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AEROBIC, MICROAEROPHILIC, AND GRAM-POSITIVE

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ABSTRACT

Isolated from horse pathologies and lesions, this research set out to identify the various microbial genera and species that are aerobic and micro-aerophilic. Between 2009 and 2014, 449 horse clinical samples were tested. Out of these, 229 (or 51% of the total) came from the respiratory tract, 121 (or 26.9%) from the skin, 40 (or 8.9%) from the GI tract, 40 (or 8.9%) from the eyes, 8 (or 1.8% of the total) from the UTI, 6 (or 1.3% of the total) from the MSK, 4 (or 0.9% of the total) from the lymph and 1 (or 0.2% of the total) from the milk. Molecular phenotypic MALDI-TOF validated the putative phenotypic identification of the isolates. With prevalence rates of 37.2%, 23.4%, and 7.6%, respectively, the most often found strains (n = 330) were Staphylococcus spp., Streptococcus spp., and Corynebacterium spp. On top of that, twenty-four more taxa were discovered, including species of Bacillus, Enterococcus, Trueperella, Aerococcus, Dermatophilus, Lysinibacillus, Nocardiopsis, and Streptomyces. The vast majority of these infections are classified as opportunistic, meaning they may infect horses. To determine antibiotic susceptibility, the disc diffusion technique was used. The antibiotics that worked best were florfenicol, amoxycillin, and clavulanic acid. Every single one of the strains of Bacillus, Lysinibacillus, Corynebacterium, Dermatophilus congolensis, Streptococcus, Enterococcus, Aerococcus, Nocardiopsis alba, and Trueperella pyogenes that were evaluated showed a complete and utter sensitivity to florfenicol. Staphylococcus aureus had a susceptibility of 96.2% to florfenicol, whereas other staphylococci had a susceptibility of 98.5%. Nocardiopsis alba, Streptococcus spp., Aerococcus spp., Streptomyces spp., Dermatophilus congolensis, Streptococcus spp., and Trueperella pyogenes were among the organisms that were completely eradicated by the combination of amoxycillin and clavulanic acid. Amoxycillin with clavulanic acid was effective against 89.8% of Staphylococcus aureus strains, 98.8% of other staphylococci strains, and 20.0% of Bacillus/Lysinibacillus spp. strains.

Keywords: frequency, harmfulness, clinical presentation, immunity, protective barrier, vulnerability

Introduction

The aim of this study was to describe the genera and species of Gram-positive aerobic and micro- aerophilic microorganisms isolated from patho- logical processes and lesions in horses and to characterise their susceptibilities to antimicrobials. Staphylococci are the most frequently isolated microorganisms from veterinary clinical material (Songer and Post 2005). Previously, Wintzer (1999)reported that staphylococcal infections were associ- ated with skin and granulomatous fistulous lesions of horses. Occasionally, *S. aureus* and other pyo- genic staphylococci, which are rather considered as secondary microflora in purulent infections, are isolated from the respiratory system and paranasal sinuses of horses and also from tracheobronchial secretions (Wintzer 1999). Songer and Post (2005)

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doi: 10.17221/107/2016-VETMED linked *S. aureus*

