staff, and from 76 environmental surfaces at three equine facilities in Denmark. MRCoNS obtained by selective isolation were characterized by 16S rDNA sequencing, PCR detection of \textit{mecA}, and pulsed-field gel electrophoresis (PFGE). MRCoNS were found in 32 (82%) horses, 20 (63%) humans and 50 (66%) environmental samples. The most common species was \textit{S. vitulinus} (n=56), followed by \textit{S. sciuri} (n=25), \textit{S. haemolyticus} (n=14). Undistinguishable PFGE patterns were observed in MRCoNS of equine, human and environmental origin. A \textit{S. vitulinus} clone and a \textit{S. haemolyticus} clone were isolated from multiple horses, staff members and environmental sites within a farm and a veterinary hospital, respectively. The results indicated that methicillin-resistant strains of these two species can be shared by and possibly exchanged between horses and personnel, either directly or through contaminated environments.

Keywords: antibiotic resistance, commensal bacteria, veterinary hospitals, contamination
Introduction

Coagulase-negative staphylococci (CoNS) comprise a diverse group of commensals found on the skin and mucosae in both animals and humans but some species are recognised as important opportunistic pathogens in humans (Martins and Cunha, 2007). *S. epidermidis* and *S. haemolyticus* are the two most important human pathogenic species, mainly associated with post surgical infections, indwelling and implanted foreign bodies (Kloos and Bannerman, 1994). The role of CoNS as veterinary pathogens is less known but some species have been implicated in mastitis, wound infections and skin abscesses in various animal species (van Duijkeren et al., 2004; Taponen et al., 2006). In a Danish study (Winther et al., 2008), CoNS were cultured from 14% (16/114) clinical specimens from horses, including wound, respiratory, ocular and uterine infections.

Methicillin-resistant staphylococci, in particular methicillin-resistant *S. aureus* (MRSA), are resistant bacteria of great concern to public health. Methicillin resistance is more common in CoNS than in *S. aureus* and approximately 50-90% of human clinical isolates are reported as being resistant (von Eiff et al., 2000; Martins and Cunha, 2007). Methicillin-resistant CoNS (MRCoNS) are common inhabitants on the nasal mucosa of healthy animals, especially horses (Yasuda et al., 2000; Vengust et al., 2006; Baegicil et al., 2007). In this cross-sectional study, the objectives were i) to determine whether MRCoNS clones can be shared by horses and veterinary or caretaker staff; ii) to identify the species involved; and iii) to assess MRCoNS contamination in the environment of equine facilities.

Materials and Methods

Sampling
The sampling sites were two equine referral hospitals on two Danish islands (Hospital A on Zealand and Hospital B on Funen) and one equine farm owned by the Danish Army. Human participation in the study was on a strict voluntary basis and personal information was kept anonymous. The 32 humans tested were healthy and not on antibiotic therapy at the time of sampling. Animal samples were randomly collected from all horses at the farm (n=20) and from accessible patients admitted at Hospitals A (n=9) and B (n=10). Horses at the farm were clinically healthy and not on antibiotic therapy at the time of sampling. Most patients at the two hospitals were subjected to antibiotic therapy. All samples were collected on a single day at each facility. Cotton swabs were inserted into each nostril, rolled on the mucosal membranes for 5 sec, and stored in Stuarts’ medium (Statens Serum Institut, Copenhagen, Denmark) prior to laboratory analysis. Environmental swabs were collected from 50 objects handled by the staff, such as sinks and taps, stable doors, equine handling equipment, door and cupboard handles, surgical equipment, computer keyboards and telephones, treatment tables, kettles and radios.

MRCoNS isolation and identification

Methicillin-resistant staphylococci were detected after overnight enrichment in MRSA selective broth (Statens Serum Institute, Copenhagen, Denmark) at 37°C, followed by plating on Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid, Hampshire, United Kingdom). After 24-48 hr incubation at 35°C, a single presumptive methicillin-resistant staphylococcal colony (blue or white on ORSAB) was isolated from each sample and confirmed as methicillin-resistant by PCR detection of mecA (Zhang et al., 2004). Diversity of mecA-positive isolates was assessed by Random Amplification of Polymorphic DNA (RAPD) analysis (Bagcigil et al., 2007) and isolates exhibiting distinct RAPD profiles were identified by partial 16S rDNA sequencing using primers 1AF: 5’- AGAGTTTGATYMTGGCT-3’ and
Nucleotide sequences were compared with those on Genbank and sequences with the highest similarity score to a type strain was deemed the species identity.

