Kafkas Univ Vet Fak Derg
24 (1): 83-90, 2018
DOI: 10.9775/kvfd.2017.18370

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
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Research Article

Isolation and Antibiotic Susceptibilities of Beta Hemolytic *Streptococcus* Species from Various Body Site Infections with Cytologic Evidences in Thoroughbred and Arabian Racehorses in Turkey

Alper METE [1]

[1] Jockey Club of Turkey İstanbul Veliefendi Racetrack Horse Hospital, TR-34144 Bakırköy, İstanbul - TURKEY

Article Code: KVFD-2017-18370 Received: 10.07.2017 Accepted: 19.10.2017 Published Online: 20.10.2017

Citation of This Article

Mete A: Isolation and antibiotic susceptibilities of beta hemolytic *Streptococcus* species from various body site infections with cytologic evidences in Thoroughbred and Arabian racehorses in Turkey. *Kafkas Univ Vet Fak Derg,* 24 (1): 83-90, 2018. DOI: 10.9775/kvfd.2017.18370

Abstract

Streptococcal microorganisms mostly beta hemolytic streptococci are the most important and widespread bacterial agents causing infectious diseases in horses and they often follow a difficult course. The goals of the present study are to determine; i) together with cytologic findings, the system distributions of the beta hemolytic streptococci as a primary pathogen, ii) the susceptibility patterns to the most commonly used antibiotics in thoroughbred and Arabian racing horses iii) the presence of multiple antibiotics resistance of beta hemolytic streptococci encountered in horses. Beta hemolytic streptococci isolated and cytologically neutrophilic inflammation confirmed samples which were submitted to Jockey Club of Turkey horse hospital laboratory between April 2014 - April 2017 were included into the study. The study was conducted on 128 samples including tracheal wash (71.9%), endometrial swabs (21.9%), abscess material (5.4%) and urine sample (0.8%). According to the isolation results, *S. equi* subsp. *zooepidemicus (S. zooepidemicus), S. dysgalactiae* subsp. *equisimilis (S. equisimilis)* and *S. equi* subsp. *equi (S. equi)* were isolated from whole samples as 80.4%, 14.1% and 5.5% respectively. Antibiotics susceptibility results demonstrated that amoxicillin/clavulanic acid (100%), amoxicillin (100%), ampicillin (100%), penicillin (100%), imipenem (100%), ampicillin/sulbactam (100%), rifampicin (97.6%), ceftiofur (100%) and ceftazidime (87.5%) were found to be effective, while enrofloxacin (14.8%), amikacin (0.8%), gentamicin (4.7%), kanamycin (0%), neomycin (0%), streptomycin (0%), oxytetracycline (7.0%) and trimethoprim/sulfamethoxazole (27.3%) were found to be mostly uneffective. The multiple antibiotics resistance rate was found to be 1.6% amongst beta hemolytic streptococci isolates in horses. These results of the present study confirmed the results of many similiar previous studies that *S. zooepidemicus* was the most frequently isolated beta hemolytic *Streptococcus* species followed by *S. equisimilis* and *S. equi.* In suspecious infections where a beta hemolytic streptococcal infection is suspected penicillin should be considered as the first line antimicrobial for horses when results of the samples submitted for bacterial culture and susceptibility test results are pending.

Keywords: *Antibiotic susceptibility, Beta hemolytic streptococci, Cytology, Horse, Lancefield group C Streptococcus spp*

Safkan İngiliz ve Arap Yarış Atlarında Görülen Beta Hemolitik *Streptococcus* Türlerinin Sitolojik Bulgular Eşliğinde Çeşitli Vücut Bölgelerinden İzolasyonu ve Antibiyotik Duyarlılıkları