with infections of wounds, skin, joints, mammary glands as well as vaginal infec- tions in various species of animals. Less frequently, coagulase-positive staphylococci such as *S. pseu- dointermedius* and *S. delphini* have been described as opportunistic pathogens in horses (Stull et al. 2014). Stull et al. (2014) also describe *S. warneri* in doves (septicaemia), *S. intermedius* (dermati- tis, otitis, mastitis) in dogs, cattle, birds and occa- sionally in horses, *S. hyicus* (dermatitis, arthritis, mastitis) in pigs, cattle and birds, as potentially pathogenic species in animals. The same authors include *S. chromogenes*, *S. sciuri* and *S. equorums* in the normal microflora of the skin and mucous membranes of cattle, pigs, horses and squirrels. Other authors have described infections of animals caused by rare coagulase-negative staphylococci, such as *S. auricularis* in canine otitis (Silva 2001). Streptococcal infections are the most important and most widespread infectious diseases in horses and often have a difficult course (Wintzer 1999). Streptococci are involved primarily in diseases of the respiratory system and joints. They can cause abortions, sterility and may be the source of sec- ondary viral infections. *S. equi* ssp. *equi*, *S. equi* ssp. *zooepidemicus* and *S. pneumoniae* are considered as pathogenic in horses. *S. dysgalactiae* ssp. *equi- similis* was described as a possible cause of chronic diseases of the upper respiratory tract (Laus et al. 2007). *Streptococcus agalactiae* is a common find- ing in cattle, sheep and goats with mastitis, as well as in humans, dogs and cats where it causes urogenital infections (Quinn et al. 1994). Strains detected in horses have similar characteristics as human isolates (Yildirim et al. 2002). Erol et al. (2012a) reported the relative percentages of differ- ent β-haemolytic streptococci in horses. The most common type was *S. equi* ssp. *zooepidemicus* (72.0% of isolates), followed by *S. dysgalactiae* ssp. *equi- similis* (21.3% of isolates), *S. equi* ssp. *equi* (5.8% of isolates), while the remainder were unidenti- fied β-haemolytic streptococci (0.9% of isolates). *Streptococcus pluranimalium* is a part of the natural microflora of the vagina, cervix and tonsils of cat- tle and can cause bovine mastitis. *Streptococcus equinus* is also isolated from bovine mastitis, as well as cases of septicaemia and arthritis in pigeons (Songer and Post 2005). Papadimitriou et al. (2014) reported the potential participation of *S. bovis* and *S. equinus* in human endocarditis and colon cancer.

Streptococcus infantis is described as a commensalof the upper respiratory tract in human medicine (Bek-Thomsen et al. 2008). The pathogenicity of *Enterococcus durans* was not clearly demonstrated in horses (Wintzer 1999), while the *E. faecalis* is as- sociated with endocarditis (Bolin et al. 2005), with the microflora of chronic wounds (Freeman et al. 2009), and with synovitis (Herdan et al. 2012). *Aerococcus viridans* tends to be associated with environmental bovine mastitis (Liu et al. 2015) and rarely with endocarditis, septicaemia and arthritis in various animals (Popescu et al. 2005), as well as with human urological infections (Leite et al. 2010). Another large group of microorganisms iso- lated from horses are coryneform microorganisms. Songer and Post (2005) described microorganisms of the *Corynebacterium* genus particularly in cattle, sheep, goats, and rodents (mastitis, dermatitis, uri- nary infections, infections of the lymphatic system and pneumonia); these species are less frequently detected in dogs, pigs, turtles and also in humans (dermatitis, otitis, vaginitis, necrotic lesions, uri- nary tract infections, diphtheria). Of these, only *C. pseudotuberculosis* and *C. diphtheriae* are equine pathogens (abscesses and ulcerative lymphangitis, wound infections). Many corynebacteria are known to produce urease, phospholipases, haemolysins, mycolic acids and diphtheric toxin (Songer and Post 2005). *C. afermentans* is associated with organ in- fections and sepsis in human patients (Funke et al. 1997), (Minkin and Shapiro 2004), *C. stationis* with bovine mastitis (Leon-Galvan et al. 2015), while *C. flavescens* may cause colour changes on the surface of ripened cheese (Masoud and Jakobsen 2003). *Trueperella pyogenes* is the most common opportunistic animal pathogen which is a producer of pyolysin with dermonecrotoxic and lethal effects (Songer and Post 2005). It may also be considered as a coryneform microorganism. *Dermatophilus congolensis* is the cause of exudative dermatitis in cattle, sheep and horses (Wintzer 1999), especially in tropical and subtropical regions (Songer and Post 2005). *Nocardiopsis alba* was discovered in bio- aerosol produced by compost for mushroom culti- vation and can cause a variety of health problems, including so-called hypersensitivity pneumonia (Pasciak et al. 2014). Nocardioform microorgana causative agent of cattle mastitis and abortions in various animals, including horses (Songer and Post 2005); *B. cereus* and *B. mycoides* can also produce enterotoxin (Bednarczyk and Daczkowska-Kozon 2007). Members of the family *Lysinibacillus* have also been described as causes of bacteraemia and sepsis (Wenzler et al. 2015).

