

# Bacterial infections in horses: A retrospective study at the University Equine Clinic of Bern

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## Summary

Bacterial infections present a major challenge in equine medicine. Therapy should be based on bacteriological diagnosis to successfully minimize the increasing number of infections caused by multidrug-resistant bacteria. The present study is a retrospective analysis of bacteriological results from purulent infections in horses admitted at the University Equine Clinic of Bern from 2004 to 2008. From 378 samples analyzed, 557 isolates were identified, of which *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus* and coliforms were the most common. Special attention was paid to infections with methicillin-resistant *S. aureus* (MRSA) ST398 and a non-MRSA, multidrug-resistant *S. aureus* clone ST1 (BERN100). Screening of newly-admitted horses showed that 2.2% were carriers of MRSA. Consequent hygiene measures taken at the Clinic helped to overcome a MRSA outbreak and decrease the number of MRSA infections.

Keywords: nosocomial, MRSA, *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus*, hygiene, antibiotic resistance

## Bakterielle Infektionen bei Pferden: Eine retrospektive Untersuchung an der Pferdeklinik der Universität Bern

Bakterielle Infektionen stellen in der Pferdemedizin eine grosse Herausforderung dar. Eine erfolgreiche Therapie stützt sich, bei zunehmender Anzahl an Infektionen mit multiresistenten Keimen, auf eine bakteriologische Abklärung. Die vorliegende retrospektive Studie untersucht die Ergebnisse bakteriologischer Proben, die zwischen 2004 und 2008 von Patienten der Pferdeklinik der Universität Bern mit einer eitrigen bakteriellen Infektion entnommen wurden. Aus 378 untersuchten Proben konnten 557 Isolate identifiziert werden, wobei *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus* und Coliforme am häufigsten isoliert wurden. Infektionen mit Methicillin-resistentem *S. aureus* (MRSA) ST398 und mit einem nicht-MRSA aber multiresistenten *S. aureus* Klon ST1 (BERN100) wurden in der Studie besonders berücksichtigt. Die Untersuchung neu aufgenommener Patienten ergab, dass 2.2% von ihnen bereits beim Klinikeintritt MRSA-Träger waren. Verschärfte Hygienemassnahmen an der Pferdeklinik konnten die Infektionen mit MRSA eindämmen.

Schlüsselwörter: nosokomial, MRSA, *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus*, Hygiene, Antibiotika-Resistenz

## Introduction

Bacterial infections remain a major challenge for the equine practitioner, even though such diseases are prevalent in equine health. Knowledge about the most commonly found isolates and their susceptibility to antimicrobials is key for successful therapy. Sample collection of infected tissue and bacteriological diagnostic procedures are usually time consuming. Therefore, antimicrobial therapy often starts prior to obtaining bacteriological results (Clark et al., 2008). Additionally, bacteriological analysis might also be omitted from a practitioner's prescription for financial reasons.

The emergence of infections by multidrug-resistant bacteria, such as *Staphylococcus aureus*, *Enterococcus faecium* and the extended spectrum  $\beta$ -lactamases (ESBL)-producing *Enterobacteriaceae*, is a major concern in both human and veterinary medicine (Li et al., 2007; Navarro et al., 2008; Pitout and Laupland, 2008; Rodriguez-Bano and Pascual, 2008; Weese and van Duijkeren, 2009; Leclercq, 2009). Infections with resistant strains lead to intensive and prolonged medical care, higher treatment costs and risk of spreading resistant strains to other patients, personnel or the environment. A judicious choice of the

adequate antimicrobial therapy protocol is considered one of the key measures to help limit the emergence of both resistant and multidrug resistant bacteria, which increases the chances for therapeutic success. Adequate sanitary protocols are also necessary to prevent patient-to-patient contamination and human-patient interaction that results from cross-contaminating each other (Smith, 2004; Weese and Lefebvre, 2007; Anderson et al., 2008). There is increasing evidence that people professionally or privately involved with animals are known to have an increased risk of being colonized with multidrug-resistant bacteria (Hanselman et al., 2006; Wulf et al., 2008; Moodley et al., 2008; Anderson et al., 2008). Official health agencies are currently addressing the issue of multidrug-resistant bacteria in veterinary settings in the context of both, livestock and companion animals through guideline development (European Food Safety Authority, 2009a; European Food Safety Authority, 2009b).

