A retrospective analysis of antimicrobial resistance in bacterial pathogens in an equine hospital (2012–2015)

J. N. van Spijk[1], A. Schoster[1], M. M. Wittenbrink[2], S. Schmitt[2]

[1]Equine Department and [2]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Switzerland

DOI 10.17236/sat00069

Received: 29.03.2016
Accepted: 24.04.2016

Abstract

Antimicrobial resistance has become an important concern in veterinary medicine. The aim of this study was to describe the rate of antimicrobial resistance in common equine pathogens and to determine the occurrence of multidrug-resistant isolates. A retrospective analysis of all susceptibility testing results from bacterial pathogens cultured from horses at the University of Zurich Equine Hospital (2012–2015) was performed. Strains exhibiting resistance to 3 or more antimicrobial categories were defined as multidrug-resistant. Susceptibility results from 303 bacterial pathogens were analyzed, most commonly *Escherichia coli* (60/303, 20%) and *Staphylococcus aureus* (40/303, 13%). High rates of acquired resistance against commonly used antimicrobials were found in most of the frequently isolated equine pathogens. The highest rate of multidrug resistance was found in isolates of *Acinetobacter baumannii* (23/24, 96%), followed by *Enterobacter cloacae* complex (24/28, 86%) and *Escherichia coli* (48/60, 80%). Overall, 60% of *Escherichia coli* isolates were phenotypically ESBL-producing and 68% of *Staphylococcus* spp. were phenotypically methicillin-resistant. High rates of acquired antimicrobial resistance towards commonly used antibiotics are concerning and underline the importance of individual bacteriological and antimicrobial susceptibility testing to guide antimicrobial therapy. Minimizing and optimizing antimicrobial therapy in horses is needed.

Keywords: horse, multidrug-resistant (MDR) bacteria, methicillin-resistant staphylococci (MRS), ESBL-producing *Enterobacteriaceae*, infection

Retrospektive Auswertung der Resistenzsituation bakterieller Pathogene bei hospitalisierten Pferden (2012–2015)

Antibiotikaresistenzen gehören zu den grössten Herausforderungen in der Tiermedizin unserer Zeit. Das Ziel dieser Studie war es, die Resistenzsituation der häufigsten bakteriellen Pathogene und das Vorkommen von multiresistenten Keimen zu untersuchen. Es wurde eine retrospektive Analyse aller Empfindlichkeitsprüfungen von bakteriellen Pathogenen von Pferden am Tierspital Zürich (2012–2015) durchgeführt. Isolate welche Resistenzen gegen 3 oder mehr Antibiotikaklassen zeigten, wurden als multiresistent definiert. Insgesamt wurden die Resultate von 303 Resistenzprüfungen ausgewertet, wobei am häufigsten Ergebnisse für *Escherichia coli* (60/303, 20%) und *Staphylococcus aureus* (40/303, 13%) vorlagen. Die grosse Mehrzahl der von Pferden isolierten Infektionserreger zeigten hohe Resistenzraten gegenüber gebräuchlichen Antibiotika, wobei *Acinetobacter baumannii* (23/24, 96%), gefolgt von *Enterobacter cloacae* complex Isolaten (24/28, 86%) und *Escherichia coli* (48/60, 80%) die höchsten Raten an Multiresistenz aufwiesen. Zusätzlich waren 60% der *Escherichia coli* Isolate phänotypisch ESBL-produzierend und 68% der Staphylokokken phänotypisch methicillin-resistent. Die hohen Resistenzraten gegenüber gebräuchlichen Antibiotika sind beunruhigend und unterstreichen die Wichtigkeit einer Kultur mit Empfindlichkeitsprüfung des Infektionserregers, um eine gezielte Therapie einleiten zu können. Minimierung und Optimierung des Antibiotikaeinsatzes beim Pferd sind von grösster Wichtigkeit.

Schlüsselwörter: Pferd, multiresistente Bakterien, Methicillin-resistente Staphylokokken (MRS), ESBL-produzierende *Enterobacteriaceae*, Infektion

Introduction

Over the last decades, acquired antimicrobial resistance has gained increasing importance in human and veterinary medicine (WHO, 2011; Weese et al., 2015). Highly resistant bacterial isolates increase morbidity, mortality and costs of treatment for horses by increasing the risk of inappropriate and delayed antimicrobial therapy (Weese et al., 2015). In addition, equine multiresistant bacteria pose a public health concern, as transmission of resistant isolates via human-horse contact and transmission of resistance genes between human and animal isolates has been shown (van Duijkeren et al., 2010; Dolejska et al., 2011).

Methicillin-resistant staphylococci (MRS) and extended-spectrum β-lactamases (ESBL)- producing *Enterobacteriaceae* are currently the most important multidrug-resistant (MDR) isolates in equine medicine (Maddox et al., 2015). Both acquired resistance towards β-lactam antibiotics, the most important antimicrobial category in veterinary medicine, and therefore substantially complicate antimicrobial therapy (Weese, 2009). Methicillin resistance in *Staphylococcus* (S.) spp. is conferred by the *mec* gene. It encodes an altered penicillin-binding protein, rendering virtually all b-lactam antimicrobials used in veterinary medicine ineffective (Poole, 2004). Staphylococci, including their methicillin-resistant subpopulations, are part of the commensal equine flora. Methicillin-resistant *S. aureus* (MRSA) have been isolated from the nares of up to 40% of hospitalized horses (Van den Eede et al., 2012) and methicillin-resistant coagulase-negative staphylococci (MR-CNS) from up to 80% of healthy horses (Corrente et al., 2009; Moodley and Guardabassi, 2009). Such previous colonization was identified as risk factor for the development of subsequent clinical disease with MRS (Weese et al., 2006). Common infections caused by these opportunistic pathogens include soft tissue, musculoskeletal and infections (Corrente et al., 2009; Maddox et al., 2010; van Spijk et al., 2016 accepted). Production of ESBL in *Enterobacteriaceae* allows the isolate to open the b-lactam ring of cephalosporins, thereby inactivating them (Paterson and Bonomo, 2005). While infections with ESBL-producing *Enterobacteriaceae* are well described in humans (Paterson and Bonomo, 2005), only small numbers of ESBL-producing bacteria (mainly *Escherichia* (*E.*) *coli*) have so far been isolated from horses causing wound infections, arthritis and sepsis in foals (Dolejska et al., 2011; Dierikx et al., 2012; Smet et al., 2012; Walther et al., 2014). Additional bacteria of increasing concern are MDR *Acinetobacter* (*A.*) *baumannii*, *Enterococcus* spp. and *Pseudomonas* (*P.*) *aeruginosa*. Their high level of intrinsic resistance together with their ability to acquire resistance towards a wide range of antimicrobial agents have led to their emergence as important pathogens in human medicine (Weese, 2009). Little is known about the role these highly resistant isolates play in equine infections (Maddox et al., 2015).

