Bacterial flora of the conjunctiva in healthy mules (Equus mulus)

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SUMMARY

This study was aimed to identify the bacterial isolates of eye surface in healthy mules and to determine the sensitivity of isolates to antimicrobial agents. A total of 100 conjunctival swabs were taken from 50 mules, ranging in age from 5 to 12 years, and without any signs of ophthalmic problems. Samples were cultured for aerobic bacteria and susceptibility assessed to 12 antibiotics. Bacterial growth was positive in 85/100 (85%) of sampled eyes. Most of the isolated bacteria (97/109: 89%) were Gram-positive pathogens. Isolates of present study were comparable with those previously reported for the ocular surface of other equine species.

Keywords: bacterial flora, eye, mule, Staphylococcus spp., antibiotic sensitivity

Introduction

Ulcerative keratitis is a common disease in horses and can be potentially sight threatening if not diagnosed early and treated properly. A prominent cornea and its susceptibility to trauma, a relatively slow rate of corneal healing, and insufficient defense mechanisms of ocular surface are considered for frequency of corneal infections in horses [3]. Most resident bacteria of the eye surface have a role in maintenance of health cornea by decreasing ability of invasive bacteria to attach and colonize the ocular surface [24]. However, these microorganisms can become potentially pathogenic if corneal ulcers/erosions due to trauma occur [3]. Hence identification of normal flora of eyes permits the clinician to anticipate the presence of certain organisms on the eye surface and, in the event of trauma, to initiate proper preventive antibacterial treatment. Many studies have described normal conjunctival bacterial flora of horses [1,10,11,28] and donkeys [9], but there is no report in the literature regarding normal ocular microflora of mules. Genera of Staphylococcus, Streptococcus, Bacillus and Corynebacterium are the most frequently isolated organisms of normal equine ocular surface [1,9,10,14]. The purposes of present study were to document of bacterial flora of conjunctiva in the healthy mules and to assess antimicrobial susceptibility of isolates for the recommendation of appropriate antibiotics for preventive treatment of corneal ulcers in mules.

Material and Methods

ANIMALS AND AREA OF STUDY

The study population included 50 healthy mules, 29 (58%) females and 21(42%) males, ranging in age from 5 to 12 years, and living in mountainous districts of Urmia (2,120 m above sea level), located West-Azerbaijan province, northwestern Iran with temperate climate. The study was approved by the ethics committee for animal experimentation by the Islamic Azad University-Urmia Branch (Serial No. 1683/2011).

SAMPLE COLLECTION

From July to August 2011, the lower cul-de-sac of 100 eyes were swabbed using a dry sterile cotton swap per eye, taking care not to contaminate the swap by contact with the eyelashes or skin of eyelids. Swabs were placed in test tubes containing sterile transport medium (peptone water; Merck, Darmstadt, Germany), and were transported immediately to

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Yersinia enterocolitica. L’ efficacité antibactérienne et la sensibilité aux antibiotiques ont été évaluées. Un total de 100 prélèvements conjonctivaux a été réalisé à partir de 50 mules, âgés de 5 à 12 ans, et ne présentant aucun signe de problèmes oculaires. Les échantillons ont été cultivés pour les bactéries aérobies et la sensibilité à 12 antibiotiques a été évaluée. Une croissance bactérienne a été obtenue chez 85 échantillons sur 100 (85%). La plupart des bactéries isolées (97/109: 89%) étaient Gram positif. Les agents bactériens identifiés étaient par ordre de fréquence Staphylococcus epidermidis, Corynebacterium spp., Bacillus cereus, Streptococcus equi subsp. zopledemicus, Escherichia coli, Citrobacter diversus et Yersinia enterocolitica. Antibactérienne efficacy of flurenicol, flumequine and gentamicin was identified against 90,82, 70,64 and 67,88 % of isolates respectively. Bacterial species isolated in the current study were comparable with those previously reported for the ocular surface of other equine species.

