

The study of aerobic bacterial flora of the upper respiratory tract of equines from Jammu and Kashmir region of India

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Abstract

Aim: To isolate aerobic bacterial micro flora residing in the upper respiratory tract of equines used by the pilgrims and tourists in Jammu & Kashmir.

Materials and Methods: 88 apparently healthy equines and 53 equines with respiratory tract diseases were used in this study. Swab samples were collected from the upper respiratory tract of equines. Isolation and identification of the bacteria was conducted under aerobic conditions. Each of the sample processed yielded at least one type of bacteria species.

Results: A total of 321 bacterial isolates were recovered from both groups of equines. The majority of the isolates were Gram positive (84.11%) and the rest were Gram negative (15.88%). Bacterial isolates identified in order of the magnitude were *Streptococcus equi* subsp. *zooepidemicus* (17.44%), *Micrococcus* spp. (9.96%), *Corynebacterium* spp. (9.65%), *Staphylococcus intermedius* (9.65%), *Staphylococcus aureus* (8.72%), *Bacillus* spp. (7.16%), *Streptococcus pneumoniae* (5.60%), *Staphylococcus chromogens* (5.60%), *Streptococcus equismilis* (5.29%), *Pseudomonas aeruginosa* (5.29%), *Rhodococcus equi* (3.73%), *Escherichia coli* (3.73%), *Klebsiella pneumoniae* (3.42%), *Proteus vulgaris* (3.42%), and *Streptococcus equi* subsp. *equi* (1.24%).

Conclusion: The present study reveals the predominance of Gram positive bacteria in both healthy and diseased animals. Bacteria were recovered at a higher rate from diseased equines than from apparently healthy animals. *Streptococcus equi* subsp. *zooepidemicus* was mainly found to be associated with respiratory tract infections.

Keywords: bacterial flora, equines, respiratory tract, *Rhodococcus equi*, *Streptococcus equi*

Introduction

India has about 20 million equids maintained under different farming systems and employed for various purposes, out of which Jammu & Kashmir (J&K) has approximately 5.3 million equines [1]. In particular, equines in this region are used by Gujjars and Bakarwals to earn their livelihood primarily through tourism. Due to their ability to survive on lesser quantity of food and due to their ability to conserve more energy, equines can be used in harsh environments and for difficult working conditions [2] such as a visit to the important religious shrines in J&K; Mata Vaishno Devi Shrine and Holy Amarnath Cave. Besides, equines are widely used by tourists for horse riding and trekking. Equines are vulnerable to many diseases and among them respiratory problems are the most severe health issues observed in working equines and have a high prevalence rate of 30% [3]. So far most of the research remained focused on viral rather than bacterial causes of respiratory tract infections because

bacteria are generally considered as secondary invaders of upper respiratory tract in equines. The most common opportunistic bacteria associated with respiratory infections include *Streptococcus equi* subsp. *zooepidemicus*, *Actinobacillus equuli*, *Bordetella bronchiseptica*, *Escherichia coli*, *Pasteurella* spp. and *Pseudomonas aeruginosa* [4].

Therefore this study was designed to explore and differentiate the bacterial flora of the diseased and apparently healthy animals in the upper respiratory tract. Moreover, our study will yield highly relevant data regarding important zoonotic bacterial pathogens that can be contracted from equines. Furthermore, our data will be helpful to determine and assess the prevalence of bacterial species in upper respiratory tract of equines in the northern temperate environment of J&K, India.

Materials and Methods

Study area: The present study was carried out in areas of Jammu and Kashmir (J&K) in which equine rearing is highly prevalent. The study was conducted from September, 2010 to August, 2011. Samples were collected from five regions of J&K viz. R.S. Pura, Katra, Tangmarg,

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Table-1. Area wise details of sample collection

| Sr. No. | Region | No. of animals examined | No. of diseased | No. of apparently healthy | Total no. of Samples |
|---------|-----------|-------------------------|-----------------|---------------------------|----------------------|
| 1. | R.S. Pura | 63 | 21 | 25 | 46 |
| 2. | Katra | 54 | 16 | 23 | 39 |
| 3. | Patnitop | 22 | 5 | 13 | 18 |
| 4. | Tangmarg | 33 | 6 | 15 | 21 |
| 5. | Pahalgam | 29 | 5 | 12 | 17 |
| | Total | 201 | 53 | 88 | 141 |