Özet

Başta beta hemolitik türler olmak üzere streptokoklar atlarda bulaşıcı hastalıklara neden olan ve en sık görülen, en yaygın bakteriyel ajanlardır ve genellikle zor bir seyir izlemektedir. Bu çalışmanın amacı; i) sitolojik bulgularla beraber birincil patojen olarak beta hemolitik streptokokların atlardaki sistem dağılımlarını ii) safkan İngiliz ve Arap yarış atlarında en sık kullanılan antibiyotiklere karşı olan duyarlılıklarını iii) Türkiye' deki atlarda görülen beta hemolitik streptokokların çoklu antibiyotik dirençlilik oranlarını belirlemektir. Çalışmaya Nisan 2014 ile Nisan 2017 arasında Türkiye Jokey Kulübü İstanbul at hastanesi laboratuvarına gelen, beta hemolitik streptokok izole edilen ve sitolojik incelemede nötrofilik inflamasyon varlığı saptanan örnekler alınmıştır. Çalışmada %71.9'u trakeal aspirat sıvısı, %21.9'u endometriyal svap, %5.4'ü abse içeriği ve %0.8'i idrar örneği olmak üzere 128 örnek incelenmiştir. Çalışmada elde edilen sonuçlara göre tüm örneklerden *S. zooepidemicus, S. equisimilis* ve *S. equi* sırasıyla %80.4, %14.1, %5.5 oranlarında izole edilmiştir. Antibiyotik duyarlılık test sonuçlarına göre amoksisilin/klavulanik asit (%100), amoksisilin (%100), ampisilin (%100), penisilin (%100), imipenem (%100), ampisilin/sulbaktam (%100), rifamisin (%97.6), seftiofur (%100) ve seftazidim (%87.5) atlardan izole edilen beta hemolitik streptokoklara karşı duyarlı bulunurken, enrofloksasin (%14.8), amikasin (%0,8), gentamisin (%4.7), kanamisin (%0), neomisin (%0), streptomisin (%0), oksitetrasiklin (%7) ve trimetoprim sulfametoksazol (%27.3) çoğunlukla etkisiz bulunmuştur. Çoklu antibiyotik direnç oranı, izole edilen beta hemolitik streptokoklar arasında %1.6 olarak saptanmıştır. Bu çalışmadaki sonuçlar önceki benzer çoğu çalışmaların sonuçlarını tastik etmiş olup, *S. zooepidemicus* en sık izole edilen beta hemolitik streptokok türü olarak saptanmıştır. *S. zooepidemicus*'u, *S. equisimilis* ve *S. equi* izlemektedir. Şüpheli infeksiyonlarda kültür ve antibiyogram analizleri sonuçlanana kadar penisilin atlarda kullanılabilecek ilk antimikrobiyal olarak düşünülmelidir.

Anahtar sözcükler: *Antibiyotik duyarlılığı, At, Beta hemolitik streptokok, Lancefield grup C, Sitoloji, Streptococcus spp*

İletişim (Correspondence)
+90 533 5523772
alpermete1985@yahoo.com

INTRODUCTION

Beta hemolytic *Streptococcus* spp., which produce complete clearing of blood agar medium due to lysis of erythrocytes in media, are the most important and widespread agents causing infectious diseases in horses [1]. Of the beta hemolytic streptococci species *Streptococcus equi* subsp. *equi (S. equi), Streptococcus equi* subsp. *zooepidemicus (S. zooepidemicus)* and *Streptococcus dysgalactiae* subsp. *equisimilis (S. equisimilis)* and are three major beta hemolytic *Streptococcus* species, those cause severe and economically important diseases in horses [2,3].

S. equi is the causative agent of strangles, a highly contagious infection of the upper respiratory tract and associated lymph nodes of horses [2,3]. *S. equi* was also isolated from intra-uterine swabs (3.2%) [4], lower respiratory tract of foals (6.3%), adult horses (6.2%), lymph node abscess of foals (41.9%), adults (49.6%) and total isolation rate was found to be 6.3% in the same study [3]. *S. equi* was isolated 15.5% [1] and 12.8% [5] from various body sites of the horses in different studies.

S. zooepidemicus probably causes more disease in the horse than any other infectious agent yet [6]. *S. zooepidemicus* was the most isolated microorganism (72.0%) followed by *S. equisimilis* (21.3%), *S. equi* (5.8%) and unidentified beta hemolytic streptococci (0.9%) in a previous study and in different studies such as 56.4% [1] and 87.2% [5] amongst beta hemolytic streptococci. The agent was isolated 81.0% and 84.2% from respiratory tract of adult horses and foals respectively in the same study [3]. *S. zooepidemicus* is considered to be a mucosal commensal of the upper respiratory tract of horses which, causes disease as an opportunistic pathogen of the lower respiratory tract [2,3]. The agent is also responsible for the disease of reproductive tract especially when it causes endometritis in horses. Beta hemolytic *Streptococcus* was found to be the most frequently isolated microorganisms amongst other bacterial agents in equine uteri during fertility problems [7]. In equine genital tract, *S. zooepidemicus* was isolated 81.0% and 64.0% from adults and foals respectively [3]. The agent was isolated 67.8% from uterine swabs amongst beta hemolytic streptococci species [4]. It has also been reported as the most common bacterial agent causing placentitis in mares [8]. The agent was isolated from an aborted equine fetus in Turkey [9]. *S. zooepidemicus* was also reported as an etiologic agent in canine infectious respiratory disease [10] and has also been reported to cause septicaemia, meningitis, nefritis and arthritis in humans [11-14]. Molecular typing methods showed that human and equine isolates were identical and closely related, according to these datas *S. zooepidemicus* should also be recognized as an emerging zoonosis [15].

S. equisimilis is considered to be an infrequent pathogenic agent isolated from horses. It has been isolated from aborted placenta, less frequently from abscessed lymph nodes and from strangle-like disease of the upper respiratory system in horses [2,3,16]. The agent was also isolated from endometrial swabs (12.9%) [4], lower respiratory tract of the foals (12.4%) [3] and adult horses (12.4%) [3].