There are numerous studies from different countries describing antimicrobial resistance in equine pathogens (Clark et al., 2008; Theelen et al., 2014; Toombs-Ruane et al., 2015). Results from these studies aid practitioners in making an evidence based antimicrobial drug choice in cases where prompt therapy is needed and culture and susceptibility testing results cannot be awaited. Bacterial resistance profiles can vary between geographical locations and change rapidly over time (Canton et al., 2008). Therefore, continuous local surveillance is of great importance. Currently, there are very few data on antimicrobial resistance and the occurrence of multidrug resistance in equine pathogens in Switzerland (Panchaud et al., 2010). The objectives of this study were to retrospectively describe the rate of resistance to clinically used antimicrobial drugs in pathogens isolated from horses and to analyze the occurrence of MDR isolates in horses presented to the Equine Hospital of the University of Zurich between 2012 and 2015.

Material and Methods

Data collection

The database of the Institute of Veterinary Bacteriology and the Equine Hospital of the University of Zurich were retrospectively analyzed for susceptibility testing results from bacterial pathogens cultured from horses presented to the Equine Hospital between March 2012 and October 2015. Date of collection, origin of sample, diagnosis, cultured bacteria and susceptibility testing results were recorded.

Classification

Diagnoses were classified into 7 groups based on the origin of the sample. Implant infections, infections of incisions, injection sites and after dental procedures were grouped as post-procedural infections. Synovial infections, osteitis and hoof abscesses were combined to musculoskeletal infections. Soft tissue infections included external abscesses, wounds, cellulitis and infected hematomas. Urinary tract infections contained upper and lower urinary tract infections and omphalitis. Respiratory tract infections included pathogens isolated from the upper and lower airways. Isolates from the uterus and from milk were summarized as reproductive infections. The rest of the samples was classified as 'diverse' and included isolates from sepsis, dental infections, peritonitis, dermatitis, otitis, neoplasia infections and hepatitis. Bacteria isolated from fecal samples were excluded. Isolates considered non-pathogenic or contamination were excluded based on information on

Table 1: Antimicrobial categories and agents analyzed for defining multidrug resistance in *A. baumannii*, *Enterobacteriaceae*, *Enterococcus* spp., *P. aeruginosa* and *S. aureus*. Table adapted from Magiorakos et al. (2012).

Bacteria	Antimicrobial category	Antimicrobial agents	Notes
Acinteobacter baumannii	Aminoglycosides	Gentamicin, Amikacin, Tobramycin	
	Extended-spectrum cephalosporins	Cefpirom	
	Carbapenems	Imipenem	
	Fluoroquinolones	Enrofloxacin, Marbofloxacin	
	Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	
	Polymyxins	Polymyxin B	
*Enterobacteriaceae**	Aminoglycosides	Gentamicin, Amikacin, Tobramycin	
	Penicillins	Ampicillin	*E. cloacae* and *Klebsiella* spp. intrinsically resistant
	Carbapenems	Imipenem	
	Extended-spectrum cephalosporins	Cefpodoxim, Ceftiofur, Cefpirom	
	Fluoroquinolones	Enrofloxacin, Marbofloxacin	
	Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	
	Non-extended spectrum cephalosporins	Cefalexin	*E. cloacae* intrinsically resistant
	Penicillins + β-lactamase inhibitors	Amoxicillin-clavulan acid	*E. cloacae* intrinsically resistant
	Phenicols	Chloramphenicole	
	Polymyxins	Polymyxin B	Only tested in *K. pneumoniae* and *E. coli*
	Tetracyclines	Tetracycline	
Enterococcus spp.	Aminoglycosides	Gentamicin (high level)	
	Penicillins	Ampicillin	
	Carbapenems	Imipenem	*Enterococcus faecium* intrinsically resistant
	Fluoroquinolones	Enrofloxacin, Marbofloxacin	
	Glycopeptides	Vancomycin	
	Tetracyclines	Tetracyclin	
Pseudomonas aeruginosa	Aminoglycosides	Gentamicin, Amikacin, Tobramycin	
	Antipseudomonal cephalosporins	Cefpirom	
	Antipseudomonal carbapenems	Imipenem	
	Antipseudomonal fluoroquinolones	Enrofloxacin, Marbofloxacin	
	Polymyxins	Polymyxin B	
Staphylococcus aureus	Aminoglycosides	Gentamicin	
	Ansamycins	Rifampicin	
	Antistaphylococcal β-lactams	Oxacillin, Cefoxitin	Resistance to either of these predicts MRSA phenotype (non-susceptibility to all categories of β-lactam antimicrobials, except of anti-MRSA cephalosporins)
	Fluoroquinolones	Enrofloxacin, Marbofloxacin	
	Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	
	Fucidanes	Fusidic acid	
	Glycopeptides	Vancomycin	
	Lincosamides	Clindamycin	
	Macrolides	Erythromycin	
	Phenicols	Chloramphenicol	
	Tetracyclines	Tetracycline	

* *Enterobacteriaceae* include: *Enterobacter cloacae* complex, *E. coli*, *K. pneumoniae* ssp. *pneumoniae* and *Proteus mirabilis*

bacterial species, origin of sample, quantity of growth, the occurrence of mixed cultures and clinical findings. If opportunistic pathogens were isolated, they were only included if a role as causative pathogen seemed reasonable based on clinical findings. When mixed infections occurred in one sample, all isolates were included. When a bacterial strain was isolated more than once within 6 months from the same site in a horse, the isolate was considered to come from a persistent infection and was only counted once in the study.