Mots-clés : fleur bactérienne, oeil, mule, Staphylococcus spp., sensibilité aux antibiotiques

RESUME

Flore bactérienne conjonctivale chez les mules en bonne santé (Equus mulus)

Cette étude visait à identifier la flore bactérienne de la surface oculaire de mules en bonne santé et à déterminer la sensibilité de ces germes à certains antibiotiques. Un total de 100 prélèvements conjonctivaux a été réalisé à partir de 50 mules, âgés de 5 à 12 ans, et ne présentant aucun signe de problèmes oculaires. Les échantillons ont été cultivés pour les bactéries aérobies et la sensibilité à 12 antibiotiques a été évaluée. Une croissance bactérienne a été obtenue chez 85 échantillons sur 100 (85%). La plupart des bactéries isolées (97/109: 89%) étaient Gram positif. Les agents bactériens identifiés étaient par ordre de fréquence Staphylococcus epidermidis, Corynebacterium spp., Bacillus cereus, Streptococcus equi subsp. zopledemicus, Escherichia coli, Citrobacter diversus et Yersinia enterocolitica. L’efficacité antibactérienne du flurenicol, de la flumequine et de la gentamicine s’est manifestée respectivement sur 90,82, 70,64 et 67,88% des souches isolées. Les espèces bactériennes identifiées dans la présente étude étaient comparables à celles rapportées pour la surface oculaire d’autres équidés.
the microbiology laboratory in a chilled thermal box.

**BACTERIOLOGY**

In the laboratory, samples were inoculated on 5% ovine blood (Merck, Darmstadt, Germany) and MacConkey (Merck, Darmstadt, Germany) plates and incubated at 37 °C for 48 h in an aerobic condition. Isolates were subjected to identification using standard microbiological methods [21].

**ANTIMICROBIAL SENSITIVITY TEST**

Antibiotic sensitivity test of all the bacterial isolates was conducted using disc diffusion technique on Mueller-Hinton agar (Merck, Darmstadt, Germany) at 37 °C for 24 h. The results of sensitivity testing with the 12 antibiotic disks including chloramphenicol 30 μg (C), tylsoin 30 μg (TY), enrofloxacin 5μg (NFX), trimethoprim 5 μg (TMP), amoxicillin 25 μg (AMX), furazolidone 100 μg (FR), flumequine 30 μg (FM), ceftiofur 30 μg (CEF), gentamicin 10 μg (GM), colistin 10 μg (CL), lincomycin 15 μg/ spectinomycin 200 μg (LS) (Padtan Teb, Tehran, Iran) and florfenicol 30 μg (FFC) (Iran Daru, Tehran, Iran) were interpreted and recorded to the Clinical Laboratory Standards Institute (CLSI) recommendations [18].

**Results**

**ISOLATES**

Out of the 100 samples, 85 (85%) were found to be positive for bacterial growth. A total of 109 bacteria belonging to 7 genera were isolated (Table I), with Gram-positive bacteria (4 genera) predominating (97/109: 89%). *Staphylococcus epidermidis* was the most commonly isolated organism, accounting for 29.35% of isolates. Among the Gram-negative bacteria (3 genera), the most frequently isolated species was *Escherichia coli* (7.33% of isolates).

**ANTIBIOTIC SENSITIVITY OF ISOLATES**

Table II exhibits the antibiotic sensitivity profile of all bacterial isolates from healthy mule’s conjunctiva. Six out of the 12 antimicrobials including florfenicol, flumequine, enrofloxacin, trimetoprim, chloramphenicol and ceftiofur were effective against all isolates. Only 14 of the 109 (12.84%) isolates were sensitive to all antibiotics tested. Florfenicol, flumequine and gentamicin were the most efficient antimicrobials with efficacy rate of 90.82, 70.64 and 67.88% against the isolates, respectively.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. of isolates</th>
<th>% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>32</td>
<td>29.35</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>27</td>
<td>24.77</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>24</td>
<td>22.01</td>
</tr>
<tr>
<td><em>Streptococcus equi subsp. zooepidemicus</em></td>
<td>14</td>
<td>12.84</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8</td>
<td>7.33</td>
</tr>
<tr>
<td><em>Citrobacter diversus</em></td>
<td>3</td>
<td>2.75</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>1</td>
<td>0.91</td>
</tr>
</tbody>
</table>