Bacterial infections at the University Equine Clinic of Bern have been closely monitored for several years with a focus on surgical site infections following colic surgery (Lippold, 2001) and on the rapid selection of multidrug-resistant bacteria following antibiotic use (Schnellmann et al., 2006). The present study uses data from different infection sites in horses admitted to the Clinic from January 2004 to December 2008 and gives an overview of the isolates with special consideration paid to *S. aureus* infections, including methicillin-resistant *S. aureus* (MRSA). MRSA is characterized by the presence of the methicillin-resistance gene *mecA*, which confers resistance to all  $\beta$ -lactam antibiotics. MRSA is also often resistant to other antibiotics, making staphylococcal infections more difficult to treat (Deurenberg and Stobberingh, 2009). By providing insight into the type and distribution of equine wound isolates, the results of this study should help to improve veterinary bacteriological diagnostic and therapy.

## Animals, Material and Methods

### Animals

For this study, 378 horses suffering from purulent infections or with postoperative discharge were sampled with swabs (Oxoid Ltd, Basingstoke, England) at the University Equine Clinic of Bern between January 1, 2004 and December 31, 2008.

### Bacteriology

Cultures and identification of the bacteria were performed at the Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance (ZOBA) at the Institute of Veterinary Bacteriology in Bern. All bacteriological analyses were routinely stored in the institute's electronic database LabControl (Ticono GmbH, Hannover, Germany).

Data include the date and identification of the sample, information about the patient (owner, address), characteristics of the samples (origin, type of sample) and information about the diagnosis (bacteria, amount, presence of antibiotic agents, comments). Samples that did not contain bacteria were not taken into consideration. From the 378 samples analyzed, 557 isolates were identified. Results were calculated in percentages of samples containing a specific bacterium. One sample can contain several isolates, and therefore, some percentages exceed 100%. Bacteria were isolated using Tryptone-Soy-Agar (TSA-SB) containing 5% sheep blood (Oxoid Limited®, Basingstoke, UK). All bacteria were gram-stained and tested for their capacity to split indole from the amino acid tryptophan. The bacteria were also observed for their reaction in the presence of oxidase and catalase and tested for potassium hydroxide (KOH) activity. These analyses provided a preliminary classification of the bacteria into families. Bacteria were then identified to the species level using an adequate API identification kit or VITEK®2 compact (bioMérieux, Marcy l'Etoile, France). Isolates diagnosed as "mixed flora" contained more than 3 different types of bacteria, which is due to excessive bacterial growth, including contaminants that make it impossible to assess a specific pathogen. The presence of MRSA in nasal cavities of horses was determined using nasal swabs spread onto MRSA-selective agar (BBL™ CHROMagar™ MRSA; Becton, Dickinson & Company, Franklin Lakes, NJ). MRSA were characterized by the presence of the *mecA* gene as detected by PCR (Schnellmann et al., 2006). Clonality between different *S. aureus* isolates was determined using a variable number tandem repeat (VNTR)-based method (Francois et al., 2005). *Spa* type was determined as previously described (Harmsen et al., 2003) and analyzed using the Ridom StaphType software (Ridom StaphType, Ridom GmbH, Würzburg, Germany). Sequence types (ST) were determined using multilocus sequence typing (MLST) (Enright et al., 2000). Clonal complex (CC) groups were identified based on ST and VNTR. Antibiotic susceptibility of *Staphylococcus* was determined using ATB® STAPH 5 galleries (bioMérieux, Marcy l'Etoile, France). The minimal inhibitory concentration of antibiotics was determined for MRSA strain KM740–07 and *S. aureus* BERN100 strain KM595–06 by broth microdilution using custom-made NLV73 plates (Trek Diagnostics Systems, East Grinstead, England; MCS Diagnostics BV, Swalmen, The Netherlands) according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2009). Antibiotic resistance genes were detected using a microarray capable of detecting more than 90 antibiotic resistance genes (Perreten et al., 2005).