Culture and susceptibility testing

Depending on the isolation site, samples were streaked on standard agars (Columbia blood agar with sheep blood, Gassner agar, Columbia CNA agar, Chocolate agar with vitox, Oxoid AG, Pratteln, Switzerland) and incubated at 37 °C for 24–48 hours under aerobic conditions. For culture of obligate anaerobes, samples were streaked on Columbia blood agar with sheep blood and Schaedler KV agar (Oxoid AG, Pratteln, Switzerland) and incubated at 37 °C under anaerobic conditions for 48–72 hours. The samples were investigated by standard bacteriological methods using gram staining, catalase test, oxidase test, oxidation-fermentation test, agglutination test to check coagulase activity, agglutination test to classify the Lancefield groups of *Streptococcus* spp., Staphaurex® plus latex agglutination test (Oxoid AG, Pratteln, Switzerland) and growth performance on different agar (Markey, 2013). Identification to species level and antimicrobial susceptibility testing for aerobic bacteria (microdilution method) was conducted using the automated VITEK® 2 Compact system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. The AST-ST01 susceptibility testing card was used for *Streptococcus* spp., the AST-GP69 susceptibility testing card for all other Gram-positive isolates and the AST-GN38 card for Gram-negative isolates. The advanced expert system (AES) of the VITEK® 2 provided minimal inhibitory concentration (MIC) and susceptibility interpretations, which were automatically edited in some phenotypes (Livermore et al., 2002). Interpretation of results referred to the guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2013) and, if information was lacking, guidelines from the European Committee on antimicrobial susceptibility testing (EUCAST, 2016) and the U.S. Food and Drug Administration (FDA, 2016) were used. Intermediate susceptibility was classified as resistant.

ESBL production was evaluated phenotypically (pESBL-producing) in *E. coli* and *Klebsiella* (*K.*) *pneumoniae* ssp. *pneumoniae* by using the VITEK® 2 ESBL-screen test on the AST-GN38 card, assessing the inhibitory effect of cefotaxime, ceftazidime, and cefepime with and without clavulanic acid (Paterson and Bonomo, 2005). The VITEK® 2 cefoxitin-screen test on the AST-GP69 card was used to detect phenotypically methicillin-resistant (pMR) staphylococci (John et al., 2009). Resistance to cefoxitin (6 µg/ml) indicated resistance to all β-lactam antibiotics (CLSI, 2013).

MDR definition

Intrinsic resistance was adopted according to current knowledge (Hollenbeck and Rice, 2012; Leclercq et al., 2013). MDR isolates were defined as isolates with acquired resistance towards ≥1 agent in ≥3 defined antimicrobial categories, according to an international expert proposal for MDR bacteria in human medicine (Magiorakos et al., 2012). If the antimicrobial category

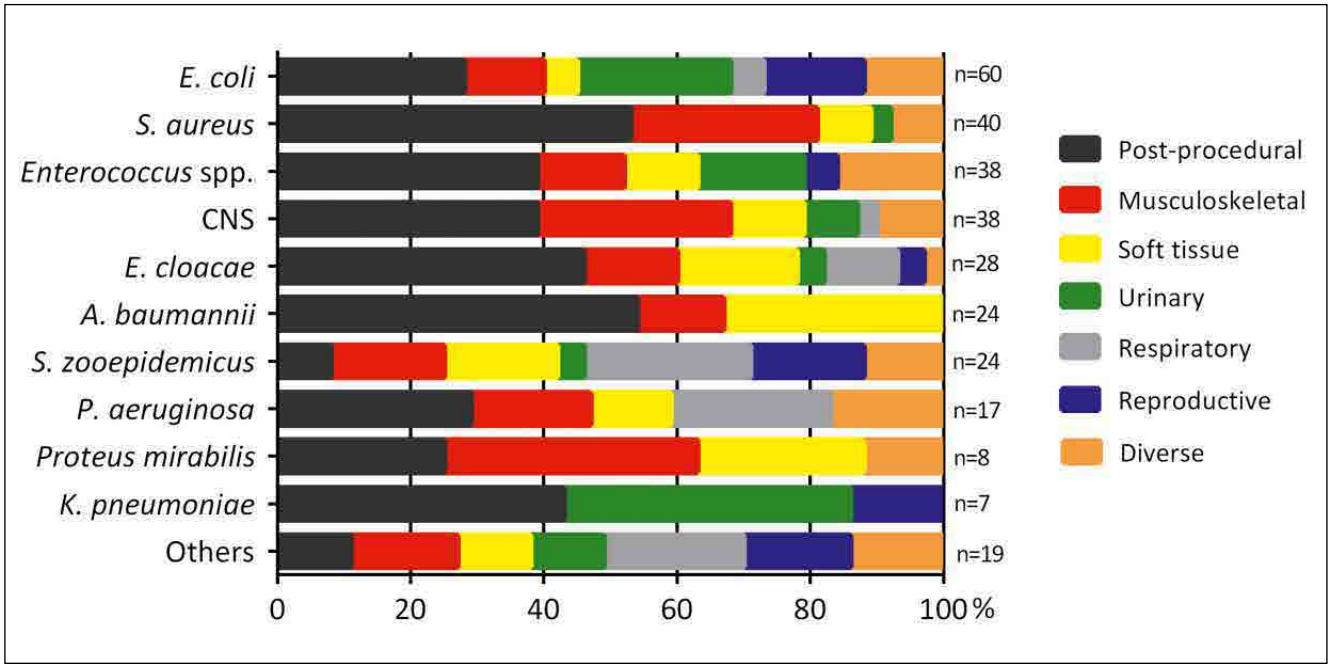

Figure 1: Overview of number and origin of bacterial isolates with antimicrobial testing results from horses at the University of Zurich (2012–2015)
Abbreviations: E. cloacae – *Enterobacter cloacae* complex, S. zooepidemicus – *Streptococcus equi* ssp. *zooepidemicus*, K. pneumoniae – *Klebsiella pneumoniae* ssp. *pneumoniae*
Others include: *Citrobacter* spp. (n=6), *Pasteurella* spp. (n=3), *Proteus vulgaris* group (n=2), *Pantoea agglomerans* (n=2), *Streptococcus uberis* (n=2), *Streptococcus equinus* (n=1), *Bordetella bronchiseptica* (n=1), *Streptococcus equi* ssp. *equi* (n=1), *Streptococcus dysgalactiae* ssp. *equisimilis* (n=1)

was not evaluated in this study (e.g. glycylcyclines), it was not taken into account for definition of MDR isolates. If available, commonly used equine antimicrobials were used for interpretation, instead of human antimicrobials (e.g. enrofloxacin instead of ciprofloxacin). The complete list of analyzed antimicrobials can be found in Table 1. This approach allowed determination of multidrug resistance prevalence in *A. baumannii*, *E. coli*, *Enterobacter cloacae* complex, *K. pneumoniae* ssp. *pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*, *Enterococcus* spp. and *S. aureus*.

