

Bacteriological Examination of Respiratory Tract of Apparently Healthy Camels in Egypt

¹Mohamed Ismail, ²Nashwa Ezz El-Deen and ²Mahmoud El-Hariri

¹General Organization for Veterinary Services, Egypt

²Department of Microbiology,

Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: Camels are important in the livestock economy by their adaptability to adverse environmental conditions as they are good sources of protein and income for developing countries. Studies conducted on the bacterial flora of the respiratory tract in camels is very rare and with fewer studies on the apparently normal nasal passage. This study was carried out on 98 apparently healthy camels. These camels were selected from different abattoirs at three different Egyptian Governorates (Cairo, Giza and El sharkia). The collected samples were blood, nasal swabs, tracheal swabs and lung samples. Seventy-seven isolates were obtained. The most frequently isolated species was *Staphylococcus aureus* (14.5 %), while *Pseudomonas aeruginosa* and *Bacillus* spp. were the second dominant bacteria (11.0 %) and (10.5 %) respectively. The lowest rate of isolation achieved by *E. coli* (3 %) and *Klebsiella* spp (0.5 %). The isolation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from the respiratory tract of apparently healthy camels in this work reflects their potential role in most common respiratory diseases encountered in small camels.

Key words: Camels • Respiratory passages • Bacteriological examination • *S.aureus* • *P.aeruginosa*

INTRODUCTION

The dromedary camel is a multipurpose animal of very high socio-economic value. Camels are adapted to the harsh environments of semiarid and arid zones, essentially kept for milk and meat production and transportation. [1, 2].

Camels were naturally considered resistant to most of the diseases commonly affecting livestock. Studies conducted on the bacterial flora of the respiratory tract of domestic camels in various parts of the world including Egypt mainly focus on the pneumonic lungs of camel with very rare studies on the microbial flora of the upper and lower respiratory passages of apparently normal camels [3, 4]. The aim of this study to identify the most common inhabitant of aerobic micro flora in respiratory tract of apparently healthy camels by using conventional microbiological standard methods and PCR for identification of pathogenic bacterial isolates.

MATERIALS AND METHODS

Animals: The study was carried out at three different abattoirs at Cairo, El-Sharkia and Giza, on 98 apparently healthy camels at ante-mortem examination.

Sample Collection

Blood: Blood samples were collected from the jugular vein with a sterile syringe. The blood were collected aseptically on citrated vacutainer, then samples were inserted into tubes containing brain heart infusion broth.

Nasal Swabs: The nasal samples were collected by inserting sterile cotton-tipped applicator sticks or swab into the nasal passage after proper cleaning and disinfection of the external nares. Each nasal swab was carefully cut and put into a labeled bottle containing 2 mL brain heart infusion broth. The swabs were transported in a cool box to the laboratory for bacterial culture.

Tracheal Swabs and Lung Tissue: After slaughtering, the trachea of each camel was opened using a sterile scalpel blade and the sample collected by inserting sterile cotton swab into the trachea, then the swabs were processed like nasal swabs. In the samples of lung tissues, the external surfaces were disinfected with 70% alcohol to minimize surface contamination using sterile scissors and tissue forceps. A pieces of the lung were transferred into sterile screw-capped bottles and transported in a cool box for further examination.

Microbiological Analysis: The totals of nasal and tracheal swab samples belong to 98 camels were streaked over the plates containing blood agar base supplemented with 7% sheep blood and McConkey agar. The streaking was further spread with inoculating loop to aid colony isolation. The plates were incubated under aerobic conditions for 24-48 h at 37°C [5]. After the incubation the cultural growth characteristics and each different colony was examined macroscopically (colony morphology, hemolysis, pigment producing) and microscopically (Gram staining). Mixed colonies and Gram negative bacteria were subculture on both blood and McConkey agars and further incubated aerobically for 24 h. Pure culture of single colony type from both blood and McConkey agars were transferred onto tryptic soya agar slants for further characterizations and Identification of microorganisms was done using conventional biochemical tests including catalase, oxidase and fermentative/oxidative tests for final identification following standard procedures [6 1 7].

Data Analysis: It was collected 200 clinical samples divided on blood, nasal, tracheal swabs and lung tissues out of examination of 98 camels. The total of isolated bacteria was 77 bacterial isolates divided into 50 Gram positive and 27 Gram negative. The recovery rate of each bacterial spp. Estimated by dividing the number of isolated bacterial spp on total number of examined samples (200) as shown in Table (1).