To date, several studies have reported the occur- rence of antibiotic resistance, as well as increas- ing rates of resistance, particularly in frequently detected microorganisms such as *S. aureus* and β-haemolytic streptococci. Rubin et al. (2011) described resistance to penicillin (43%) and tet- racycline (10%) in *S. aureus* strains isolated from equines. Peyrou et al. (2003) published a compari- son of the resistance of veterinary hospital strains identified in horses in the period 1986–1989, and described resistance in the period 1996–1998. A rise of resistant coagulase-positive staphylococci was recorded in the case of cotrimoxazole, from 0% to 33%, while in response to penicillin a decrease in the number of resistant strains was recorded, from 70% to 41%. Uwaezuoke and Aririatu (2004) reported resistance rates of 95.8% to penicillin, 87.5% to tetracycline and 33.3% to streptomycin, in human strains of *S. aureus*.

Erol et al. (2012a) published a very detailed work about β-haemolytic streptococci in horses and their susceptibility to antimicrobial agents. They assessed the susceptibility of microorganisms to 11 antimicrobial substances and found varia- tion between different streptococci. For example, penicillin susceptibility ranged between 97.1% and 99.2%, susceptibility to bacitracin between 79.4% and 100%, tetracycline susceptibility between 44.0% and 98.8%, erythromycin between 87.8% and 99.2%, gentamicin between 82.8% and 91.2% and cotri- moxazole between 30.9% and 94.4%.

MATERIAL AND METhODS

All samples were collected from pathological le- sions and processes of clinically diseased animals and they included the following materials:

Samples from the digestive tract. Faeces, rec- tal swabs, and swabs taken from the stomach lin- ing were examined routinely using conventional methods of cultivation on meat peptone blood agar (MPBA), endo agar (EA) and xylose lysine de- oxycholate citrate agar (XLD; Trios s.r.o., Prague,Czech Republic) and plates were incubated aero- bically at 37 ± 1 °C for 24 h. Parallel cultivation focusing on organisms of the genus *Salmonella* was also carried out by non-selective enrichment of 1 g of the material in 9 ml buffered peptone water (BPW) at 37 ± 1 °C for 18 h, followed by a selective enrichment of 0.1 ml of the incubated BPW on semisolid Rappaport-Vassiliadis agar (MSRV) at 41.5 ± 1 °C for 24 h and subsequent double inocula- tion onto XLD agar and Rambach agar (RA; Trios s.r.o., Prague, Czech Republic). The incubation of plates was again conducted at 37 ± 1 °C for 24 h.

Samples from the skin and urinary appara- tus. The cultivation of hair, swabs, scrapings of skin, urine and swabs of the urinary tract was performed on MPBA, EA, Edward's agar (EDW) and Sabouraud agar with chloramphenicol (SAC; Trios s.r.o., Prague, Czech Republic) and the plates were again incubated aerobically at 37 ± 1 °C for 24 h. SAC was incubated for 120 h at 21 ± 1 °C. In indicated cases, these materials also underwent microaerophilic cultivation.

Samples from the oral cavity, eyes, respiratory, musculoskeletal and lymphatic system. Swabs and the lavage of the respiratory tract, pharynx, conjunctiva, oral mucosa, and the puncture of chest, lymph nodes and joints were all cultured as materials from the skin. In addition, micro- aerophilic incubation of inoculated plates with MPBA and Haemophilus testing medium (HTM; Trios s.r.o., Prague, Czech Republic) was per- formed. Microaerophilic cultivation took place in a plastic box for microaerophilic cultivation (GEN box) of 2.5-litre volume (BioMerieux, Marcy l'Etoile, France) with the CO_2 GEN box microaer (BioMerieux, Marcy l'Etoile, France) for 48 h at 37 ± 1 °C.

Mammary gland and milk samples. Colostrum was cultivated on MPBA, and incubation was car- ried out at 37 ± 1 °C for 42–48 h. Samples were concurrently cultivated on SAC. These plates were incubated for 120 h at 21 ± 1 °C. In parallel, milk samples were incubated after inoculation on MPBA in culture tubes at 37 ± 1 °C for 18–24 h; after in- oculation onto EDW, plates were incubated at 37 ± 1 °C for a further

18–24 h.

Bacteriological confirmation and susceptibility determination. All types of colonies on plates were isolated, and the growth of suspected a MALDI-TOF mass detector (Bruker Daltoniks GmbH, Bremen, Germany). Clinical strains were tested for antibiotic susceptibility using the disc diffusion method. Muller Hinton agar (Trios s.r.o., Prague, Czech Republic) and antibiotic discs were used for testing (Oxoid Ltd., Basingstoke, UK). The tested antibiotics were penicillin G, streptomycin, neomycin, gentamicin, florfenicol, tetracycline, erythromycin, clindamycin, amoxycillin/clavulan- ic acid, enrofloxacin, bacitracin, cephalothin and cotrimoxazole. Tests were assessed after 18–24 h of incubation at 37 ± 1 °C. The interpretation of values was performed accordance to CLSI stand- ards. All used discs and mediums were tested with *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) reference strains.