Results

The database included 303 susceptibility testing results from equine pathogens: 53/303 (17%) from 2012, 107/303 (35%) from 2013, 83/303 (27%) from 2014, and 60/303 (20%) from 2015. Most results were available for *E. coli* (60/303, 20%) and *S. aureus* (40/303, 13%), followed by *Enterococcus* (*E.*) spp. (containing *E. faecalis* and *E. faecium*) and coagulase-negative staphylococci (CNS) (each 38/303, 13%). Most isolates were sampled from post-procedural (108/303, 36%), musculoskeletal (54/303, 18%), and soft tissue infections (37/303, 12%). An overview of bacterial isolates and their origin is shown in Figure 1.

Resistance rates against common antimicrobials for bacteria with at least 7 isolates tested for antimicrobial susceptibility are shown in Table 2. High rates of acquired antimicrobial resistance were found in Gram-negative isolates. Amikacin was the only effective drug inhibiting growth of all Gram-negatives. Marbofloxacin resistance was rare in Gram-negative isolates, except for *A. baumannii*. *A. baumannii* generally showed high resistance rates and the highest multidrug resistance prevalence of all bacteria (23/24, 96%). *Enterobacter cloacae* complex isolates and *E. coli* also had high multidrug resistance rates of 86% (n=24/28) and 80% (n=48/60), respectively. The ESBL screen test was positive in 36/60 (60%) *E. coli*. Minimal changes in this percentage were seen between years (55–67%). Only a small number of *K. pneumoniae* ssp. *pneumoniae* was tested and 3/7 (43%) were pESBL-producing; all of them isolated from post procedural infections. Prevalence of pESBL-producing *E. coli* and *K. pneumoniae* ssp. *pneumoniae* in different organ systems is shown in Figure 2. *P. aeruginosa* is intrinsically resistant towards a wide range of antimicrobials, but resistance towards other drugs, with the exception of enrofloxacin, was moderate.

In Gram-positive isolates, high resistance rates were found in *Staphylococcus* spp. and *Enterococcus* spp., while *Streptococcus equi* ssp. *zooepidemicus* showed low resistance rates towards most tested antimicrobials, especially towards β-lactam antibiotics. *Staphylococcus* spp. had high resistance rates to penicillin, ampicillin, ampicillin/sulbactam, cephalosporins and tetracycline. Rifampicin and chloramphenicol were effective in inhibiting growth in a high percentage of staphylococci. In addition, most *S. aureus* were susceptible towards erythromycin and fluorochinolons, while CNS were more commonly susceptible to gentamicin. Multidrug-resistance prevalence in *S. aureus* was 65% (n=26/40). The cefoxitin-screen test was positive for 53/78 (68%) tested *Staphylococcus* spp. CNS were more commonly pMR (27/38, 71%, 50–75% during study period) than *S. aureus* (26/40, 65%, 50–80% during study period). A disproportionately high percentage of *Staphylococcus* spp. from post-procedural infections was pMR (14/15, 93% of CNS and 18/21, 86% of *S. aureus*). In Figure 2 occurrence of pMR staphylococci in different organ systems is presented. *Enterococcus* spp. are intrinsically resistant towards all cephalosporins, trimethoprim-sulfamethoxazole (TMPS) and erythromycin. In addition, acquired resistance was common for fluoroquinolons and tetracycline. Ampicillin and ampicillin/sulbactam, followed by chloramphenicol, were most effective against *Enterococcus* spp.

Table 2: Resistance rate towards common antimicrobials and prevalence of multidrug resistance in bacterial pathogens from horses at the University of Zurich (2012–2015)
Only bacterial species with at least 7 tested isolates are shown n.a. not assessed

		Ampicillin	Amox/Clav	Penicillin	Cefalexin (1th Gen)	Ceftiofur (3rd Gen)	Cefpirom (4th Gen)	Gentamicin	Amikacin	TMPS	Enroflox.	Marboflox.	Erythro.	Rifampicin	Tetrac.	Chloramph.	MDR rate
GRAM NEGATIVES																	
A. baumannii	n=24	100%	100%	n.a.	100%	100%	100%	100%	0%	92%	96%	96%	n.a.	n.a.	100%	100%	96%
E. coli	n=60	73%	60%	n.a.	60%	60%	60%	68%	0%	75%	48%	37%	n.a.	n.a.	72%	60%	80%
E. cloacae	n=28	100%	100%	n.a.	100%	86%	86%	86%	0%	86%	68%	14%	n.a.	n.a.	79%	86%	86%
K. pneumoniae	n=7	100%	57%	n.a.	43%	43%	43%	57%	0%	43%	29%	0%	n.a.	n.a.	43%	43%	57%
Proteus mirabilis	n=8	75%	38%	n.a.	38%	25%	25%	63%	0%	75%	63%	38%	n.a.	n.a.	100%	75%	75%
P. aeruginosa	n=17	100%	100%	n.a.	100%	100%	47%	41%	0%	100%	88%	35%	n.a.	n.a.	100%	100%	47%
GRAM POSITIVES		Amp/Sulb															
S. aureus	n=40	83%	65%	83%	65%	65%	65%	60%	n.a.	60%	30%	28%	8%	3%	65%	3%	65%
CNS	n=38	87%	84%	82%	71%	71%	71%	29%	n.a.	47%	47%	50%	50%	8%	63%	16%	n.a.
Enterococcus spp.	n=38	21%	21%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	100%	68%	76%	100%	n.a.	76%	37%	50%
S. zooepidemicus	n=24	0%	n.a.	0%	0% *	0% *	0% *	n.a.	n.a.	33%	n.a.	n.a.	0%	n.a.	21%	n.a.	n.a.

intrinsic resistant | low level intrinsic resistant | 50-100% resistant | 25-49% resistant | 0-24% resistant

* Cefotaxim and Ceftriaxone tested
Abbreviations: for bacteria see Figure 1; Amox/Clav – amoxicillin/clavulanic acid, Amp/Sulb – ampicillin/sulbactam, 1th Gen – first generation cephalosporin, 3rd Gen – third generation cephalosporin, 4th Gen – fourth generation cephalosporin, TMPS – trimethoprim/sulfamethoxazole, Enroflox. – enrofloxacin, Marboflox. – marbofloxacin, Erythro. – erythromycin, Tetrac. – tetracyclin, Chloramph. – chloramphenicol, MDR – multidrug-resistant