### Hygiene measures

From the onset of the MRSA problem at the Clinic of Bern in May 2007, affected patients were isolated in special isolation stalls. Handling and caretaking of MRSA

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carriers were performed while wearing disposable full body protections suits, gloves and overshoes. Material used on and around these horses (e.g., nasogastric tubes, diagnostic apparatus, disposable materials and stable tools, such as forks and brooms) were stored separately for each horse and were disinfected or disposed of separately after use. Appropriate instructions were also given to all owners who came to visit their horses. In December 2007, disposable protective gloves were worn by all clinic personnel during every patient interaction. Additionally, glove changing and hand disinfection (Sterillium®, BODE Chemie, Hamburg, Germany) occurred between all patients visits. Separate stabling in the isolation barn of MRSA-positive horses was continued.

### Results

In total, 378 cases of bacterial purulent infections of horses were analyzed between 2004 and 2008 (Tab. 1). Approximately one-third of the samples (34.1%) con-

tained more than 3 different types of bacteria (“mixed flora”), which prevented the isolation of specific known pathogens. In all other samples, opportunistic pathogenic bacteria could be identified. Among them, *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooeconomicus* and coliforms were the most commonly found (Tab. 1). Twenty-six percent of the samples contained *S. aureus* isolates, 24.9% *S. equi* subsp. *zooeconomicus*, 4.8% *S. equi* subsp. *equi* and 11.9% *E. coli*. The group labeled “others” includes the less frequently found isolates (10 or less isolates found in the overall population, for a frequency of < 2% over the 5 year period) and contains the following bacteria: other streptococci species, *Actinomyces* sp., *Clostridium* sp., *Klebsiella oxytoca*, *Pasteurella* sp., *Actinobacillus* sp. other than *A. equuli* sp. *Proteus* sp., *Serratia* sp., *Bacteroides fragilis*, *Moraxella* sp., *Propionibacterium* sp., other *Enterobacteriaceae*, *Stenotrophomonas maltophilia*, *Morganella morganii*, *Fusobacterium* sp., *Prevotella* sp., *Pantoea* sp. and *Citrobacter freundii*. Between 2005 and 2007, a specific dominant multi-drug-resistant *S. aureus* clone named BERN100 was

Table 1: Results of bacteriological analysis from 378 sampled horses between 2004 and 2008.

	Years					Overall 2004–2008
	2004	2005	2006	2007	2008	
Number of samples	48	45	110	93	82	378
Mixed flora*	29.2%	37.8%	30.9%	25.8%	48.8%	34.1%
<i>Staphylococcus aureus</i> :						
– <i>S. aureus</i> (excl. BERN100- & MRSA-strains)	16.7%	11.1%	17.3%	14.0%	8.5%	13.8%
– <i>S. aureus</i> BERN100-strain	n.c.	6.7%	12.7%	9.7%	0.0%	6.9%
– methicillin-resistant <i>S. aureus</i> (MRSA)	n.c.	0.0%	0.0%	8.6%	14.6%	5.3%
<i>Streptococcus equi</i> subsp. <i>zooeconomicus</i>	18.8%	24.4%	31.8%	23.7%	20.7%	24.9%
Coliforms (including <i>Escherichia coli</i> )	14.6%	8.9%	8.2%	17.2%	11.0%	11.9%
<i>Enterococcus</i> sp.	6.3%	2.2%	2.7%	3.2%	12.2%	5.6%
<i>Staphylococci</i> excl. <i>S. aureus</i>	0.0%	8.9%	5.5%	5.4%	4.9%	5.3%
<i>Pseudomonas</i> sp.	10.4%	6.7%	4.5%	2.2%	7.3%	5.0%
<i>Streptococcus equi</i> subsp. <i>equi</i>	4.2%	4.4%	2.7%	3.2%	9.8%	4.8%
<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i>	4.2%	2.2%	2.7%	6.5%	2.4%	4.5%
<i>Actinobacillus equuli</i> **	2.1%	0.0%	3.6%	5.4%	8.5%	3.7%
<i>Acinetobacter</i> sp.	2.1%	6.7%	2.7%	4.3%	1.2%	3.4%
<i>Enterobacter</i> sp.	2.1%	0.0%	0.9%	9.7%	2.4%	3.2%
Others***	29.2%	13.3%	17.3%	7.5%	13.4%	15.1%