**PFGE analysis**

PFGE was performed according to Murchan et al. (2003) with minor modifications (Bagcigil et al., 2007). A low-range PFGE Marker (New England Biolabs, Ipswich, England) was used as a molecular weight marker and for normalisation between gels. Gels were analysed using GelCompar II software (Applied Maths, Kortrijk, Belgium), and cluster analysis was performed by UPGMA based on the Dice similarity coefficient, with optimization and position tolerance set at 0.5% and 1.5%, respectively. A similarity cut-off value of 90% was used to define PFGE types. Within each PFGE type, isolates exhibiting minor band differences were assigned to distinct subtypes.

**Results**

MRCoNS were isolated from 32 (82%) horses, 20 (63%) humans and 50 (66%) environmental samples (Table 1). We identified 12 RAPD types and 16S rDNA sequencing of isolates representative of each type revealed the occurrence of six species: *S. vitulinus* (n=56), *S. sciuri* (n=25), *S. haemolyticus* (n=14), *S. equorum* (n=4), *S. epidermidis* (n=2) and *S. saprophyticus* (n=1). *S. vitulinus* was the most common MRCoNS species in horses (46%), humans (28%) and the environment (38%). The species distribution differed greatly between the three equine facilities. *S. vitulinus* accounted for 80% and 70% of MRCoNS isolates from the equine farm and Hospital B, respectively, but was less frequent among isolates from Hospital A (22%). *S. haemolyticus* and *S. sciuri* was isolated more frequently at Hospital A (30% and 49%, respectively) than at the other two sampling sites (< 10%). *S. epidermidis* and
\textit{S. saprophyticus} were only isolated from humans at the farm. \textit{S. equorum} was only isolated at Hospital B, from a horse, a veterinarian and samples from the stall and radio. MRCoNS were isolated from all environmental sites and the most contaminated sites were wash and treatment areas (Table 1).

High variability of PFGE patterns was observed within the three major species. The degree of inter-species diversity in band patterns was notably greater for \textit{S. vitulinus} (up to 60\% and 58\%, respectively) than for \textit{S. haemolyticus} (up to 25\%). Twenty-four distinct PFGE types were identified among the 56 \textit{S. vitulinus} isolates (See Figure S1, supplementary file). A dominant \textit{S. vitulinus} clone (Type A) was isolated from four horses, one human and various environmental sites within the farm, including wash areas, door handles, horse restraints, and stall bars (Figure 1a). Other less frequent indistinguishable PFGE patterns were shared between equine or human and environmental \textit{S. vitulinus} isolates from the same equine facility. One indistinguishable \textit{S. vitulinus} clone (Type E) was isolated from a veterinarian at Hospital B and a horse and stall bar at the Farm. At Hospital A, clonal spread of \textit{S. haemolyticus} and \textit{S. sciuri} was observed. Detection of the \textit{S. sciuri} clone was limited to equine and environmental samples, whereas the \textit{S. haemolyticus} clone occurred in all sample types (Figures 1b-c). The four \textit{S. equorum} isolates from Hospital B and the two \textit{S. epidermidis} isolates from the farm displayed genetically unrelated PFGE patterns (data not shown).

\textbf{Discussion}

MRCoNS were widely disseminated among horses, humans and environmental sites at the three equine facilities. The PFGE results demonstrated that specific methicillin-resistant \textit{S. vitulinus} and \textit{S. haemolyticus} clones were shared by various horses and humans with the same farm or hospital environment, indicating possible interspecies transmission, either directly or
through the contaminated environment. Although methicillin-resistance in staphylococci is a public health concern, not all staphylococcal species are equally important as human or veterinary pathogens. *S. haemolyticus* is one of the most important pathogenic species among CoNS isolated from human infections (Kloos and Bannerman, 1994). This species is also sporadically isolated from equine infections (van Duijkeren et al., 2004). On the contrary, *S. vitulinus* has not been associated with human nor animal infections.