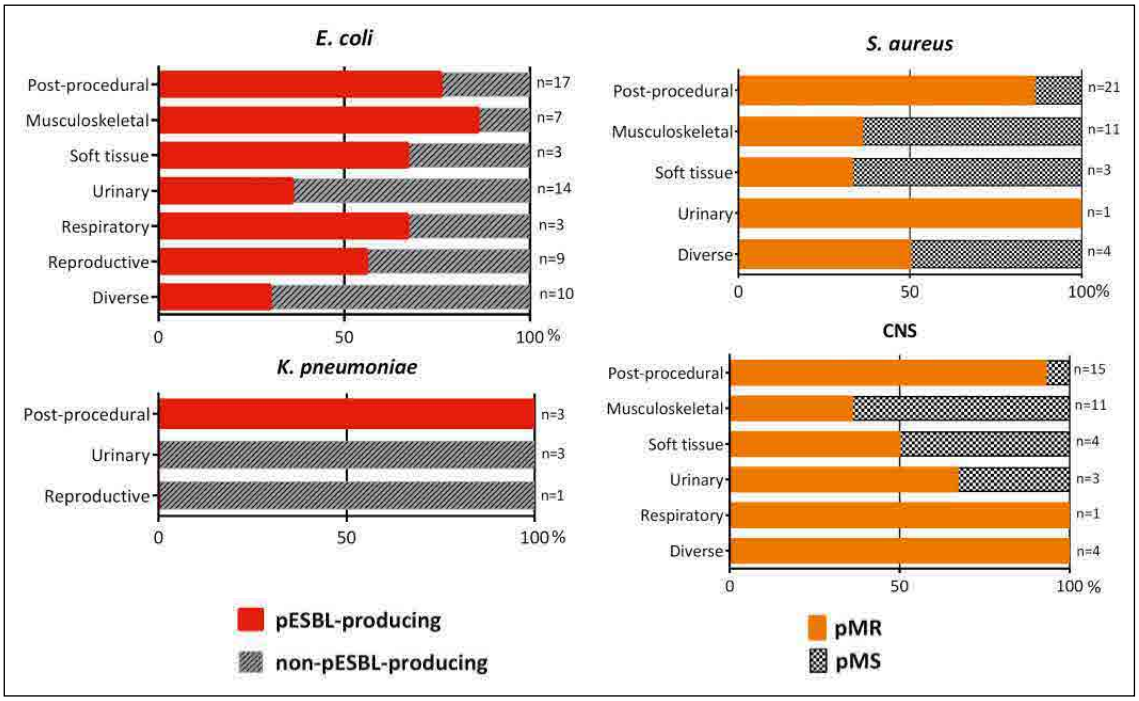

Figure 2: Organ distribution of pESBL-producing *E. coli* and *K. pneumoniae* ssp. *pneumoniae* and pMR *Staphylococcus* spp. from horses at the University of Zurich (2012–2015).
Abbreviations: for bacteria see Figure 1; pESBL-producing – phenotypically ESBL-producing, pMR – phenotypically methicillin-resistant, pMS – phenotypically methicillin-susceptible

Discussion

The results of this study show a high level of antimicrobial resistance in common bacterial pathogens and a high prevalence of MDR pathogens isolated from patients at the Equine Hospital of the University of Zurich. Production of β-lactamase enzymes is the major mechanism of b-lactam resistance in *Enterobacteriaceae* (Poole, 2004). High prevalence rates (up to 100%) of ESBL-producing *E. coli* in feces of hospitalized horses treated with extended-spectrum cephalosporins are present (Dolejska et al., 2011; Damborg et al., 2012). Few studies have reported isolation of ESBL-producing *Enterobacteriaceae* from equine infections such as infected wounds, septic arthritis and sepsis (Dolejska et al., 2011; Dierikx et al., 2012; Smet et al., 2012; Walther et al., 2014). Resistance rates against 3rd or 4th generation cephalosporins in *Enterobacteriaceae* from clinical samples are 5–14% and an increase over time was noted (Clark et al., 2008; Dierikx et al., 2012; Theelen et al., 2014). In our collection, the prevalence of pESBL-producing *E. coli* and *K. pneumoniae* ssp. *pneumoniae* was 60% and 43%, respectively.

In this study *Staphylococcus* spp. showed high resistance rates against β-lactam antimicrobials. Resistance towards penicillin is frequent in equine isolates (Clark et al., 2008; Theelen et al., 2014), while resistance towards other β-lactams, especially cephalosporins, is rarely reported (Clark et al., 2008; Theelen et al., 2014). Here, 68% of staphylococci showed cephalosporin resistance due to methicillin resistance. Methicillin resistance precludes therapy with all β-lactam antimicrobials, including all penicillins, cephalosporins and carbapenems (Poole, 2004). In previous equine studies MRSA prevalence was 25–37% (Cuny et al., 2006; van Duijkeren et al., 2010), but in our study it was 65% MRSA and 71% MR-CNS. An outbreak of infections with a MDR *S. aureus* as reported in another hospital (Panchaud et al., 2010) did not occur and isolation rate remained stable between 2012 and 2015. Possible reasons for the increased prevalence include population and environmental factors, antimicrobial treatment practices and diagnostic methods. In another study an increase in prevalence of MRS was noted over time and therefore a higher occurrence can be expected in more recent data (van Duijkeren et al., 2010). Additionally, several staphylococcal species with different prevalence of methicillin resistance are included in the MR-CNS group (Becker et al., 2014). Members of this group are increasingly important opportunistic pathogens in human medicine (Becker et al., 2014), but there are few reports of equine infections (Trostle et al., 2001; Corrente et al., 2009). The increasing number of CNS in clinical samples at our hospital (van Spijk et al., 2016 accepted) indicates that these species are potentially emerging in equine disease.

Acinetobacter spp., especially *A. baumannii*, are opportunistic pathogens involved in various equine infections (Weese, 2009). In recent years, they gained attention because of their ability to acquire high rates of antimicrobial resistance and the occurrence of pan-resistant *A. baumannii* (Valencia et al., 2009; Weese, 2009). An increasing occurrence of *Acinetobacter* spp. from equine infections was recently shown in our hospital (van Spijk et al., 2016 accepted) and isolates showed very high resistance rates (96% MDR). Amikacin, enrofloxacin and imipenem are generally thought to remain effective (Weese, 2009). While fluorochinolons showed poor efficacy in our isolates, no amikacin resistance was observed and only a single isolate was imipenem-resistant (data not shown). Only little is known about the clinical importance of MDR *A. baumannii* infections in horses. Together with case reports of horses with similar infections (Jokisalo et al., 2010) and human literature, our results suggest that clinicians should be aware of this highly resistant pathogen, especially in hospitalized horses.

