Antimicrobial susceptibility patterns of blood culture isolates from foals in Switzerland

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Abstract

We report blood culture results of 43 foals admitted to an equine hospital for medical or surgical disorders and determine minimal inhibitory concentrations (MIC) of different antibiotics. Eleven foals had a positive blood culture result despite prior administration of antibiotics in 10 of these animals. MIC values above EUCAST and/or CLSI breakpoints were identified in coagulase-negative *staphylococci*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecium*. Gram-negative isolates were less frequently identified and did not appear to exhibit increased MIC values. This study shows that bloodstream infections in Switzerland are caused by diverse bacteria including Gram-positive bacteria which exhibit resistance to several classes of antibiotics.

Keywords: Antibiotics, Bacteremia, Horse, Minimal inhibitory concentration, Sepsis

Resistenzprofile bakterieller Pathogene in Blutkulturen von Fohlen in der Schweiz

Im Rahmen dieser Studie präsentieren wir Resultate von Blutkulturen von 43 Fohlen, die aufgrund einer internistischen oder chirurgischen Erkrankung in der Pferdeklinik vorgestellt wurden. Elf dieser Fohlen zeigten ein bakterielles Wachstum in der Blutkultur obwohl 10 von ihnen bereits vom Privattierarzt mit Antibiotika vorbehandelt wurden. Coagulase-negative *Staphylokokken*, Methicillin-resistente *Staphylococcus aureus* und *Enterococcus faecium* zeigten minimale Hemmstoffkonzentrationen oberhalb der EUCAST und/oder CLSI Referenzen. Gram-negative Bakterien wurden seltener identifiziert und zeigten keinen Anstieg minimaler Hemmstoffkonzentrationen. Diese Studie zeigt, dass septische Infektionen in der Schweiz durch ein breites Spektrum an Bakterien verursacht werden können. Unter Anderem kommt in dieser Studie Gram-positiven Bakterien eine besondere Bedeutung zu aufgrund der erhöhten Resistenzen gegen diverse Antibiotika.

Schlüsselwörter: Antibiotika, Bakteriämie, Pferd, Minimale Hemmstoffkonzentration, Sepsis

Selection of appropriate antimicrobials for treatment of bacterial infections is a challenging procedure in veterinary medicine due to the number of specifications that need to be considered. Drug dosages are defined on the basis of pharmacokinetic and -dynamic studies which need to be performed specifically in every species, weight and age group. Bacterial diversity and susceptibility patterns may change over time (Theelen et al., 2014; Theelen et al., 2014) which impedes re-evaluation of drug dosage for target organisms on a regular basis. Furthermore, defined resistance breakpoints are sparse for veterinary pathogens and are often extrapolated from those set in human medicine (Turnidge et al., 2007), potentially making interpretation of susceptibility tests difficult. It is therefore imperative to have guidelines for antibiotic susceptibility tests available for veterinary medicine to permit targeted therapy and to perform studies of bacterial prevalence and evaluate and report minimal inhibitory concentrations on a regular basis (Theelen et al., 2014). This is especially important in populations such as foals where bacterial infections and sepsis often have detrimental consequences (Cohen, 1994). The objective of this study was to determine which bacterial species are present in blood cultures from foals in Switzerland and to assess their minimal inhibitory concentrations to relevant antibiotics.

A total of 43 foals admitted to the equine hospital between 2014 and 2016 were included in the study on the basis that they had an intravenous catheter inserted for the treatment of a medical or surgical disorder. Ten milliliters of blood were collected aseptically into a 10-ml Isolator™ tube (Isolator™ 10 Tube Blood Culture System, Thermo Fisher Scientific, Pratteln, Switzerland) and processed according to the manufacturer's protocol to provide bacteria for further isolation, identification, and susceptibility testing. After centrifugation, 100 µl of the lysed blood concentrate was plated on trypticase soy agar plate containing 5% defibrinated sheep blood (TSA-SB) (BD™ Trypticase™ Soy Agar II with 5% Sheep Blood, Becton

Dickinson, Allschwil, Switzerland) for the cultivation of aerobic and capnophilic bacteria, selective BROLAC agar (Thermo Fisher Scientific, Pratteln, Switzerland) for Enterobacteriaceae, Brucella Blood Agar with Hemin and Vitamin K1 for anaerobic bacteria (Becton Dickinson, Allschwil, Switzerland) and in thioglycolate medium for enrichment (Becton Dickinson, Allschwil, Switzerland). All the media were incubated at 37°C for 48h under appropriate atmospheres. The resulting cultures were subcultivated on TSA-SB and isolates were identified by Matrix-Assisted-Laser-Desorption/Ionization-Time-Of-Flight-Mass-Spectrometry (MALDI-TOF MS) (Microflex LT, Bruker Daltonics GmbH, Bremen). Antimicrobial susceptibility to antibiotics representing major drug classes was determined by microbroth dilution in Müller-Hinton broth using different sensititre plates (Sensititre™ Complete Automated System, Thermo Fisher Scientific, CH- Reinach) and according to the EUCAST guidelines. (www.eucast.org). Minimal inhibitory concentrations (MIC) of antibiotics were tentatively interpreted using CLSI (CLSI, 2017) and EUCAST (EUCAST, 2018) criteria set for human bacteria as no criteria exist for bacteria isolated from blood cultures of horses in the CLSI guidelines for bacteria from animals (CLSI, 2015) (Table 2 and Table 3). The use of these criteria is only indicative of the presence of a possible resistance mechanism in the bacteria under test and may not be appropriate for clinical use. Characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) was performed as previously described (Sieber et al., 2011). In addition to blood culture results, the following information was recorded: age, sex, breed, diagnosis, and antibiotic treatment prior to presentation.

