

Conjunctival Bacterial and Fungal Isolates in Clinically Healthy Working Horses in Iran

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Summary

This study was conducted to identify bacterial and fungal isolates of the normal eyes in working horses in Iran. Ninety swabs were taken from the conjunctival sac of 45 clinically healthy horses. Aerobic bacterial and fungal cultures were plated. A total of 9 different bacterial species (3 Gram-positive, 6 Gram-negative) and 7 different fungal species (6 molds, 1 yeast) were recovered. *Bacillus* spp., *Staphylococcus* spp. and *Klebsiella* spp. were the most frequently isolated bacteria. *Aspergillus* spp. and *Penicillium* spp. were the most frequently recovered fungi. The microbial species isolated are comparable with studies performed on horses in other areas.

Keywords: Bacteria, Fungi, Conjunctiva, Working horse, Iran

İran'da Klinik Olarak Sağlıklı Yük Atlarından İzole Edilen Konjunktival Bakteriyel ve Fungal Ajanlar

Özet

Bu çalışma, İran'da yaşayan sağlıklı yük atlarının gözünde bulunan bakteri ve mantar izolatlarının identifikasyonu amacıyla yapıldı. Klinik olarak sağlıklı görünen 45 atın konjunktival kesesinden 90 sürüntü örneği toplandı. Aerobik bakteri ve mantarlar yönünden ekimler yapıldı. 9 farklı bakteri (3 Gram-pozitif, 6 Gram-negatif) ve 7 farklı mantar türü (6 küf, 1 maya) izole edildi. En fazla izole edilen bakterinin *Bacillus* spp., *Staphylococcus* spp. ve *Klebsiella* spp. olduğu belirlendi. En yaygın izole edilen mantar türlerinin ise *Aspergillus* spp. ve *Penicillium* spp. olduğu tespit edildi. Bu mikroorganizmaların farklı ülkelerde yaşayan atlardan izole edilenler ile benzer olduğu anlaşıldı.

Anahtar sözcükler: Bakteri, Mantar, Konjunktiva, Yük atı, İran

INTRODUCTION

Most reports of conjunctival bacterial flora in the healthy horses show predominance of Gram-positive organisms. The most commonly isolated bacteria include *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. [1-3]. From fungi genera of *Aspergillus*, *Cladosporium*, *Penicillium* and *Mucor* predominate in most studies [2-4]. When a corneal erosion/ulceration occurs, resident and transient ocular surface microbes penetrate the sub-epithelial tissue and result in infection [5]. Knowledge of normal conjunctival microflora is important to adopt proper treatment of corneal ulcers. This investigation was conducted to determine the fungal and aerobic bacterial flora in the conjunctival sac of healthy working horses in Iran.

MATERIAL and METHODS

Forty-five working horses, of different sexes (23 males and 22 females) and ages (1-20 years), without signs of ophthalmic abnormality, situated in rural areas of Urmia (northwest of Iran) were included in this study. During summer of 2012, samples from the conjunctival sac of both eyes of horses were obtained using a dry cotton swab and transported to the laboratory in tubes containing sterile transport medium. Swabs were plated in 5% ovine blood agar (Merck, Germany, catalog No. 940986), MacConkey agar (Merck, Germany, catalog No. 138521) and sabouraud dextrose agar (Merck, Germany, catalog No. 426638) and incubated at 37°C for 48 h for bacterial growth and at 25°C for 4 weeks for fungal growth. Colonies isolated from all plates were identified using standard microbiological and



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biochemical methods [6]. The study was approved by the ethics committee for animal experimentation by the Islamic Azad University-Urmia Branch (Serial No. 1714/2012).

RESULTS

The isolated organisms and the frequencies of isolation are shown in [Table 1](#) and [Table 2](#).

Bacterial Isolates

Gram-positive organisms were the predominant bacteria, comprising 59.51% of isolates. *Bacillus* spp. (27.68%) was the most frequent isolate, followed by *Staphylococcus* spp. (24.22%). Gram-negative bacteria comprised 40.49% of all isolates, with *Klebsiella* spp. being the most prevalent (12.58%).

Table 1. Isolated bacteria from normal conjunctiva of healthy working horses

Tablo 1. Sağlıklı iş atlarının normal konjunktivalarından izole edilen bakteriler

Bacteria	Number of Isolates	Percent of Isolates
Gram-positive		
<i>Bacillus cereus</i>	80	27.68
<i>Staphylococcus epidermidis</i>	70	24.22
Beta-hemolytic streptococci	22	7.61
Gram-negative		
<i>Klebsiella oxytoca</i>	36	12.58
<i>Escherichia coli</i>	23	7.95
<i>Providencia alcalifaciens</i>	17	5.88
<i>Enterobacter aerogenes</i>	16	5.53
<i>Citrobacter diversus</i>	13	4.49
<i>Proteus</i> spp.	12	4.15
Total	289	100

Table 2. Isolated fungi from normal conjunctiva of healthy working horses

Tablo 2. Sağlıklı iş atlarının normal konjunktivalarından izole edilen funguslar

Fungi	Number of Isolates	Percent of Isolates
Mold		
<i>Penicillium</i> spp.	65	25.59
<i>Aspergillus fumigatus</i>	50	19.68
<i>Aspergillus niger</i>	44	17.32
<i>Alternaria</i> spp.	35	13.77
<i>Aspergillus flavus</i>	28	11.02
<i>Mucor</i> spp.	24	9.44
Yeast		
<i>Candida</i> spp.	8	3.14
Total	254	100

Fungal Isolates

Molds were the predominant fungi, comprising 96.85% of isolates. *Aspergillus* spp. (48.03%) was the most frequent isolate, followed by *Penicillium* spp. (25.59%). *Candida* spp. (3.14%) was the only isolated yeast species.

DISCUSSION

Bacillus spp. was the most frequently isolated bacterial organism in our study. This organism has been reported in several different studies as the most common isolate of conjunctival sac in healthy horses [1,3,7]. *Bacillus* spp. is usually considered as a non-pathogenic organism that isolate from healthy and diseased eyes [8].

Other Gram-positive isolates of this study especially Beta-hemolytic streptococci as potentially pathogens have been reported frequently from equine infectious keratitis [8,9].

Gram-negative bacteria are the most commonly isolated organisms from infectious keratitis of horses in various studies which include *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp., *Escherichia coli*, *Moraxella* spp. and *Providencia* spp. [8,10]. Most of these potentially pathogenic organisms were isolated from eye of healthy horses in our study.

In various studies, molds vs. yeasts have been reported to be the predominant components of fungal flora in equine normal ocular surface by *Aspergillus* spp. having first rate [1,3,4]. In our study filamentous fungi with predominance of *Aspergillus* spp. were also the most frequently isolated fungi. Fungal genera isolated in this report are considered saprophytic; however, all of them had reported from equine keratomycosis [11,12].

The microbial species isolated in our study are comparable with studies performed on healthy horses in other countries. Most of these isolates are potentially pathogenic organisms.

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