An increase in resistant enterococci was recently found in foal sepsis isolates and cases of MDR *Enterococcus* spp. causing musculoskeletal infections have been reported (Herdan et al., 2012; Theelen et al., 2014). Ampicillin alone or in combination with sulbactam and chloramphenicol remain reasonable treatment options (Weese, 2009; Theelen et al., 2014). Low-level resistance towards penicillin and gentamicin is intrinsic to enterococci, but combinations of both drugs can remain effective (Weese, 2009; Hollenbeck and Rice, 2012). However, very high MICs for gentamicin, so-called high level gentamicin resistance, in 68% of our enterococci isolates precludes such a therapeutic approach (data not shown). Resistance towards vancomycin is of critical concern in *Enterococcus* spp. in human medicine (Giguère et al., 2013; Maddox et al., 2015). Fortunately, vancomycin-resistant enterococci were not present in our samples (data not shown).

Similarly to other studies, *P. aeruginosa* isolates showed low resistance towards gentamicin, amikacin, and marbofloxacin, but high resistance rates towards enrofloxacin (Clark et al., 2008; Theelen et al., 2014). The use of fluorchinolons in general should be minimized because of their classification as critically important drugs in human medicine (WHO, 2011).

A low-level of antimicrobial resistance was seen in *S. equi* ssp. *zooepidemicus* isolates. In accordance to other studies, full susceptibility towards b-lactam antibiotics was seen and they remain the most important drugs in treating infections caused by streptococci (Erol et al., 2012; Theelen et al., 2014). In order to reduce the use of critically important antimicrobials for human medicine (WHO, 2011), penicillin should be preferred to later-generation cephalosporins in these cases. Combinations of penicillin with streptomycin are contraindicated due to widespread resistance against streptomycin and its common side effects (Giguère et al., 2013).

A disproportionally high rate of pMR staphylococci (89%) was isolated from post-procedural infections, compared to 68% in staphylococci from all samples. MDR *A. baumannii* and pESBL-producing *Enterobacteriaceae* were other frequent pathogens from post-procedural infections. Factors like antimicrobial therapy, hospitalization, duration of therapy and hospitalization, as well as local or systemic immunosuppression favor the development of resistant isolates in these infections (Struelens, 1998). Bacteriological culture and susceptibility testing are particularly important to guide antimicrobial therapy in these cases.

The high resistance rates against commonly used antimicrobials found in this study, would suggest a high percentage of prophylactic and therapeutic treatment failure. However, prevalence of post-operative infections at our hospital is comparable to other studies, e.g. confirmed bacterial incisional infections occurred in 7.4% of colic surgeries (data not shown) compared to 9.3% in other studies (Torfs et al., 2010). Furthermore, the general clinical perception at our hospital is that bacterial infections, other than sepsis, are not generally associated with a high mortality rate, although outcome was not specifically analyzed in this study. Results of the present study show that antimicrobial therapy is frequently ineffective and likely unnecessary as no increase in therapy failure seemed to occur. Depending on the affected organ, additional therapy such as local therapy is considered to be of major importance to successfully treat bacterial infections.

Caution needs to be applied when comparing to results from older studies, which mainly used formerly common disc diffusion testing methods and out-dated CLSI guidelines. In this study only samples from hospitalized horses were included and extrapolation of results to the field is difficult. Our hospital population is frequently treated with antimicrobials prior to referral and samples from complicated cases might be overrepresented. However, primary cases were also included. Definition of MDR isolates is inconsistent in medical literature, precluding reliable comparison of data. In order to reach a standardized definition, we implemented the multidrug resistance definition from human medicine (Magiorakos et al., 2012). This adaption was limited by unavailability of certain susceptibility results and differing antimicrobial agents in human and veterinary medicine. Therefore, establishment of a standard definition of MDR bacteria in veterinary medicine should be sup-

ported. Identification of ESBL-producing *Enterobacteriaceae* and MRS was limited to phenotypical analysis. Genotypic analysis is not common for routine clinical diagnostics, but would allow confirmation of results and provide epidemiological information.

Conclusion

In conclusion, high levels of resistance in equine pathogens were present, complicating therapy with commonly used antimicrobial agents, like penicillins, cephalosporins, gentamicin or TMPS. This underlines the importance of individual bacteriological and antimicrobial susceptibility testing as well as minimizing and optimizing the antimicrobial therapy in horses to reduce further development of resistance.

Acknowledgements

The study was supported by a Forschungskredit of the University of Zurich, grant no. [FK-14-052].

Conflict of interest

The authors have no conflict of interest to declare.

Analyse rétrospective des résistances rencontrées chez les bactéries pathogènes isolées sur des chevaux hospitalisés (2012–2015)

Les résistances aux antibiotiques font partie des plus grands défis rencontrés par la médecine vétérinaire de notre temps. Le but de la présente étude était d'étudier la situation des résistances chez les bactéries pathogènes les plus communes ainsi que la survenance de germes multi-résistants. On a procédé à une analyse rétrospective de tous les tests de sensibilité effectués sur des germes pathogènes isolés sur des chevaux à l'Hôpital vétérinaire de Zürich entre 2012 et 2015. Les isolats présentant des résistances vis-à-vis de trois classes d'antibiotiques ou plus ont été définis comme multi-résistants. Au total on a exploité les résultats de 303 tests de sensibilité, parmi lesquels on trouvait particulièrement des résultats concernant *Escherichia coli* (60/303, 20%) et *Staphylococcus aureus* (40/303, 13%). La grande majorité des germes pathogènes isolés sur des chevaux présentaient un taux de résistances élevé face aux antibiotiques usuels, *Acinetobacter baumannii* (23/24, 96%), suivi par des isolats complexes d'*Enterobacter cloacae* (24/28, 86%) et par *Escherichia coli* (48/60, 80%) montrant la plus haute proportion de résistances multiples. En outre, 60% des isolats d'*Escherichia coli* étaient phénotypiquement producteurs d'ESBL et 68% des staphylocoques étaient phénotypiquement résistant à la méthicilline. Ces taux élevés de résistances vis-à-vis des antibiotiques usuels sont inquiétants et soulignent l'importance d'une culture avec test de sensibilité pour pouvoir mettre en œuvre un traitement ciblé. La réduction et l'optimisation de l'usage des antibiotiques chez le cheval sont d'une grande importance.