Besides the isolation of a microorganism from clinical samples, determining the inflammation by cytological examination in concordance with bacterial isolation is also detrimental in order to separate contamination from real infection in the diagnosis of bacterial infections [17,18].

An increased attention has been placed upon antimicrobial resistance in both medical and veterinary field [19]. *Streptococcus* group C agents isolated from endometrial swabs yielded 82.7% susceptibility to amoxicillin/clavulanic acid, 67.7% ampicillin, 8.4% enrofloxacin, 6.7% gentamicin, 65.2% penicillin, 51.3% rifampicin, 17.0% trimethoprim/sulfamethoxazole and 15.1% tetracycline in a study performed in Italy [7]. Ampicillin, gentamicin, neomycin, oxytetracycline, penicillin G, trimethoprim/sulfamethoxazole yielded 100%, 19%, 13%, 29%, 100% and 90% efficacy against beta hemolytic streptococcal isolates in a different study respectively [4]. Penicillin showed 100% efficacy against *S. equisimilis*, *S. equi* and *S. zooepidemicus* [1]. The efficacy of streptomycin were determined as 33.3%, 0%, and 10.0%, neomycin 50.0%, 0% and 23.5%, gentamicin 100%, 100% and 97.4%, tetracycline 47.1%, 60.0% and 60.0%, amoxicillin/clavulanic 100% against the three pathogens, while the efficacy of enrofloxacin was found to be 73.3%, 81.8% and 86.8% against *S. equisimilis*, *S. equi* and *S. zooepidemicus* respectively in the same study [1]. Erol et al.[3] reported that gentamicin efficacy was 83.3%, 82.8% and 87.5% against *S. equi*, *S. equisimilis* and *S. zooepidemicus* respectively where penicillin efficacy was found to be 98.7%, 99.2%, 98.9%, tetracycline efficacy was found to be 98.8%, 50.9%, 44.0% and trimethoprim/sulfamethoxazole efficacy was found to be 50.2%, 94.4%, 30.9% against the same agents respectively. Results from these studies enlight practitioners in making an evidence based antimicrobial medicine choice where rapid treatment is needed and culture-susceptibility results cannot be awaited. Bacterial resistance profiles can vary over time. Thus continuous local surveillance has great importance [20].

There has been also a great concern of multidrug resistant bacteria in veterinary field. Multidrug resistance was defined as resistance to three or more antimicrobial classes in pathogens isolated from animals [19]. In New Zealand, multidrug resistance rate of *Streptococcus* spp. was found to be 3.9% and age of the horse was determined to be significantly associated with multidrug resistance statistically [19].

The goals of the present study are to determine; i) together with cytologic findings, the system distributions of the beta hemolytic streptococci as a primary pathogen, ii) the susceptibility to the most commonly used antibiotics in

thoroughbred and Arabian racing horses iii) the presence of multiple antibiotics resistance of beta hemolytic streptococci encountered in horses.

MATERIAL and METHODS

Samples

Only beta hemolytic streptococci isolated and cytologically neutrophilic inflammation confirmed samples were included into the study. The study was conducted on 128 samples. All the samples were submitted between April 2014 and April 2017 into the Jockey Club of Turkey Istanbul Veliefendi Racetrack Horse Hospital. The samples, from which beta hemolytic streptococci isolated, were consisted of 71.9% (n=92) tracheal wash, 21.9% (n=28) endometrial swabs, 5.4% (n=7) abscess material and %0.8 (n=1) urine sample. Nasal swabs were not included to the study due to high content of beta hemolytic streptococci and other bacterial agents as normal flora of upper airways in horses [2,16]. Tracheal wash samples were collected according to the previous study [18]. Briefly, 40 mL sterile physiologic saline solution was instilled from proximal trachea and immediately aspirated back to a 60 mL sterile injector with a flushing catheter elongated via the biopsy channel of the endoscopy. Maximum precautions were taken to reduce the risk of upper airway contamination. After collecting the samples, they were transferred to a plane tube for bacteriology and EDTA tube for cytologic examination. Endometrial swabs were collected according to the previous study [21]. Briefly double guarded swabs were used to collect the samples. The instrument was protected with the gloved hand of the veterinarian when introduced in the vagina and directed to the cervix. The inner sheath of the instrument was advanced through the cervix and the swab was rolled several times against the endometrium. Approximately 30 sec later the swab was retracted in the inner sheath while the outer one was left in the place within the cervix for the second sampling. Abscess materials were collected with sterile needles into sterile 5 mL syringes and they were immediately sent to the laboratory for culture. Horses' age information were also recorded. 0-2 years old animals were classified as foals. Horses older than 3 years old≤ were classified as adult horses [3].