Table-2. Bacterial species isolated from the upper respiratory tract of equines

| Sr. No. | Type of bacteria | Diseased | | Apparently Healthy | |
|---------|--|--------------------------|---------------|--------------------------|---------------|
| | | Total number of isolates | % of isolates | Total number of isolates | % of isolates |
| | Gram-positive | 105 | 82.7 | 165 | 85.05 |
| 1. | <i>Bacillus</i> spp. | 4 | 3.14 | 19 | 9.79 |
| 2. | <i>Streptococcus zooepidemicus</i> | 37 | 29.13 | 19 | 9.79 |
| 3. | <i>Streptococcus pneumonia</i> | 7 | 5.51 | 11 | 5.67 |
| 4. | <i>Streptococcus equismilis</i> | 11 | 8.66 | 6 | 3.09 |
| 5. | <i>Streptococcus equi</i> subsp. <i>equi</i> | 4 | 3.14 | 0 | 0 |
| 6. | <i>Rhodococcus equi</i> | 7 | 5.51 | 5 | 2.57 |
| 7. | <i>Staphylococcus aureus</i> | 17 | 13.38 | 11 | 5.67 |
| 8. | <i>Staphylococcus intermedius</i> | 8 | 6.29 | 23 | 11.85 |
| 9. | <i>Corynebacterium</i> spp. | 4 | 3.14 | 27 | 13.91 |
| 10. | <i>Micrococcus</i> spp. | 6 | 4.72 | 26 | 13.4 |
| 11. | <i>Staphylococcus chromogens</i> | 0 | 0 | 18 | 9.27 |
| | Gram-negative | 22 | 17.3 | 29 | 14.94 |
| 1. | <i>Escherichia coli</i> | 4 | 3.14 | 8 | 4.12 |
| 2. | <i>Proteus vulgaris</i> | 5 | 3.93 | 6 | 3.09 |
| 3. | <i>Pseudomonas</i> spp. | 6 | 4.72 | 11 | 5.67 |
| 4. | <i>Klebsiella pneumoniae</i> | 7 | 5.51 | 4 | 2.06 |
| | Total | 127 | 100 | 194 | 100 |

Pahalgam and Patnitop (Table-1).

Ethical approval: The experiment was done as per guideline of CPCSEA and Institutional Animal Ethics Committee.

Samples: Samples were collected from both clinically affected animals and apparently healthy animals. A total of 141 nasal swab samples were collected from five regions of J&K. The swab samples were collected from both nostrils after cleaning the anterior part of nostrils of equines using manually made 15 inch long sterile swabs.

Bacteriological isolation was done as soon as possible. Brain heart infusion (BHI) broth was used for the primary enrichment of bacterial isolates. The growth in BHI was streaked onto sheep blood agar with 5% (v/v) defibrinated sheep blood. All cultures were incubated aerobically at 37°C for 48 hrs. Representative colonies from culture positive plates were subcultured on the 5% sheep blood agar for isolation of pure colonies. Colony morphology, colour, size, elevation, status of haemolysis were recorded. Pure colonies were transferred to blood agar slants for further biochemical tests. These culture isolates were subjected to Gram's staining procedure, and tests for primary and secondary biochemical tests for identification of culture [5]. Characterisation of Streptococcal isolates was done using Histrep identification kit (Himedia, Mumbai, India) and Histrep Latex agglutination kit (Himedia, Mumbai, India) and the results were interpreted as per the manufacturer's instructions.

Results and Discussion

All the samples (88 nasopharyngeal swabs from

apparently healthy equines and 53 nasopharyngeal swabs from equines with respiratory disease) collected for aerobic bacterial isolation yielded at least one type of bacteria. Except for a few, *Bacillus* spp., *Staphylococcus intermedius* and *S. aureus* which were isolated as pure cultures in samples collected from nasopharynx, the majority of cultures on plates revealed mixed infections with bacteria. A total of 321 isolates were obtained from the 141 nasal swabs, of which 270 (84.11%) were Gram-positive and 51 (15.88%) were Gram-negative. The isolation rate of Gram positive bacteria was significantly higher ($P < 0.01$) than Gram negative bacteria, a finding that is in good agreement with previous report [6]. The data regarding the bacterial isolates recovered from nasopharynx of equines with respiratory disease and apparently healthy equines are shown in Table-2.