Valutazione retrospettiva della prevalenza della resistenza ai batteri patogeni nei cavalli ricoverati (2012–2015)

La resistenza agli antibiotici fa parte delle più grandi sfide nel campo della medicina veterinaria ai nostri giorni. Lo scopo di questo studio era di valutare la prevalenza della resistenza dei batteri patogeni più comuni e la presenza di germi multi-resistenti. Un'analisi retrospettiva di tutti i test di sensibilità dei batteri patogeni nei cavalli è stata eseguita presso l'Ospedale veterinario di Zurigo (2012–2015). Gli isolati che mostrano resistenze verso tre o più classi di antibiotici sono stati definiti come multi-resistenti. Complessivamente, sono stati valutati i risultati di 303 prove di resistenza, tra cui ricorrevano di frequente *Escherichia coli* (60/303, 20%), e *Staphylococcus aureus* (40/303, 13%). La grande maggioranza degli agenti infettivi isolati dei cavalli hanno mostrato alti tassi di resistenza agli antibiotici usati comunemente, quali *Acinetobacter baumannii* (23/24, 96%), seguito da *Enterobacter cloacae* isolati complessi (24/28, 86%) e *Escherichia coli* (48/60, 80 %) che ha ottenuto il più alto tasso di resistenza. Inoltre, il 60% degli isolati di *Escherichia coli* erano fenotipicamente produttori di ESBL e il 68% degli stafilococchi erano fenotipicamente meticillino-resistenti. Gli alti tassi di resistenza agli antibiotici usati comunemente sono preoccupanti e sottolineano l'importanza di una cultura con test di sensibilità dell'agente infettivo, al fine di avviare una terapia mirata. La minimizzazione e l'ottimizzazione dell'uso di antibiotici nel cavallo sono della massima importanza.

References

Becker, K., Heilmann, C., Peters, G.: Coagulase-negative staphylococci. Clin. Microbiol. Rev. 2014, 27: 870–926.

Canton, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., Coque, T. M.: Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. Clin. Microbiol. Infect. 2008, 14 Suppl 1: 144–153.

Clark, C., Greenwood, S., Boison, J. O., Chirino-Trejo, M., Dowling, P. M.: Bacterial isolates from equine infections in western Canada (1998–2003). Can. Vet. J. 2008, 49: 153–160.

CLSI, 2013. VET01-S2 Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals.

Corrente, M., D'Abramo, M., Latronico, F., Greco, M. F., Bellacicco, A. L., Greco, G., Martella, V., Buonavoglia, D.: Methicillin-resistant coagulase negative staphylococci isolated from horses. New Microbiol. 2009, 32: 311–314.

Cuny, C., Kuemmerle, J., Stanek, C., Willey, B., Strommenger, B., Witte, W.: Emergence of MRSA infections in horses in a veterinary hospital: strain characterisation and comparison with MRSA from humans. Euro. Surveill. 2006, 11: 44–47.

Damborg, P., Marskar, P., Baptiste, K.E., Guardabassi, L.: Faecal shedding of CTX-M-producing *Escherichia coli* in horses receiving broad-spectrum antimicrobial prophylaxis after hospital admission. Vet. Microbiol. 2012, 154: 298–304.

Dierikx, C. M., van Duijkeren, E., Schoormans, A. H., van Essen-Zandbergen, A., Veldman, K., Kant, A., Huijsdens, X. W., van der Zwaluw, K., Wagenaar, J. A., Mevius, D. J.: Occurrence and characteristics of extended-spectrum-beta-lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. J. Antimicrob. Chemother. 2012, 67: 1368–1374.

Dolejska, M., Duskova, E., Rybarikova, J., Janoszowska, D., Roubalova, E., Dibdakova, K., Maceckova, G., Kohoutova, L., Literak, I., Smola, J., Cizek, A.: Plasmids carrying blaCTX-M-1 and qnr genes in *Escherichia coli* isolates from an equine clinic and a horseback riding centre. J. Antimicrob. Chemother. 2011, 66: 757–764.

Erol, E., Locke, S. J., Donahoe, J. K., Mackin, M. A., Carter, C. N.: Beta-hemolytic *Streptococcus* spp. from horses: a retrospective study (2000–2010). J. Vet. Diagn. Invest. 2012, 24: 142–147.

EUCAST 2016. Breakpoint tables for interpratation of MICs and zone diameters, Version 6.0. http://www.eucast.org

FDA 2016. U.S. Food and Drug Administration. http://www.fda.gov/

Giguère, S., Prescott, J. F., Dowling, P. M.: Antimicrobial therapy in veterinary medicine, 5th Edition. Wiley Blackwell, Ames, Iowa, USA.

Herdan, C. L., Acke, E., Dicken, M., Archer, R. M., Forsyth, S. F., Gee, E. K., Pauwels, F. E.: Multi-drug-resistant *Enterococcus* spp. as a cause of non-responsive septic synovitis in three horses. N. Z. Vet. J. 2012, 60: 297–304.

Hollenbeck, B. L., Rice, L. B.: Intrinsic and acquired resistance mechanisms in *Enterococcus*. Virulence. 2012, 3: 421–433.

John, M. A., Burden, J., Stuart, J. I., Reyes, R. C., Lannigan, R., Milburn, S., Diagre, D., Wilson, B., Hussain, Z.: Comparison of three phenotypic techniques for detection of methicillin resistance in *Staphylococcus* spp. reveals a species-dependent performance. J. Antimicrob. Chemother. 2009, 63: 493–496.

Jokisalo, J., Bryan, J., Legget, B., Abbott, Y., Katz, L. M.: Multiple-drug resistant *Acinetobacter baumannii* bronchopneumonia in a colt following intensive care treatment. Equine Vet. Educ. 2010, 22: 281–286.

Leclercq, R., Canton, R., Brown, D. F., Giske, C. G., Heisig, P., MacGowan, A. P., Mouton, J. W., Nordmann, P., Rodloff, A. C., Rossolini, G. M., Soussy, C. J., Steinbakk, M., Winstanley, T. G., Kahlmeter, G.: EUCAST expert rules in antimicrobial susceptibility testing. Clin. Microbiol. Infect. 2013, 19: 141–160.

Livermore, D. M., Struelens, M., Amorim, J., Baquero, F., Bille, J., Canton, R., Henning, S., Gatermann, S., Marchese, A., Mittermayer, H., Nonhoff, C., Oakton, K. J., Praplan, F., Ramos, H., Schito, G. C., Van Eldere, J., Verhaegen, J., Verhoef, J., Visser, M. R.: Multicentre evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests. J. Antimicrob. Chemother. 2002, 49: 289–300.