Cytology

Slide preparations of the tracheal wash samples were made by centrifugation of the samples at 1500 rpm. for 5 min. The slides were prepared from the sediment of the centrifuged samples as described before [17]. The air dried slides were stained with May Grunwald Quick stain (Bio Optica 20134 Milano Italy) that was used according to the manufacturer's instructions. The slides were examined under 100x magnification microscope and percentage (%) of the neutrophils, macrophages, lymphocytes, eosinophils and mast cells were determined by counting 300 cells [18]. Inflammation of the lower airways was considered positive when neutrophils made up ≥30% of all cells and the presence of degeneration in neutrophils and likely to have intracellular bacteria *(Fig. 1)* [17]. Cytological examination of the intrauterine swabs were performed according to the previous study [22]. Endometrial swabs were smeared on a microscope glass slide after streaking to the appropirate agars for culture. The slides were then stained using May Grunwald Giemsa stain (Bio Optica 20134 Milano Italy). All slides were examined under the microscope (40x magnification) for the presence of polimorphonuclear cells (PMNs). The sample was considered as positive for inflammation when PMNs made up ≥0.5% of all cells. Cytologic examination of the urine samples were performed by centrifuging the urine at 3.000 rpm for 5 min. After the centrifuge, the supernatant was discarded and sediment was examined microscopically using high dry objective (40X). The sample was accepted as positive, if white blood cells are observed 3< high power field [23].

Bacteriology and Antibiotics Susceptibility Testing

Isolation of beta hemolytic streptococci from tracheal wash samples, intrauterine swabs and abscess materials were carried out as described before [5,24]. Briefly, the samples were inoculated in 5% sheep blood agar in both aerobic and microaerobic (5% CO_2) conditions at 37°C. The media were incubated 24-48 h for beta hemolytic streptococci growth.

The criteria used for reporting a culture for beta hemolytic

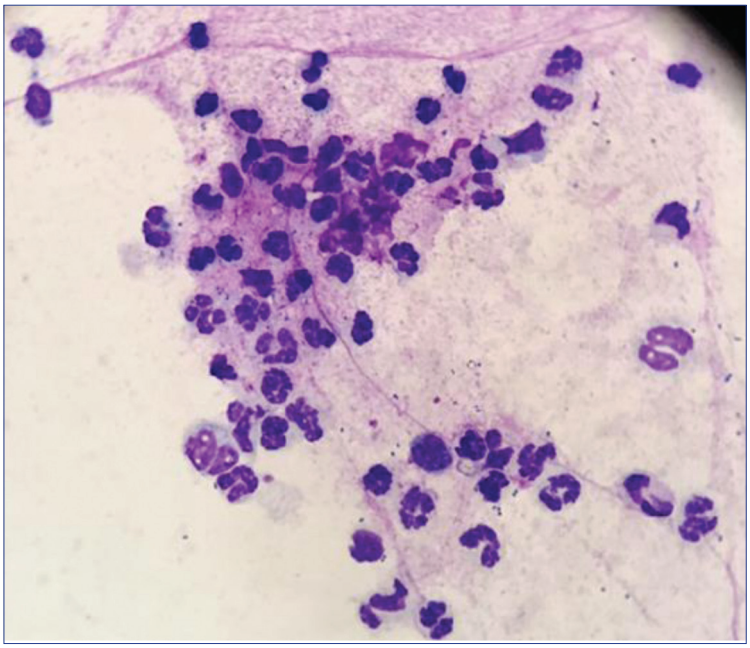

Fig 1. Septic neutrophilic inflammation in a tracheal wash sample (100x magnification)

streptococci growth was the isolation of the micro-organisms in pure culture or predominating on the agar plate as previously described [8].

Identification of the suspected colonies were carried out by subjecting them to Gram stain (GBL, İstanbul), catalase test, latex agglutination test (Oxoid Ltd., Basingstoke, UK) to determine serological group and then finally determining their biochemical profile using API 20 Strep ID kit according to the manufacturer's instructions (bioMerieux S.A., Marcy l'Etoile, France) respectively.

In vitro antibiotics susceptibility testings were performed by using disc diffusion method (25) according to the standards of the Clinical Laboratory Standards Institute (CLSI). Isolates were reported as susceptible to an antimicrobial if the diameter of the zone of inhibition was greater than the breakpoint for that drug, according to the CLSI Standards [26]. Amikacin (30 µg), amoxicillin/clavulanic acid (30 µg), amoxicillin (25 µg), ampicillin (10 µg), ceftiofur (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), neomycin (10 µg), oxytetracycline (30 µg), penicillin (10 IU), streptomycin (10 µg), ampicillin sulbactam (20 µg), trimethoprim/sulfamethoxazole (25 µg) and ceftazidime (30 µg) antimicrobial discs (Oxoid Ltd., Basingstoke, UK) were included in the susceptibility tests. Multidrug antimicrobial resistance was also assessed which was described as an isolate being resistant to three or more of the following antimicrobial agents: enrofloxacin, gentamicin, ceftiofur, penicillin, oxytetracycline and trimethoprim-sulfonamide combination [19].