RESULTS

In the period of 2009–2014, 449 clinical samples from horses were examined, out of which 229 (51.0%) were collected from the respiratory tract, 121 (26.9%) from the skin, 40

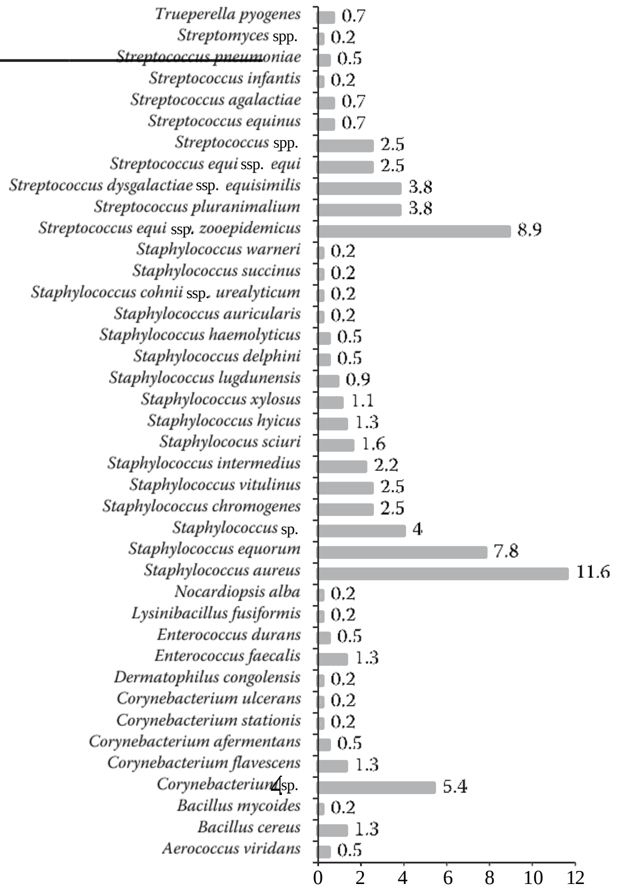
Gram-positive organisms was subsequently confirmed by a phenotypic molecular method usingdoi: 10.17221/107/2016-VETMED

(8.9%) from the diges- tive tract, 40 (8.9%) from the eyes, eight (1.8%) from the urinary tract, six (1.3%) from the musculoskel- etal system, four (0.9%) from the lymphatic system and one (0.2%) from milk. The detailed list of tested samples is shown in Table 1. From these samples330 bacterial strains were isolated: *Staphylococcus* in 167 cases (37.2% prevalence), *Streptococcus* spp. in 105 cases (23.4%), *Corynebacterium* spp. in 34 cases (7.6%), *Enterococcus* spp. in eight cases (1.8%), *Bacillus* spp. in seven cases (1.6%), *Trueperella pyogenes* in three cases (0.7%), *Aerococcus viridans* in two (0.5%), and *Dermatophilus congolensis*, *Lysinibacillus* spp., *Nocardiopsis alba* and *Streptomyces* spp., all in one case (0.2%). Out of the 71 strains of β-haemolytic streptococci detected were species including *S. equi* ssp. *zooepidemicus* (40 strains, 8.9% prevalence), *S. dysgalactiae* ssp. *equisimilis* (17 strains, 3.8% prevalence), *S. equi* ssp. *equi* (11 strains, 2.5% prevalence) and *S. agalactiae* (three strains, 0.67% prevalence). More detailed data are listed in Figure 1.