Maddox, T. W., Clegg, P. D., Williams, N. J., Pinchbeck, G. L.: Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance. Equine Vet. J. 2015, 47: 756–765.

Maddox, T. W., Scantlebury, C. E., Clegg, P. D., Dawson, S., Pinchbeck, G. L., Williams, N. J.: A review of the characteristics and treatment of methicillin-resistant Staphylococcus aureus (MRSA) in the horse and a case series of MRSA infection in four horses. Equine Vet. Educ. 2010, 22: 91–102.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., Monnet, D. L.: Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012, 18: 268–281.

Markey, B.K.: Clinical veterinary microbiology, 2nd Edition. Elsevier, Edinburgh.

Moodley, A., Guardabassi, L.: Clonal spread of methicillin-resistant coagulase-negative staphylococci among horses, personnel and environmental sites at equine facilities. Vet. Microbiol. 2009, 137: 397–401.

Panchaud, Y., Gerber, V., Rossano, A., Perreten, V.: Bacterial infections in horses: a retrospective study at the University Equine Clinic of Bern. Schweiz. Arch. Tierheilk. 2010, 152: 176-182.

Paterson, D. L., Bonomo, R. A.: Extended-spectrum beta-lactamases: a clinical update. Clin. Microbiol. Rev. 2005, 18: 657–686.

Poole, K.: Resistance to beta-lactam antibiotics. Cell. Mol. Life Sci. 2004, 61: 2200–2223.

Smet, A., Boyen, F., Flahou, B., Doublet, B., Praud, K., Martens, A., Butaye, P., Cloeckaert, A., Haesebrouck, F.: Emergence of CTX-M-2-producing *Escherichia coli* in diseased horses: evidence of genetic exchanges of bla(CTX-M-2) linked to ISCR1. J. Antimicrob. Chemother. 2012, 67: 1289–1291.

Struelens, M. J.: The epidemiology of antimicrobial resistance in hospital acquired infections: problems and possible solutions. Bmj. 1998, 317: 652-654.

Theelen, M. J., Wilson, W. D., Edman, J. M., Magdesian, K. G., Kass, P. H.: Temporal trends in in vitro antimicrobial susceptibility patterns of bacteria isolated from foals with sepsis: 1979–2010. Equine Vet. J. 2014, 46: 161–168.

Toombs-Ruane, L. J., Riley, C. B., Kendall, A. T., Bolwell, C. F., Benschop, J., Rosanowski, S. M.: Antimicrobial Susceptibilities of Aerobic Isolates from Respiratory Samples of Young New Zealand Horses. J. Vet. Intern. Med. 2015, 29: 1700–1706.

Torfs, S., Levet, T., Delesalle, C., Dewulf, J., Vlaminck, L., Pille, F., Lefere, L., Martens, A.: Risk factors for incisional complications after exploratory celiotomy in horses: do skin staples increase the risk? Vet. Surg. 2010, 39: 616–620.

Trostle, S. S., Peavey, C. L., King, D. S., Hartmann, F. A.: Treatment of methicillin-resistant Staphylococcus epidermidis infection following repair of an ulnar fracture and humeroradial joint luxation in a horse. J. Am. Vet. Med. Assoc. 2001, 218: 554–559, 527.

Valencia, R., Arroyo, L. A., Conde, M., Aldana, J.M., Torres, M. J., Fernandez-Cuenca, F., Garnacho-Montero, J., Cisneros, J. M., Ortiz, C., Pachon, J., Aznar, J.: Nosocomial outbreak of infection with pan-drug-resistant *Acinetobacter baumannii* in a tertiary care university hospital. Infect. Control Hosp. Epidemiol. 2009, 30: 257–263.

Van den Eede, A., Hermans, K., Van den Abeele, A., Flore, K., Dewulf, J., Vanderhaeghen, W., Crombe, F., Butaye, P., Gasthuys, F., Haesebrouck, F., Martens, A.: Methicillin-resistant *Staphylococcus aureus* (MRSA) on the skin of long-term hospitalised horses. Vet. J. 2012, 193: 408–411.

van Duijkeren, E., Moleman, M., Sloet van Oldruitenborgh-Oosterbaan, M. M., Multem, J., Troelstra, A., Fluit, A. C., van Wamel, W. J., Houwers, D. J., de Neeling, A. J., Wagenaar, J. A.: Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. Vet. Microbiol. 2010, 141: 96–102.

van Spijk, J. N., Schmitt, S., Fuerst, A. E., Schoster, A.: A retrospective analysis of bacterial pathogens in hospitalized horses (1988–2014). Schweiz. Arch. Tierheilk. 2016 accepted.

Walther, B., Lubke-Becker, A., Stamm, I., Gehlen, H., Barton, A. K., Janssen, T., Wieler, L. H., Guenther, S.: Suspected nosocomial infections with multi-drug resistant E. coli, including extended-spectrum beta-lactamase (ESBL)-producing strains, in an equine clinic. Berl. Munch. Tierarztl. Wochenschr. 2014, 127: 421–427.

Weese, J. S.: Antimicrobial therapy for multidrug resistant pathogens. Equine Vet. Educ. 2009, 21: 328–334.

Weese, J. S., Giguere, S., Guardabassi, L., Morley, P. S., Papich, M., Ricciuto, D. R., Sykes, J. E.: ACVIM Consensus Statement on Therapeutic Antimicrobial Use in Animals and Antimicrobial Resistance. J. Vet. Intern. Med. 2015, 29: 487–498.

Weese, J. S., Rousseau, J., Willey, B. M., Archambault, M., McGeer, A., Low, D. E.: Methicillin-resistant *Staphylococcus aureus* in horses at a veterinary teaching hospital: frequency, characterization, and association with clinical disease. J. Vet. Intern. Med. 2006, 20: 182–186.

WHO 2011. Critically important antimicrobials for human medicine.

Corresponding author

Dr. med. vet. Sarah Schmitt
FVH (Bakteriologie), Fachtierärztin für Mikrobiologie
Laborleiterin, Wissenschaftliche Mitarbeiterin
Institut für Veterinärbakteriologie
Vetsuisse-Fakultät Universität Zürich
Winterthurerstrasse 270
8057 Zürich
Tel.: +41 44 635 86 09
Fax: +41 44 635 89 12
E-Mail: sarah.schmitt@vetbakt.uzh.ch