Statistical Analysis

The statistical analysis of the association between age groups (including ≤2 years old and 3≤ years old) and the amount multidrug resistant isolates were evaluated using the Pearson Chi-Square (X^2) test with Statistical Package for Social Sciences (SPSS) [27].

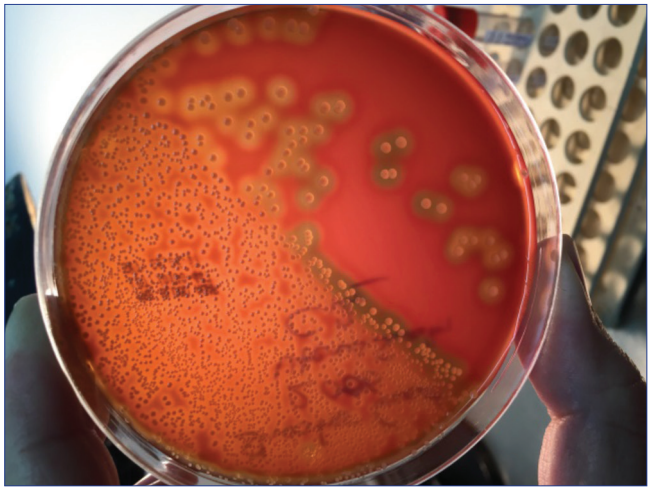

Fig 2. Beta hemolytic Streptococci colonies on 5% sheep blood agar. The isolated colonies in the picture were identified *as Streptococcus equi* subsp. *equi*

RESULTS

Beta hemolytic streptococci isolated from 128 samples including tracheal washes, endometrial swabs, abscess material and urine samples were *S. equi* (5.5%) *(Fig. 2)*, *S. equisimilis* (14.1%) *(Fig. 3)* and *S. zooepidemicus* (80.4%) *(Fig. 4)*, which were identified according to their biochemical profile.

When the sites of isolations were examined, *S. zooepidemicus* (88.0%), *S. equisimilis* (10.9%) and *S. equi* (1.1%) were

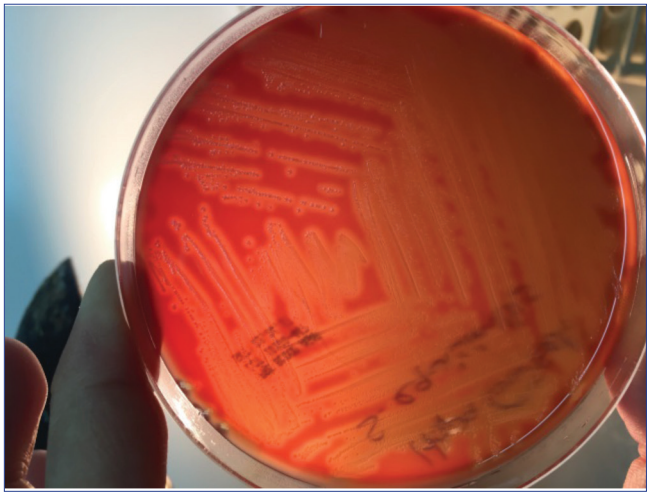

Fig 3. *Streptococcus dysgalactiae* subsp. *equisimilis* colonies isolated from endometrial swab

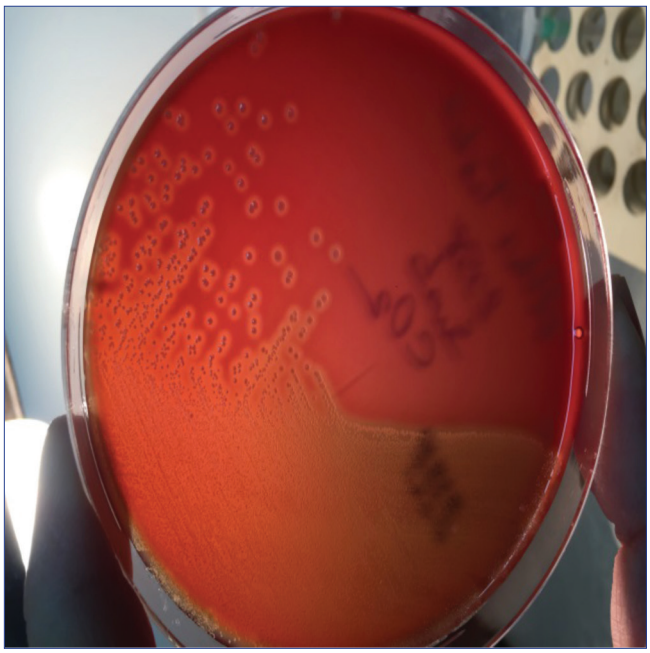

Fig 4. *Streptococcus equi* subsp. *zooepidemicus* colonies isolated from endometrial swab