n.c., not characterized

\*Mixed flora being defined as plates on which differentiation of isolates became impossible due to increased growth of different types of bacteria

\*\**Actinobacillus equuli* may contain both subspecies *A. equuli* subsp. *equuli* and *A. equuli* subsp. *haemolyticus*

\*\*\*Others sums up the less frequently found isolates (10 or less isolates found in the overall population, for a frequency < 2% over the 5 years) and contains the following bacteria: other streptococci species, *Actinomyces* sp., *Clostridium* sp., *Klebsiella oxytoca*, *Pasteurella* sp., *Actinobacillus* sp. other than *A. equuli*, *Proteus* sp., *Serratia* sp., *Bacteroides fragilis*, *Moraxella* sp., *Propionibacterium* sp., other *Enterobacteriaceae*, *Stenotrophomonas maltophilia*, *Morganella morganii*, *Fusobacterium* sp., *Prevotella* sp., *Pantoea* sp. and *Citrobacter freundii*.

Table 2: Clonal lineage and resistance profile of methicillin-resistant *Staphylococcus aureus* (MRSA) (strain KM740 - 07) and *S. aureus* BERN100 (strain KM595 - 06) isolated from wound infections of horses.

	MRSA (ST398, CC398, spa-type <i>t011</i> )		BERN100 (ST1, CC1, spa-type <i>t2863</i> )	
	MIC <sup>a</sup>	Gene	MIC	Gene
Oxacillin	>8	<i>mecA</i> <sup>b</sup>	1	–
Penicillin	>8	<i>blaZ</i>	8	<i>blaZ</i>
Gentamicin	32	<i>aac(6′)-Ie-aph(2′)-Ia</i> <sup>b</sup>	>32	<i>aac(6′)-Ie-aph(2′)-Ia</i> <sup>b</sup>
Streptomycin	>32	<i>str</i>	>32	<i>str</i>
Tetracycline	>32	<i>tet(m)</i>	≤1	–
Trimethoprim	>32	<i>dfr(k)</i>	>32	<i>dfr(a)</i>
Erythromycin	>32	<i>erm(c)</i> <sup>b</sup>	≤0.25	–

<sup>a</sup>MIC, minimal inhibitory concentration

<sup>b</sup>*mecA* confers resistance to all β-lactam antibiotics; *aac(6′)-Ie-aph(2′)-Ia* also confers resistance to neomycin and kanamycin; *erm(C)* confers resistance to macrolides, lincosamides and streptogramins B.

responsible for 9.7% of all infections (Tab. 1). Infections caused by *S. aureus* BERN100 occurred in wounds after surgery (57.1%), injuries (28.6%) or jugular catheterization (14.3%). *S. aureus* BERN100 type strain KM595–06 belongs to the clonal complex CC1 (ST1, *spa* type *t2863*). All of the *S. aureus* BERN100 isolates shared the same genetic profile as determined by VNTR analysis, indicating that they belong to the same CC group 1. They all also displayed resistance to penicillin, gentamicin, streptomycin and trimethoprim, as well as decreased susceptibility to oxacillin (MIC, 1 µg/ml) (Tab. 2). However, BERN100 is not a MRSA because it does not contain the methicillin-resistance gene *mecA*.