Although clone sharing suggests that MRCoNS transmission between horses and humans may have occurred at two of the three equine facilities, the present study cannot be used to infer the direction of transmission. However, some speculations can be made based on the natural habitats of the species involved. Methicillin-resistant *S. vitulinus* is known to be a frequent commensal in the nasal cavity of horses (Bagcigil et al., 2007) and is not associated with carriage in healthy humans (Nagase et al., 2002; Busscher et al., 2006). Therefore, the *S. vitulinus* clone isolated from humans is likely to have originated from the horses or from the contaminated environment. In contrast, the *S. haemolyticus* clone at Hospital A could have originated from either horses or humans since this species is part of the normal nasal flora of both hosts.

Two previous studies (Nagase et al., 2002; Busscher et al., 2006) have reported *S. sciuri* as the most prevalent CoNS in horses. This observation is in contrast with our findings since *S. vitulinus* was the frequently isolated from horses at all three equine facilities. This discrepancy could be due to methodological factors. It should be noted that *S. vitulinus* and *S. sciuri* are members of the *S. sciuri* group and discrimination between the two species is difficult by conventional phenotypic methods (Stepanovic et al., 2004) such as those used in the two earlier studies (Nagase et al., 2002; Busscher et al., 2006). The high frequency of *S. vitulinus
detected in this study by 16S rDNA sequencing suggests that isolates belonging to this species could have been misidentified as *S. sciuri* in the previous studies.

Historically, the presence of CoNS in clinical specimens is thought to be a result of contamination in both human and veterinary medicine. During the last decade, some species, mainly *S. epidermidis* and *S. haemolyticus*, have gained increasing attention as nosocomial pathogens in humans. Although no information is available on the frequency of nosocomial pathogens in veterinary hospitals, postoperative infections are a common complication in equine surgery and can result in intensive postoperative care, prolonged hospitalization, increased costs and mortality (Santschi, 2006). Schnellmann et al. (2006) showed that horses entering a hospital become readily colonised by CoNS after hospitalisation. Persistence and clonal spread of methicillin-resistant *S. haemolyticus* have been demonstrated within a veterinary teaching hospital in Norway (Sidhu et al., 2007). CoNS are able survive on many hospital surfaces such as floors, tables, fabric, gloves and medical devices (Neely et al., 2000). The property of CoNS to adapt to veterinary hospital environments was confirmed by our study. Both veterinary hospitals were found to be widely contaminated with CoNS, including surgical and medical equipment (Table 1).

Our study illustrates that MRCoNS clones, including clinically-relevant species such as *S. haemolyticus*, can be shared by and possibly exchanged between horses and humans in close contact, and that the environment of veterinary hospitals can be widely contaminated with these bacteria. Although human and animal health risks associated with these findings remain unknown, CoNS human infections are usually caused by the patient’s own colonizing strain (Frebourg et al. 1999 and Costa et al. 2004). The study also provides interesting information
on ecology and epidemiology of MRCoNS within veterinary hospitals, and underlines the importance of infection prevention and control best practices in equine medicine.

Acknowledgments

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References


Tables

Table 1: Occurrence and species distribution of methicillin-resistant coagulase-negative staphylococci (MRCoNS) among horses, humans and environmental sites at the three equine facilities under study.
Figure legend

Figure 1: PFGE dendrogram of indistinguishable or closely-related equine, human and environmental methicillin-resistant staphylococci identified as *S. vitulinus* (A), *S. haemolyticus* (B) and *S. sciuri* (C). PFGE types were defined based on a 90% similarity cut-off. Bands included in the cluster analysis ranged from 48.5 kb- 679kb. The line indicates the lower limit of bands used in the cluster analysis.
<table>
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<td>Environment</td>
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<td>Wash area</td>
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<td>Equine</td>
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<td></td>
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<td>Farm</td>
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