Table 1. *Distribution of recovered microorganisms according to the sample type*

Isolated Microoganisms	Tracheal Wash (n=92)	Endometrial Swab (n= 28)	Abscess Material (n=7)	Urine Sample (n=1)
S. equi subsp. *zooepidemicus*	81 (88.0%)	16 (57.1%)	5 (71.4%)	1 (100%)
S. dysgalactiae subsp. *equisimilis*	10 (10.9%)	7 (25.0%)	1 (14.3%)	0 (0%)
S. equi subsp. *equi*	1 (1.1%)	5 (17.9%)	1 (14.3%)	0 (0%)

Table 2. *Distribution of isolated microorganisms according to the isolation year*

Isolated Microorganisms	2014 (April-December) (n= 39)	2015 (n=53)	2016 (n=25)	2017 (January-April) (n=11)
S. equi subsp. *zooepidemicus*	33 (84.6%)	50 (94.3%)	17 (68.0%)	3 (27.3%)
S. dysgalactiae subsp. *equisimilis*	5 (12.8%)	2 (3.8%)	5 (20.0%)	7 (63.6%)
S. equi subsp. *equi*	1 (2.6%)	1 (2.6%)	3 (12.0%)	1 (9.1%)

Table 3. *Distribution of isolated microorganisms according to the age groups*

Isolated Microorganisms	Foals (0-2 age) (n=51)	Adult Horses (3≤age) (n=77)
S. equi subsp. *zooepidemicus*	43 (84.3%)	60 (77.9%)
S. dysgalactiae subsp. *equisimilis*	7 (13.7%)	11 (14.3%)
S. equi subsp. *equi*	1 (2.0%)	6 (7.8%)

Table 4. *Antibiotic susceptibilities of the isolated microorganisms*

Antimicrobials	Beta Hemolytic Streptococci (n=128)	*S. equi* subsp. *zooepidemicus* (n=103)	*S. dysgalactiae* subsp. *equisimilis* (n=18)	*S. equi* subsp. *equi* (n=7)
Amoxycillin/Clavulonic acid	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Amoxycillin	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Ampicillin	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Penicillin G	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Imipenem	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Ampicillin/Sulbactam	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Enrofloxacin	19 (14.8%)	18 (17.5%)	1 (5.5%)	0 (0%)
Rifampicin	125 (97.6%)	100 (97.1%)	18 (100%)	7 (100%)
Ceftiofur	128 (100%)	103 (100%)	18 (100%)	7 (100%)
Ceftazidime	112 (87.5%)	90 (87.4 %)	16 (88.9%)	6 (85.7%)
Amikacin	1 (0.8%)	1 (0.9%)	0 (0%)	0 (0%)
Gentamicin	6 (4.7%)	6 (5.8%)	0 (0%)	0 (0%)
Kanamycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Neomycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Streptomycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Trimethoprim/Sulfamethoxazole	35 (27.3%)	23 (22.3%)	8 (44.4%)	4 (57.1%)
Oxytetracycline	9 (7.0%)	6 (5.8%)	2 (11.1%)	1 (14.3%)

isolated from 92 tracheal wash samples. While examining 28 endometrial swabs *S. zooepidemicus* (57.1%), *S. equisimilis* (25.0%) and *S. equi* (17.9%) were isolated respectively. From 7 abscess materials *S. zooepidemicus* (71.4 %), *S. equisimilis* (14.3%) and *S. equi* (14.3%) were isolated. From 1 urine sample *S. zooepidemicus* (100%) was isolated *(Table 1)*.

Distribution of the isolated beta hemolytic streptococci according to the isolation years revealed that *S. zooepidemicus* was the most isolated pathogen in 2014, 2015, 2016. *S. zooepidemicus* was followed by *S. equisimilis* and *S. equi* respectively. *S. equisilimis* seemed to be the most isolated microorganism between January 2017

and April 2017 to the contrary of 2014, 2015 and 2016 *(Table 2)*.

When examining horse age groups in terms of beta hemolytic streptococci isolation, it has been shown that isolation rate of *S. zooepidemicus* was 84.3% in young horses/foals (0-2 years old) while the isolation rate of *S. equisimilis* and *S. equi* were 13.7% and 2.0% respectively. In adult horse group (2 years old<) *S. zooepidemicus*, *S. equisimilis* and *S. equi* were isolated 77.9%, 14.3% and 7.8% respectively *(Table 3)*.

The present study's antibiotics susceptibility test results showed that all beta hemolytic streptococci agents were susceptible to amoxicillin/clavulanic acid, amoxicillin, ampicillin, penicillin, imipenem, ampicillin/sulbactam and ceftiofur antibiotics. They were resistant to kanamycin, streptomycin and neomycin *(Table 4)*.