The antibiotics susceptibility tests shows that the most effective antibiotics were florfenicol and amoxicillin with clavulanic acid. The susceptibil- ity rate to florfenicol was 100% in tested strains of

Table 1. Clinical samples from horses examined in the period of 2009–2014

Sample type



Bacillus spp., *Lysinibacillus* spp., *Corynebacterium* spp., *Dermatophilus congolensis*, *Streptococcus* spp., *Enterococcus* spp., *Aerococcus viridans*, *Nocardiopsis alba* and *Trueperella pyogenes*. The susceptibilities of *Staphylococcus aureus* and other staphylococci to florfenicol were 96.2% and 98.5% of tested strains, respectively. Amoxycillin with clavulanic acid was 100% effective against the tested strains of *Corynebacterium* spp., *Dermatophilus congolensis*, *Streptococcus* spp., *Aerococcus viridans*, *Enterococcus* spp., *Streptomyces* spp., *Nocardiopsis alba* and *Trueperella pyogenes*. In *Staphylococcus aureus*, other staphylococci and *Bacillus*/*Lysinibacillus* spp. susceptibilities to amoxycillin with clavulanic acid were 89.8%, 98.8% and 20.0% of the tested strains, respectively. Among β-haemolytic streptococci strains resistant to streptomycin, neomycin, gentamicin, tetracycline, bacitracin, clindamycin, erythromycin, enrofloxacin and cotrimoxazole were found. For example out of the 65 tested β-haemolytic streptococci, 29 (44.6%) were resistant to tetracycline. Sixteen of these strains were *S. equi* ssp. *zooepidemicus*, nine strains were *S. dysgalactiae* ssp. *equisimilis*, two strains were *S. equi* ssp. *equi* and two strains were *S. agalactiae* species. Two strains (6.7%) were also resistant to bacitracin. These isolates belonged to the species *S. equi* ssp. *zooepidemicus* and *S. dysgalactiae* ssp. *equisimilis*. Among the β-haemolytic streptococci, 12 strains (18.7%) were resistant to clindamycin. Nine of them were from *S. equi* ssp. *zooepidemicus*, two from *S. equi* ssp. *equi* and one from *S. dysgalactiae* ssp. *equisimilis* species. From the same group five strains (7.9%) were resistant to erythromycin. Three isolates were from *S. dysgalactiae* ssp. *equisimilis*, one strain was *S. equi* ssp. *equi* and one strain was a *S. agalactiae* species. Four strains (66.7%) of β-haemolytic streptococci were also resistant to cotrimoxazole. Two of these strains were *S. equi* ssp. *zooepidemicus* and two strains were from *S. equi* ssp. *equi* species. Table 2 shows the results of the antimicrobial susceptibility testing in more details.

DISCUSSION

Clinical samples available for analysis were most frequently from the respiratory tract of horses, fol- lowed by the skin. Samples from the digestive tract and eyes were third and fourth in occurrence, respectively. In contrast, samples of urine, muscu- loskeletal system, lymphatic organs and milk were lower in number.

Our study shows that Gram-positive micro-organisms isolated from pathological lesions in horses predominated among cocci (282 isolates, 62.8% prevalence), which correlates with data in the literature (Songer and Post 2005). In addition, corynebacteria were also found relatively often in our study (34 isolates, 7.6% prevalence). It is in- teresting that staphylococci, headed by *S. aureus*, dominated not only numerically but also by the diversity of species. We often found staphylococci which have been more frequently described in other species of livestock, particularly cattle, pigs, carnivores, birds (*S. chromogenes*, *S. vitulinus*, *S. in- termedius*, *S. hyicus*, *S. sciuri*, *S. xylosus*; Songer and Post 2005). Species such as *S. delphini* and *S. auricularis* were also detected (Silva 2001; Stull et al. 2014). The situation for streptococci was not surprising, as equine species, such as *S. equi* ssp. *equi*, *S. equi* ssp. *zooepidemicus* and *S. pneumoniae*, *S. dysgalactiae* ssp. e*quisimilis*, were mostly isolated (Songer and Post 2005; Laus et al. 2007). The percentage of each species of β-haemolytic streptococci is in agreement with the literature. The most frequently isolated species were *S. equi* ssp. *zooepidemicus* (40 isolates, 8.9% prevalence), *S. dysgalactiae* ssp. *equisimilis* (17 isolates, 3.8% prevalence), *S. equi* ssp. *equi* (11 isolates, 2.5% prevalence) and *S. agalactiae* (three isolates, 0.67% prevalence). Erol et al. (2012a) found the same relative preva- lences. It is interesting that we found a relatively high prevalence of *S. pluranimalium* (3.8%), which is common in bovines (Songer and Post 2005); we also made isolated findings of *S. agalactiae*, *S. in- fantis*, *S. equinus*, and *A. viridans*, which are related to bovine mastitis and human infection (Quinn et al. 1994; Songer and Post 2005; Bek-Thomsen et al. 2008; Liu et al. 2015). Enterococci were identi- fied only in eight cases from the total number of examined samples (prevalence 1.8%) in the report- ing period. According to some authors, *E. faecalis* may participate in pathological processes of hors- es (Bolin et al. 2005). Another large group which was identified were coryneform microorganisms (34 strains, 7.6% prevalence). Except for *C. flavescens* which tends to be associated with col- our changes on cheese during ripening (Masoud and Jakobsen 2003), the other detected species of corynebacteria and *T. pyogenes* are associated with