Forty-three foals of various breeds were included in the three-year study period. Foals were aged between 1 and 146 days (9.4 ± 23.8 [mean ± SD]) and there were 20 fillies and 23 colts. Reasons for hospitalization included failure of transfer of passive immunity, sepsis, herniation, colic, meconium impaction, perinatal asphyxia syndrome, pneumonia, choke,

lameness, injury, renal disease, neonatal isoerythrolysis and prematurity. Hospitalization time ranged from 1 to 30 days. Thirty foals survived and were discharged. Five died and eight were euthanized. Eleven foals had a positive blood culture. Ten of them received an antibiotic treatment prior to presentation, including cefquinome, penicillin, gentamicin, amikacin or doxycycline (Table 1). None of the 32 foals with a sterile blood culture were pre-treated with antimicrobials (Table 1). Out of the 11 foals with positive blood culture, one one microorganism was cultured in seven animals (foals 5, 7, 11, 12, 14, 25, 43), whereas in four animals (foals 1, 35, 37 and 41) several bacteria could be cultured. The blood culture of two foals contained both Gram-positive and Gram-negative bacteria. Overall 12 Gram-positive and four Gram-negative bacteria were isolated and revealed 14 different species of *Staphylococcus, Enterococcus, Streptococcus, Macrococcus, Acinetobacter, Escherichia* and *Actinobacillus* (Table 1).

Staphylococci were the most frequent Gram-positive bacteria. Among them, two *S. aureus* were cultured, of which one was confirmed as MRSA sequence type ST 398 based on an MIC > 8 mg/l for oxacillin sodium, presence of the *mecA* gene, and multilocus sequence typing. In addition to resistance to beta-lactams, this MRSA showed markedly increased MIC values for other antibiotics including gentamicin, tetracycline and trimethoprim (Table 2). Among the coagulase-negative staphylococci, decreased susceptibility was observed for several antibiotics including penicillin, erythromycin, trimethoprim, sulfamethoxazole and fusidic acid (Table 2). *Enterococcus faecium* showed increased MIC values for ampicillin, tetracycline, erythromycin, trimethoprim and for high-level resistance to gentamicin. None of the Gram-negative isolates exhibited MIC above EUCAST or CSLI breakpoints when these values were available. Additionally MICs were frequently situated below the lowest concentrations of the antibiotics tested suggesting the absence of acquired resistance against these antibiotics (Table 3).

This study, although including a low number of cases, gives an overview of species diversity and antibiotic susceptibility patterns of bacteria cultured in blood samples of diseased foals in Switzerland. Bacterial diversity and resistance patterns were previously published including a higher number of septic foals in the US (Marsh et al., 2001; Theelen et al., 2014; Theelen et al., 2014), Australia (Russell et al., 2008), New Zealand (Toombs-Ruane et al., 2016), the UK (Corley et al., 2007) and the Czech Republic (Hytychová et al., 2015), but MIC values were only reported in one study (Theelen et al., 2014). These studies reported increasing prevalence of Gram-positive bacteria over recent years (Hytychová et al., 2015; Russell et al., 2008; Theelen et al., 2014; Theelen et al., 2014) although, with the exception of a single study looking at bacterial cultures from foals in general (Toombs-Ruane et al., 2016), more Gram-negative than Gram-positive bacteria were cultured. This reflects the general bacterial diversity in the equine population as reflected by culture results from a Swiss equine hospital (van Spijk et al., 2016). The low number of positive blood samples in this study did not allow the description of bacterial prevalence over time. However, we observed more Gram-positive than Gram-negative isolates in the blood cultures from foals, comparable to trends in human medicine (Martin et al., 2003). A possible explanation for the predominance of Gram-positive bacteria in our study group is the potential influence of prior antimicrobial treatment on bacterial distribution in the blood cultures. Antimicrobial treatment has been shown to influence the selection of resistant *E. coli* in horses (Dunowska et al., 2006) but there is, to our knowledge, no report about the influence of antimicrobial treatment on bacterial diversity in septic foals. The presence of multidrug resistant bacteria defined as isolates with acquired resistance towards ≥1 agent in ≥3 defined antimicrobial categories (Magiorakos et al., 2012), is of more concern since treatment of infections caused by such bacteria may require the use of so-called human medicine “last resort” antibiotics like vancomycin (Weese, 2009). The

occurrence of multidrug resistant bacteria with clinical significance (also including *S. aureus*, coagulase-negative *staphylococci* and *enterococci*) has previously been reported in Switzerland (van Spijk et al., 2016). It is therefore not surprising to observe the presence of such antibiotic-resistant isolates in blood cultures of foals.

The major limitation of this report is the low number of positive blood cultures in the study population and the fact that contamination cannot be excluded, especially if polymicrobial growth with bacteria of the normal skin flora occurs.

In conclusion, blood stream infections in this geographical area of Switzerland are caused by a diversity of Gram-negative and Gram-positive bacteria, some of the latter exhibiting resistance to several classes of antibiotics. MIC values above EUCAST and/or CLSI breakpoints were identified in coagulase-negative *staphylococci*, MRSA and *Enterococcus faecium* whereas the four Gram-negative isolates did not appear to exhibit increased MIC values. This study emphasizes again the importance of vigilant use of antimicrobial drugs in veterinary medicine and use of antimicrobial susceptibility testing to identify isolates with increased MIC values.

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