When the recovered microorganisms were examined individually *S. zooepidemicus*, *S. equisimilis* and *S. equi* isolates were all susceptible to amoxicillin/clavulanic acid, amoxicillin, ampicillin, penicillin G, imipenem, ampicillin/sulbactam and ceftiofur antibiotics, *S. equisimilis* and *S. equi* isolates were all also susceptible to rifampicin. All of the isolates of *S. zooepidemicus*, *S. equisimilis* and *S. equi* were resistant to kanamycin, neomycin and streptomycin. Additionally all *S. equisimilis* and *S. equi* isolates were resistant to amikacin and gentamicin *(Table 2)*. In the present study multi drug resistant isolates were cultured from 2 (1.6%) samples. Both of these isolates were resistant to gentamicin, oxytetracycline and trimethoprim/sulfamethoxazole.

DISCUSSION

Beta hemolytic streptococci species were known to be the primary bacterial pathogens of the horse. In the present study *S. zooepidemicus*, *S. equisimilis* and *S. equi* were isolated as 80.4%, 14.1% and 5.5% from clinical samples respectively. Neither, unidentified beta hemolytic streptococci nor, any other beta hemolytic streptococci species were isolated. *S. zooepidemicus*, *S. equisimilis*, *S. equi* and unidentified beta hemolytic streptococci were isolated as 72.0%, 21.3%, 5.8% and 0.9% in the same study respectively [3]. A different study determined the isolation rates of *S. zooepidemicus* 56.4%, *S. equisimilis* 23.9%, *S. equi* 15.5% and *S. agalactiae* 4.2% from a wide range of sample types including tracheal swabs, urine [1]. These results showed that the isolation rates of these agents were similar to the present study and *S. zooepidemicus* was the most isolated pathogenic agent amongst beta hemolytic streptococci in all studies. *S. equisimilis* was the second most isolated agent and *S. equi* was the third most isolated one in the present study, which was similar to the previous studies [1,3].

Analysing the results according to age groups revealed that *S. zooepidemicus*, *S. equisimilis* and *S. equi* were isolated 84.3%, 13.7% and 2.0% from the foals, 77.9%, 14.3% and 7.8% from the adult horses respectively in the present study. In a previous study, *S. zooepidemicus*, *S. equisimilis* and *S. equi* were isolated 77.5%, 11.5% and 10.4% from foals, 74.1%, 18.1% and 6.9% from adult horses respectively [3]. Despite isolation rate differences between two studies, which might had been due to the geographic differences and population difference, both of these studies suggested that *S. zooepidemicus* was the most frequently isolated agent both in foals and adult horses followed by *S. equisimilis* and *S. equi* respectively.

In a study examining the mares with fertility problems, a total of 31 beta hemolytic streptococci consisted of 67.8% *S. zooepidemicus*, 12.9% *S. equisimilis*, 3.2% *S. equi* and 16.1% unidentified agents were isolated from endometrial swabs [4]. In the present study *S. zooepidemicus*, *S. equisimilis* and *S. equi* were isolated 57.1%, 25.0% and 17.9% from 28 endometrial swabs. There were differences in the isolation rates of the agents but it could be concluded that *S. zooepidemicus* was the most frequently isolated agent followed by *S. equisimilis* and *S. equi* amongst beta hemolytic streptococcal agents from intrauterin infections of mares in both studies. Additionally *S. zooepidemicus* was also isolated from an urine sample with the presence of leukocytes that supports the presence of inflammation. From all these datas, it could be concluded that beta hemolytic streptococcal agents should not be ignored in horses' uro-genital infections besides of other microbial agents.

In the present study *S. zooepidemicus* (88.0%) is the most frequently isolated agent from respiratory tract samples in both foals and adult horses. This result is in agreement with results of a retrospective study performed in U.S. [3]. The reason of high isolation rate of *S. zooepidemicus* might had been due to being a normal mucosal flora bacteria of the upper respiratory tract in healthy horses and for any reason that caused immuno compromisation such as stress, transportation, racing or anatomical predisposition could let the agent migrate to lower respiratory tract and finally might had caused infection [2]. According to the present study, *S. equisimilis* (10.9%) is the second most frequently isolated agent followed by *S. equi* (1.1%) which showed agreement with the other retrospective study [3].

Beta hemolytic streptococci species were found to be 100% susceptible against amoxycillin/clavulanic acid, amoxycillin, ampicillin, penicillin, imipenem, ampicillin/sulbactam and ceftiofur according to the present study in vitro. These results are mostly in agreement with previous studies [1,3-5,19].

Ceftazidime and rifampicin were also effective against beta hemolytic streptococci species that were isolated in the study (87.5% and 97.6% respectively). Unfortunately no work at the literature was found that use disc diffusion method to compare the suspectibility testing results of

the present study for ceftazidime in horses. Efficiency of rifampicin against *Streptococcus* spp. group C was found to be 51.3% in endometrial swabs [7]. However, in the present study the efficiency of rifampicin against the same microbials was found as 96.4% in endometrial swabs. The variations between the results of these two studies could be due to the frequency of the use of rifampicin in two different countries/regions.