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Table 2. Percentages of strains susceptible to the tested antimicrobials

Microorganism	Examined number/susceptible number (susceptible %)												
	penicillin G	streptomycin	neomycin	gentamicin	florfenicol	tetracycline	erythromycin	clindamycin	amoxycillin/ clavulanic acid	enrofloxacin	bacitracin	cephalothin	cotrimoxazole
Bacillus and *Lysinibacillus* spp.	3/1 (33.3)	5/4 (80)	2/2 (100)	4/4 (100)	4/4 (100)	5/5 (100)	4/3 (75)	5/5 (100)	5/1 (20)	4/4 (100)	1/0 (0)	5/1 (20)	NT
Corynebacterium spp.	7/6 (85.7)	6/1 (16.7)	4/3 (75)	10/9 (90)	8/8 (100)	9/9 (100)	8/7 (87.5)	9/5 (55.6)	9/9 (100)	9/7 (77.8)	3/3 (100)	9/9 (100)	1/1 (100)
Staphylococcus aureus	22/13 (59.1)	21/14 (66.7)	40/37 (92.5)	51/48 (94.1)	52/50 (96.2)	49/38 (77.6)	48/44 (91.7)	49/46 (93.9)	49/44 (89.8)	51/49 (96.1)	40/21 (52.5)	48/47 (97.9)	3/2 (66.7)
Dermatophilus congolensis	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	NT
Other staphylococci	37/27 (73)	35/33 (94.3)	33/31 (93.9)	85/85 (100)	65/64 (98.5)	103/93 (90.3)	82/72 (87.8)	82/75 (91.5)	84/83 (98.8)	84/83 (98.8)	30/20 (66.7)	87/84 (96.6)	8/7 (87.5)
Streptococcus agalactiae	3/3 (100)	3/0 (0)	3/0 (0)	3/3 (100)	3/3 (100)	3/1 (33.3)	3/2 (66.7)	3/3 (100)	3/3 (100)	3/2 (66.7)	3/3 (100)	3/3 (100)	0/0 (0)
Streptococcus dysgalactiae ssp. *equisimilis*	7/7 (100)	6/2 (33.3)	4/2 (50)	15/15 (100)	12/12 (100)	17/8 (47.1)	16/13 (81.3)	16/15 (93.8)	15/15 (100)	15/11 (73.3)	14/13 (92.9)	15/15 (100)	0/0 (0)
Streptococcus equi ssp. *equi*	5/5 (100)	5/0 (0)	3/0 (0)	11/11 (100)	8/8 (100)	5/3 (60)	5/4 (80)	5/3 (60)	11/11 (100)	11/9 (81.8)	1/1 (100)	9/9 (100)	2/0 (0)
Streptococcus equi ssp. *zooepidemicus*	23/23 (100)	20/2 (10)	17/4 (23.5)	38/37 (97.4)	29/29 (100)	40/24 (60)	39/39 (100)	40/31 (77.5)	36/36 (100)	38/33 (86.8)	12/11 (91.7)	36/36 (100)	4/2 (50)
Other streptococci and *Aerococcus* spp.	14/14 (100)	12/4 (33.3)	3/1 (33.3)	27/24 (88.9)	11/11 (100)	28/27 (96.4)	22/22 (100)	25/23 (92)	27/27 (100)	27/18 (66.7)	4/4 (100)	22/22 (100)	6/3 (50)
Enterococcus spp.	NT	1/1 (100)	1/1 (100)	2/2 (100)	1/1 (100)	3/3 (100)	1/1 (100)	2/1 (50)	3/3 (100)	1/1 (100)	1/0 (0)	3/2 (66.7)	2/2 (100)