In 2007, MRSA emerged in infection sites, and since then, *S. aureus* BERN100 has not been isolated from infections caused by *S. aureus*. Between 2007 and 2008, MRSA represented 11.6% of all infections, especially those after surgery (93.7%) and injuries (6.3%). The MRSA isolates were also all genetically related, displaying the same genetic VNTR profile and belonged to the clonal complex CC398 (ST398-*t011*) (Tab. 2). Besides resistance to β-lactam antibiotics, they also display resistance to tetracycline, macrolides, lincosamides, streptogramins B, trimethoprim and aminoglycosides (Tab. 2). A screening of the nasal cavities of 135 horses entering the Equine Clinic over a 3-month period (January to March 2008) showed that 3 horses (2.2%) were carriers of MRSA belonging to CC398. This short screening period identified horses that could have introduced MRSA into the Clinic.

## Discussion

*Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus* and coliforms were the most frequent pathogens

causing wound infections in horses. These bacterial species are known pathogens in horses (Vo et al., 2007; Clark et al., 2008; Weese and van Duijkeren, 2009). Although these bacteria are part of the normal flora of horses, *S. equi* subsp. *zooepidemicus* causes respiratory tract infections, metritis and wound infections in horses (Timoney, 2004; Clark et al., 2008). Additionally, *E. coli* is known to be associated with wound infections and neonatal sepsis in foals (Sanchez, 2005; Clark et al., 2008). Over the three past years, MRSA infections have been increasingly reported in horses from different countries (Burton et al., 2008; Weese and van Duijkeren 2009; Anderson et al., 2009; Van den Eede et al., 2009). *S. aureus* strains belonging to specific clonal complexes (i.e., CC1, CC8 and CC398) that have the potential to colonize both animals and humans have been found in numerous equine clinics (Moodley et al., 2008; Cuny et al., 2008; Weese and van Duijkeren 2009; Walther et al., 2009; van Duijkeren et al., 2009). Horses that were carriers of MRSA upon admission have been shown to contribute to the nosocomial transmission of the infection in a Dutch clinic (van Duijkeren et al., 2009). A similar phenomenon may explain the emergence of MRSA at the Equine Clinic in Bern because some of the horses entering the hospital were also found to be positive for MRSA.

Many samples contained mixed flora, a result that may have negative consequences for the clinic. First, an antibiogram cannot be performed and targeted antibiotic therapy is not possible with this large diversity of bacteria present. Second, it is impossible to determine whether nosocomial bacteria, such as MRSA and ESBL-producing bacteria, are present in the mixed flora. As a practical consequence, the mixed flora may delay identification and isolation of carriers of resistant strains, which increases the risk of spreading nosocomial bacteria to other patients, personnel and owners. It is therefore important

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to use an aseptic sampling method to avoid contaminants and execute meticulous and improved bacteriological diagnostic procedures in veterinary medicine. Enrichment and selective media should be introduced for diagnosis, and installing molecular detection methods into routine diagnostics could be envisaged (Busscher et al., 2006; Van den Eede et al., 2009; van Duijkeren et al., 2009). Additional measures, such as the regular surveying of patients with unapparent infections, maintaining complete information on owners and referring veterinarians and screening horses for MRSA that were once positive and have since re-entered the clinic, have been instituted in our practice. The use of antibiotics has also been limited because this practice has been shown to rapidly select for antibiotic resistant flora in hospitalized horses (Schnellmann et al., 2006).

Another important question is whether medical and caretaking personnel of the clinic should also be tested for multidrug-resistant bacteria on a regular basis because they may act as a reservoir (European Food Safety Authority, 2009b). These additional measures may become time-consuming and very expensive. Evidence was gained by other studies suggesting that simple measures could have a positive impact on the veterinary environment (Smith, 2004; Traub-Dargatz et al., 2006; Anderson et al., 2008). In December 2007, the introduction of using new gloves and hand disinfection between all patients as well as isolation of carriers helped to overcome the MRSA outbreak at the Equine Clinic. The frequency of infections caused by MRSA did not increase over the fol-

lowing 6 months, and no MRSA were responsible for the infections registered from July to December 2008.