Aminoglycoside group antimicrobials (amikacin, gentamicin, kanamycin, neomycin, streptomycin) showed very low or zero efficacy against beta hemolytic streptococci species in the present study. These results are mostly in agreement with the previous studies [1,3,5,77,19] except gentamicin. Gentamicin showed high antimicrobial efficacies such as 84.5% [3], 99.1% [1], 90.0% [5] against beta hemolytic *Streptococcus* spp. and 91.6% against *Streptococcus* spp. in previous studies. But on the other hand some studies demonstrated low efficacies such as 19% [4] and 6.7% [7]. In the present study efficacy of gentamicin was found 4.7% which is similar with low efficacy determined studies. Variations in the efficacy rate of gentamicin might had been due to the wide spread empirical use of gentamicin in clinical practice.

Trimethoprim/sulfamethoxazole and enrofloxacin antimicrobial efficacy results of the present study revealed lower efficacy rates such as 27.3%, 22.3%, 44.4%, 57.1% for trimethoprim/sulfamethoxazole and 14.8%, 17.5%, 5.5%, 0% for enrofloxacin against beta hemolytic streptococci, *S. zooepidemicus*, *S. equisimilis* and *S. equi* respectively than previous studies [1,3,5,19]. But higher efficacy rates for sulphamethaxzole/trimethoprim and similar efficacy rates for enrofloxacin were also obtained when comparing the present study with a previous one [7].

The susceptibility results of oxytetracycline showed an efficacy of 7.0%, 5.8%, 11.1% and 14.3% against beta hemolytic *Streptococcus* spp., *S. zooepidemicus*, *S. equisimilis* and *S. equi* respectively in the present study. However in two different studies, the efficacy of tetracycline was found to be higher than the present study such as 64.6%, 44.0%, 50.9%, 98.8% and 55.7%, 60%, 47.1%, 60.0% against beta hemolytic *Streptococcus* spp., *S. zooepidemicus*, *S. equisimilis* and *S. equi* respectively [1,3]. On the contrary in a different study performed in Italy showed *Streptococcus* group C microorganisms showed low susceptibility (15.1%) against tetracycline [7]. From all these results it could be concluded that efficacy rate of oxytetracycline could change between countries/regions due to the frequency of use in clinical practice and local demonstration of oxytetracycline efficacy like other antimicrobials against beta hemolytic streptococci has paramount importance.

Based upon the antimicrobial susceptibility results of the present study, multidrug resistance was found 1.6% (2/128) in beta hemolytic streptococci isolates and no significant association could be found for the presence of the multidrug resistant bacteria in different age groups (≤2 years old and ≥3 years old). In New Zealand multidrug resistance was found to be 3.9% (12/310) amongst streptococci isolates and a significant association was found for the presence of multidrug resistant bacteria in 2 years old horse group [19]. More extensive studies including high numbers of horses and isolates should be set in order to deeply highlight multidrug resistance status of bacterial agents in Turkey.

In conclusion, the current study yielded that *S. zooepidemicus* was found to be the most frequently isolated beta hemolytic streptococci in Turkey. *S. zooepidemicus* has also zoonotic significance so that care must be taken by the practitioners and related persons to horses while handling suspected or confirmed cases with this microbial agent. Beta hemolytic *Streptococcus* spp. demonstrated high susceptibility to amoxycillin/clavulanic acid, amoxycillin, ampicillin, penicillin, imipenem, ampicillin/sulbactam, ceftiofur, rifampicin and ceftazidime while showing low susceptibility or resistance to enrofloxacin, amikacin, gentamicin, kanamycin, streptomycin, neomycin, oxytetracycline and trimethoprim/sulphamethaxzole in the present study. It can also be concluded that penicillin can be considered as a first line antimicrobial for use in horses in Turkey where a beta hemolytic streptococcal infection is suspected, when results of the samples submitted for bacterial culture and susceptibility testing are pending. No significant association was found between age groups (≤2 years old and ≥3 years old) and isolation of multidrug resistant beta hemolytic streptococci.

Acknowledgements

The author would like to thank Prof. Dr. N.Yakut Özgür for her critical review of the manuscript. The author would like to thank DVM. PhD. Cihan Kumaş, DVM. Hülya Haratoka, DVM. Gökhan Parker DVM. Öyküm Kaya, DVM. Dursun Doğan, DVM. Emrah Yiğittürk and DVM. Muhlis Arslan for sample collection and for submisson processes to the laboratory. The author also would like to thank Laboratory Technician Uygar Ündeğer and Biologist MSc. Beliz Tezel Yetiz for their assistance during the laboratory analysis.

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