Streptomyces spp.	(0)	(100)	(100)	(100)	NT	(100)	(0)	(0)	(100)	(100)	NT	(0)	
Nocardiopsis alba	(0)	(0)	NT	(100)	(100)	(100)	(0)	(0)	(100)	(100)	NT	(100)	NT
Trueperella pyogenes	2/2 (100)	2/2 (100)	1/1 (100)	2/2 (100)	3/3 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	1/1 (100)	2/2 (100)	1/0 (0)

NT = not tested

animal diseases (Funke et al. 1997; Minkin and Shapiro 2004; Songer and Post 2005; Leon-Galvan et al. 2015), as are nocardioform microbes (Erol et al. 2012b; Pasciak et al. 2014) as well as *D. con- golensis* (Wintzer 1999; Songer and Post 2005). Data from several sources indicate that also repre- sentatives of the genus *Bacillus* and *Lysinibacillus* can be agents of various diseases such as abortion,of species. The literature does not always give a full report of the occurrence of microorganisms and their role in relation to macroorganisms; the reality is always more diverse. Furthermore, even microorganisms that are commonly regarded as non-pathogenic may under certain circumstances contribute to the pathogenesis of diseases. In such cases, this is most likely to be due to an insuffi- ciency of natural barriers or of somatic compo- nents of the immune system. Therefore, we suggest that when bacteriological aetiology is suspected, it is important to have close cooperation and com- munication between doctors, and microbiologists and immunologists, similar to human medicine. As the most significant Gram-positive organisms, we compared the resistance in *S. aureus* and in β-haemolytic streptococci. We found 40.9% resist- ance to penicillin, which is very similar to the data of Rubin et al. (2011), who reported 43% and Peryou et al. (2003), who indicated 41% resistance in the period from 1996 to 1998. However, our data dif- fer significantly from the data of Uwaezuoke and Aririatu (2004) who found 95.8% resistance to peni- cillin in human strains of *S. aureus*. We recorded a higher percentage of tetracycline-resistant *S. aureus* strains (22.4%) than reported by Rubin et al. (2003) (10%) but also lower than the value of Uwaezuoke and Aririatu (2004), who found about 87.5% resistance in human strains. The resistance of *S. aureus* to cotrimoxazole that we found is iden- tical to the values of 33 and 33.3% reported previ- ously (Peryou et al. 2003; Uwaezuoke and Aririatu 2004). For β-haemolytic streptococci, we recorded 100% susceptibility to penicillin, while Erol et al. (2012a) reported a small proportion of resistant strains (susceptibility 97.7–99.2%). Although the same authors reported large variations in the sus- ceptibility of β-haemolytic streptococci to tetracy- cline (44–98.8%) and cotrimoxazole (30.9–94.4%), our data revealed resistance rates of 55.4 and 33.3% for the same antimicrobial compounds. A smaller percentage range was reported by the same authors for bacitracin, erythromycin and gen- tamicin (79.4–100%, 87.8–99.2% and 82.8–91.2%);

our average values of susceptibility to the same

antibiotics in the period 2009–2014 are 93.3%, 92.1% and 98.5%. In our study, we also examined the annual prevalence of the resistance, especially in staphylococci and streptococci. However, no sys- tematic and sustained increase in resistance could be confirmed. It would be informative to test andcompare the susceptibility of a larger number of detected strains. However, the above data indicate that it would be useful to limit the usage of antimi- crobials, and where possible to prefer local antisep- tics or natural medicaments, which can prevent the emergence of undesirable resistant microorganisms in both animal and human populations.

In the current work, we have described the presence of a relatively broad spectrum of Gram- positive aerobic and microaerophilic microor- ganisms isolated from clinical material of horses. Gram-positive cocci and corynebacteria clearly dominated in the examined period. In the litera- ture, most of taxa that were isolated are associ- ated with pathological processes in various animal species. Therefore, infection with these micro- organisms is possible in equids. We suggest that damage to natural somatic barriers function and weakened immune system may be decisive factors in the pathogenesis of infectious diseases. A sound knowledge of the microorganisms that may be pre- sent and participate in pathological lesions and pro- cesses in the horse, closer cooperation of clinical specialists with microbiologists and immunolo- gists, and rich practical experience can, together, lead to more accurate diagnosis, proper selection of therapy and a potential reduction in antimicro- bial administration. Regarding the susceptibility of microorganisms, we succeeded in mapping the susceptibility and resistance of individual groups of microorganisms, isolated from pathological pro- cesses and lesions in horses and compared these resistance rates with literature data. No annual increase in resistance to any of the tested antimi- crobials could be confirmed.

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