**Table I:** Bacteria recovered from normal conjunctival sac of mules

<table>
<thead>
<tr>
<th>Isolates</th>
<th>FFC</th>
<th>LS</th>
<th>NFX</th>
<th>C</th>
<th>CEF</th>
<th>CL</th>
<th>FR</th>
<th>TY</th>
<th>AMX</th>
<th>GM</th>
<th>TMP</th>
<th>FM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staph. epidermidis</em></td>
<td>100</td>
<td>75.00</td>
<td>75.00</td>
<td>50.00</td>
<td>12.50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46.87</td>
<td>84.37</td>
<td>50.00</td>
<td>68.75</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>92.59</td>
<td>74.07</td>
<td>25.92</td>
<td>29.62</td>
<td>37.02</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11.11</td>
<td>88.88</td>
<td>37.03</td>
<td>74.07</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>83.33</td>
<td>58.33</td>
<td>25.00</td>
<td>33.33</td>
<td>29.16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>70.83</td>
<td>83.33</td>
</tr>
<tr>
<td><em>Strep. equi subsp. zooepidemicus</em></td>
<td>85.71</td>
<td>78.57</td>
<td>42.85</td>
<td>50.00</td>
<td>42.00</td>
<td>14.28</td>
<td>7.14</td>
<td>7.14</td>
<td>35.71</td>
<td>85.71</td>
<td>42.00</td>
<td>57.14</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>75.00</td>
<td>0</td>
<td>62.50</td>
<td>62.50</td>
<td>12.50</td>
<td>37.50</td>
<td>0</td>
<td>0</td>
<td>62.50</td>
<td>87.50</td>
<td>62.50</td>
<td>62.50</td>
</tr>
<tr>
<td><em>Citrobacter diversus</em></td>
<td>100</td>
<td>66.66</td>
<td>66.66</td>
<td>66.66</td>
<td>33.33</td>
<td>0</td>
<td>33.33</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>66.66</td>
<td>33.33</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table II:** Percentage distribution of antibiotic sensitivity pattern among bacteria recovered from conjunctival sac of healthy mules

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Discussion

To our knowledge, this is the first published report of the conjunctival bacterial isolates in healthy mules. In the current study Gram-positive bacteria (89% of all isolates) were predominant in conjunctival sac of mules. This finding is in agreement with those reported for horses (52-77%) [11,14] and donkeys (65%) [9]. Isolation of a higher incidence of Gram-negative bacteria compared to Gram-positive ones [5], was reported in only one study of normal conjunctival flora in Italian horses, which may reflect geographic, seasonal or environmental differences [14].

In our study, Staphylococcus spp. was found to be the most frequently isolated bacterial species. Similar result has been found in studies of horses [28], donkeys [9] and other animal species such as water buffalos [27], dogs [19], cats [8], domestic rabbits [4], Asian elephants [26], mule deers [7], raccoons [22], opossums [20], iguanas [25] and birds [6]. It seems that the genus Staphylococcus is an important component of conjunctival bacterial flora in many domestic and wild animal species.

We found a higher number of opportunistic bacteria, i.e., coagulase-negative Staphylococcus, Corynebacterium and B. cereus, than previously reported from both normal [1,14] and diseased eyes of horses [13,16,29].

Additionally, a low prevalence of potential pathogens, such as beta-hemolytic Streptococcus [2,12,15,23,29] and Enterobacteriace genera [13,16] was identified in our study. Potential pathogens have also been isolated in low frequency in other studies of normal equine eye flora [1,16].

Because pathogenic bacteria are ubiquitous in the environment, the low isolation rates of these organisms from healthy ocular surface suggest that the normal flora may control or suppress colonization of pathogens.

Superficial ulcers generally heal quickly without complications with application of broad spectrum antibiotics and some other drugs. However, infected ulcers may progress to corneal perforation in as little as 24 h [3]. Hence, immediate use of antimicrobial is recommended for treatment of corneal ulcers to prevent the establishment of infection following the trauma. Recommended antimicrobials for the preventive treatment of corneal ulcers in horses include topical gentamicin and chloramphenicol [11,14]. In the current study, high efficacy (more than 90% of isolates sensitive) was displayed by florfenicol, but this drug is not licensed for use in equine species. Flumequine (with efficacy on more than 70% of the isolates) has toxic effects on corneal epithelial cells [17]. Antimicrobials gentamicin and chloramphenicol (available for ophthalmic topical use) were effective on only 67.88% and 43.11% of isolates, respectively. It is clear that none of the assessed antimicrobials are recommendable for preventive treatment of corneal ulcers in mules and further studies are needed for finding of appropriate antibiotics.

Genera of bacteria identified in this study were similar to genera isolated from other studies of normal equine eyes. Many of these isolates were non-pathogenic or weakly pathogenic but among them highly pathogenic also could be found.

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Conflict of interest

The authors acknowledge no conflict of interest in this study.

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