Major proportions of bacterial isolates recovered from both apparently healthy and sick equines were Gram-positive. In the present study, detailed investigation was carried out to explore the resident flora of bacteria inhabiting the upper respiratory tract of apparently healthy equines and equines with upper respiratory tract diseases in order to determine the associated risks to the persons handling them, especially the owners, tourists and pilgrims. It was found that equines in this part of India are a reservoir of some zoonotically important bacterial pathogens like *Rhodococcus equi* and *S. zooepidemicus* and pose threat to tourists and handlers to contract the infection [7-8]. In this study, the quality and quantity of bacterial isolates from both apparently healthy equines and equines with respiratory diseases was compared. The

quality of bacterial isolates recovered was not much different except for *S. equi* subsp. *equi* which was isolated only from diseased equines implying its pathogenic role in respiratory tract diseases in Jammu and Kashmir. The recovery of the *S. equi* subsp. *equi* was very low as compared to other reports [9] which could be because of that fact that we did employ any invasive methods for sampling of sites like guttural pouch which is an ideal site for bacterial recovery from chronically infected animals. Further the organism is highly sensitive to desiccation and the mucocilliary apparatus of horses is very efficient in removal of this pathogen from the site sampled in the study. Also *Staphylococcus chromogens* was isolated only from healthy equines. Out of a total 321 bacterial isolates, 270 (84.11%) were Gram-positive bacteria while the other 51 (15.88%) were Gram-negative bacteria which is in accordance with the earlier reports [10]. Of 194 bacterial isolates from apparently healthy equines, majority of the bacteria (85.05%) were Gram positive while as rest (14.94%) were Gram-negative.

The higher recovery rate of Gram-positive bacteria from apparently healthy equines has also been reported by other workers [10,11]. Out of 127 bacterial isolates from equines with respiratory diseases, 105 were Gram-positive while only 22 were Gram-negative. Predominance of Gram-positive bacteria from diseased equines shows their significant role in being opportunistic equine pathogens. However, the recovery rate in diseased equines (2.4%) was slightly greater than the rate for apparently healthy (2.2%) equines. These results are comparable to previous findings of Kester et al. [11]. The streptococcal isolates were found in both diseased and apparently healthy equines, 46.45% and 18.55% respectively. The predominant bacterial isolate found in diseased equines was *S. zooepidemicus* (29.13%) which is in accordance with other reports [12,13]. Recovery of this bacterium frequently without any other primary infection suggests its primary involvement in respiratory tract diseases of equines [14] and that it is an important upper respiratory tract pathogen causing serious inflammatory reaction and mucous deposition in the respiratory tract [15]. However Wood et al. [16] have reported it as a secondary bacterial pathogen in horses suffering from primary viral infection.

Corynebacterium spp. was encountered as a second predominant bacterium among those recovered from apparently healthy equines. They were isolated at a rate of 13.91% from apparently healthy equines, and 3.14% from equines with respiratory tract diseases. These species were also recovered as common isolates from upper respiratory tract infections in recently weaned foals [17]. They are known to cause a variety of suppurative conditions in equines [18,19]. *S. aureus* was isolated at a rate of 13.3% from apparently healthy equines, and 13.3% from equines with respiratory problems. This is lower as compared to Sweeny et al. study [19] but comparable to study of Dessica et al.

[10]. This may be due to differences in the range of animals used and different environmental factors in their study. *S. aureus* has been isolated from respiratory tracts of healthy and pneumonic cases of domestic animals in many other cases as well [20,21]. *S. aureus* is the main inhabitant of the mucous membranes in the upper respiratory tract of animals. It can be involved as an opportunistic bacterium, following pathologic role of stress conditions such as viral infections, and other causes of infection in immunosuppressed hosts [22].