Besides the consequences for human and animal health, infections with multidrug-resistant strains can generate increasing costs to the owner. Stabling the horses under increased hygienic measures in the clinic is expensive. Additionally, isolation of horses may have consequences on their physical and psychological health. Close collaboration and exchange of information between clinicians, bacteriologists and horse owners are necessary for optimal therapy. Private equine practitioners, as well horse owners, must understand the latest information concerning infections, risks and therapies, as well as the advantages of modern bacteriological diagnostic tests.

## Conclusion

All of these efforts, but in particular increased bacteriological surveillance combined with strict hygienic measures, may help to control infections and spread of multidrug-resistant strains. This study has demonstrated that many different bacterial species, including multidrug-resistant *S. aureus*, may be involved in equine infections. Outbreaks caused by multidrug-resistant *S. aureus* could be suppressed by the application of aggressive hygienic measures. However, these measures taken must be continuously maintained to prevent the introduction of resistant strains into the clinic, a permanent threat with every new patient.

### Infection bactérienne chez les chevaux: une étude rétrospective à la clinique équine de l'Université de Berne

Les infections bactériennes représentent un défi dans la médecine équine. Une thérapie réussie se base, vu le nombre croissant d'infections causées par des germes multi-résistants, sur un examen bactériologique. La présente étude rapporte les résultats d'échantillons bactériologiques prélevés entre 2004 et 2008 sur des patients de la clinique équine de l'Université de Berne souffrant d'une infection bactérienne purulente. 557 souches ont pu être identifiées sur les 378 échantillons examinés, *Staphylococcus aureus*, *Streptococcus equi* subsp. *zooepidemicus* ainsi que des coliformes étant isolés le plus fréquemment. On a particulièrement été attentif, dans cette étude, aux infections à *S. aureus* résistant à la méthicilline (MRSA) ST 398 et à celles causées par un *S. aureus* non MRSA mais multi-résistants, clone ST1 (BERN100). L'examen de patients à leur arrivée montre que 2.2% d'entre eux étaient déjà porteur de MRSA à ce moment. Des mesures d'hygiène renforcées ont pu contrôler les infections avec des MRSA à la clinique équine.

### Infezioni batteriche nei cavalli: un'analisi retrospettiva alla clinica per equini dell'Università di Berna

Le infezioni batteriche rappresentano una grande sfida nella medicina equina. Per ottenere dei successi concreti è necessario utilizzare esami batteriologici in caso di un aumento delle infezioni con germi multiresistenti. Questo studio retrospettivo esamina i risultati dei campioni batteriologici raccolti tra il 2004 e il 2008 in pazienti affetti da un'infezione batterica purulenta nella clinica per equini dell'Università di Berna. Da 378 campioni esaminati si sono potuti identificare 557 isolati tra i quali i più frequenti erano lo *Staphylococcus aureus*, lo *Streptococcus equi* subsp. *zooepidemicus* e il Coliforme. Nello studio sono state considerate specialmente le infezioni da *S. aureus* (MRSA) ST398 resistente alla meticillina e da *S. aureus* Klon ST1 (BERN100) con un non-MRSA ma multi-resistente. Dall'esame dei nuovi pazienti risultava che al momento dell'entrata in clinica il 2.2% erano già portatori di MRSA. Solo dei radicali provvedimenti di igiene nella clinica per equini potrebbero contenere l'infezione di MRSA.

## Acknowledgments

We are grateful to Christina Schnellmann, Daniel Kunz, Sandra Sieber and Vendula Jakesova for assistance; and Gudrun Overesch and Andreas Thomann for bacteriological advice. We also thank the interns, residents and other personnel of the Equine Clinic who contributed to the sample collection, as well as the personnel of the Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance (ZOBA).

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Received: 27 November 2009

Accepted: 2 February 2010