Coagulase negative *Staphylococcus* was the commonly recovered bacterial isolate in this study. It included *S. intermedius* and *S. chromogens*. They were isolated at a rate of 21.02% from apparently healthy equines, and 6.29% from equines with respiratory problems. The isolation rate of these bacteria was higher in apparently healthy equines as compared to the diseased. However, Boguta et al. [17] did not find any variation between the two cases. But in study of Fernandes et al. [23], coagulase negative *Staphylococcus* bacteria were isolated at a much higher rate of 36.6% from upper respiratory tract samples. This could be due to a smaller sample size in their study. *Bacillus* spp. was isolated at a rate of 9.79% from apparently healthy equines and 3.14% from equines with respiratory problems. Several researchers have isolated *Bacillus* spp. at different rates of recovery from different species of animals [24,25]. Most of the *Bacillus* spp. are saprophytes that are widely distributed in air, soil and water [18]. The recovery rate of *Bacillus* spp. was lower in diseased equines as compared to study of Sweeny et al. [25]. This may be due to the improved method of sample collection in our study. In some previous studies, sampling was done with artificially available cotton swabs which were shorter in length and could only reach anterior portions of the long nostrils of equines which is exposed to open environment, therefore they isolated *Bacillus* at higher rate than what we found. Contrary to this, we used 15 inch long manually made swab. During our study, we first cleaned the anterior portions of the nostril with 70% alcohol and then sampled the posterior portion of the nostril.

Klebsiella pneumoniae was the dominant isolate among Gram-negative, with a recovery rate of 2.1% from apparently healthy equines and 12.7% from equines with respiratory problems. It was recovered more frequently from diseased animals (30%) than apparently healthy ones (6%). *K. pneumoniae* was isolated in study of Savitha [21] at a rate of 5.51% from horses with pneumonia. Since *K. pneumoniae* inhabits the intestinal tracts of animals, fecal contamination of the environment may account for the wide distribution of the organism, and contributes to the occurrence of opportunistic infections [18]. *Escherichia coli* was isolated as a second dominant bacterium among Gram-negative bacteria, with the isolation rate of 4.12% from apparently healthy equines, and 3.14% from equines with respiratory problems. *E. coli* has been also

isolated from the nasal tract of other animals [26,27].

R. equi was isolated at a rate of 2.57% from apparently healthy animals, and 5.51% from equines with respiratory problems. The bacterium is an opportunistic pathogen and common soil inhabitant [18]. Severe equine pneumonia, usually found with abscess formation in the lung tissue, is most often associated with *R. equi* infection [28,29]. *R. equi* is one of the most common isolates from equine respiratory tracts [30,31]. But in our study the isolation rate was less compared to Ode et al. [32] which can be attributed to the fact that we did not use tracheo-bronchial lavaging for sampling. *Micrococcus* spp. was isolated at a higher rate from apparently healthy equines (13.4%) while a low recovery rate was observed from diseased animals signifying that these are non pathogenic species found in upper respiratory tract of equines. *Pseudomonas* spp. was the predominant Gram negative bacteria recovered from apparently healthy equines (5.67%). Similar reports of high recovery rate of *Pseudomonas* spp. were recorded by Cabbasi et al. [33] during their study on apparently healthy animals.

Conclusions and recommendations

Our study revealed a variety of bacteria; predominantly Gram positive bacteria residing in their upper respiratory tract. On a closer examination it was found that bacteria in apparently healthy equines can become opportunistic pathogens during stress to animals and thus resulting in endogenous infection. On a closer examination we found that bacteria in apparently healthy equines can become opportunistic pathogens during stress to animals and thus resulting in endogenous infection. In our study, one of the important finding was that *S. equi* subsp. *zooepidemicus* was found invariably in higher numbers in diseased equines suggesting its primary role in establishing respiratory infection. Also *R. equi*, important zoonotic bacteria was recovered in apparently healthy and diseased equines which suggest that equines can be an important reservoir for this bacterium and that humans can get infection with apparently healthy ones.

We recommend further investigation particularly in the areas of;

1. Investigation of the drug resistance of the isolates of bacteria recovered.
2. Analysis of clonal diversity to establish the source of infection particularly of zoonotic bacteria viz. *R. equi* and *S. equi* sub spp. *zooepidemicus*.
3. Isolation of anaerobic bacteria, fungal and parasitic agents associated with respiratory tract infection of equines.
4. Virulence characterization of important equines pathogens associated with respiratory tract infections.

Authors' contributions

IAM carried out sampling, isolation and manuscript preparation, BK guided the research plan and manuscript revision, AT participated in scientific discussion and coordination, NW participated in

scientific discussion and manuscript writing, FNF participated in scientific discussion, drafting and revision of manuscript, SAD helped in sampling, SG helped in Lab work of bacterial isolation and identification, GAB helped in lab work, AAZ helped in sampling and AI participated in drafting and revision of manuscript. All authors read and approved the final form of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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