Bacterial Genomic Dna Extraction: A single colony of bacteria was inoculated into 10ml of peptone water and incubated at 37°C for 18 hours. The cells were collected by centrifugation at 5000rpm for 5 minutes and washed with TE buffer. The washed cells were resuspended in 500ml of TE buffer and mixed with 10µl of Lysozyme (50mg/ml), 3µl of RNase(10mg/ml) and incubated at 37°C for 30 minutes. 500µl of Lysis buffer (Guanidine thiocyanate 60% w/v, 100mM EDTA sodium salt pH 8.0, N-Lauryl sarcosine

0.5% w/v) was added and incubated at 37°C for 10 minutes for complete cell lysis. The mixture was thoroughly mixed after the addition of 0.25ml of cold 7.5M ammonium acetate and then kept on ice for 10 minutes. 0.5ml of chloroform: Isoamyl alcohol (24:1) was mixed thoroughly with the reaction mixture. The mixture was centrifuged at 13000 rpm for 10 minutes. To the 0.7ml of the supernatant 0.37ml of cold isopropanol was added and mixed gently by inverting the tube. The mixture was centrifuged at 13000 rpm for 3 minutes and the precipitated DNA was then resuspended in 200µl of TE pH 7.4. After the addition of 0.5ml of cold absolute ethanol to the DNA solution the mixture was kept at -80°C for 15 minutes and then centrifuged at 13000rpm for 3 minutes. The supernatant was removed, the precipitate was air dried and dissolved in 100µl of TE and stored at -20°C.

Molecular Detection of *Staph Aureus* and *Pseudomonas Aeruginosa*: Polymerase chain reaction was applied on suspected isolates of *Staph aureus* and *Pseudomonas aeruginosa* to confirm detection of such pathogens on mucous membrane and respiratory passage and tissues of apparently healthy camels. The oligonucleotide primers for *Staph aureus nuc* gene specific detection were 5'-GCGATTGATGGTGATACGGTT-3' and 5'-AGCCAAGCCTTGACGAACTAAAGC-3' according to Brakstad et al. (1992) [8]. While the primers which were used for detection of *pseudomonas* isolates the specific *groE* heat-shock protein 5'-ATGAAGCTTCGTCCTCTGCAT-3' and 5'-GTCTTT CAGCTCGAT-3' [9]. The PCR amplification was performed in a thermal cycler (Swift MiniPro, ESCO.) by using a 2X DreamTaq DNA PCR master Mix (Thermo Scientific). The reaction mixture consisted of 25 µl master mix, 30 ng bacterial DNA, 0.5 µl of each primer in concentration (50 pmol) and nuclease free water up to 50 µl. Thermal cycling begin by initial denaturation 98 °C for 5 min followed by a total of 37 PCR cycles were run under the following conditions: DNA denaturation at 94°C for 1 min, primer annealing at 53°C for 0.5 min and DNA extension at 72°C for 1.5 min. After the final cycle, the reaction was terminated by keeping it at 72°C for 3.5 min. The PCR products were stored in the cycler at 4°C until they were collected.

Visualization of PCR Amplicons: Following amplification, aliquots (15 µl) were removed from each reaction mixture and examined by electrophoresis (90 V, 30 min) in gels composed of 1.5 % (w/v) agarose in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3), stained with

ethidium bromide (0.05 µg ml⁻¹). Gels were visualized under UV illumination by using a gel image analysis system (Cleaver MicroDoc).

RESULTS

A total of 77 bacterial isolates were detected on sheep blood agar plates, 50 (94.9 %) isolates observed from healthy camels were Gram positive and 27 (35%) were Gram negative. Five different bacterial genera including *Staphylococcus*, *Bacillus*, *Escherichia*, *Pseudomonas* and *Klebsiella* were isolated. The most frequently isolated species from the respiratory passages of the healthy animals sampled were *Staphylococcus aureus* (14.5%), *Pseudomonas aeruginosa* (11.0%) and

Bacillus spp. (10.5 %). The other species isolated and identified from healthy camels were shown in Table 1.

As shown in Table (2), the highest rate of bacterial isolation obtained from the lung samples and nostril swabs by a percent for positive bacterial isolates (50 %) followed by tracheal swabs (48 %), while the lowest percent for bacterial isolation was appeared for blood samples as (10 %).

In Fig. (1 and 2) all the examined samples showed the same PCR amplicons for *nuc* gene for examined *Staph aureus* isolates and produced 270 bp PCR product. On the other hand the *groES* gene was detected for all *pseudomonas* isolates and produced amplicons with average size 536 for all tested samples.

Table 1: Types of bacterial isolates from blood and respiratory passages of apparently healthy camels

Bacterial organism	Blood 50 samples	Nasal swabs 50 samples	Tracheal swabs 50 samples	Lung tissues 50 samples	Bacterial isolate / Total number of sample (%)
<i>Staphylococcus aureus</i>	1	9	9	10	29 / 200 (14.5)
<i>Pseudomonas aeruginosa</i>	3	8	8	3	22 / 200 (11.0)
<i>Bacillus spp</i>	1	5	6	9	21 / 200 (10.5)
<i>E. coli</i>	-	3	1	2	4 / 200 (3.00)
<i>Klebsiella spp</i>	-	-	-	1	1 / 200 (0.50)

Table 2: Prevalence of bacterial isolates from apparently healthy camels respiratory passages

Type of samples	No of samples examined	No of positive cases	%
Blood	50	5	10
Nostril swabs	50	25	50
Tracheal swabs	50	24	48
Lung samples	50	25	50

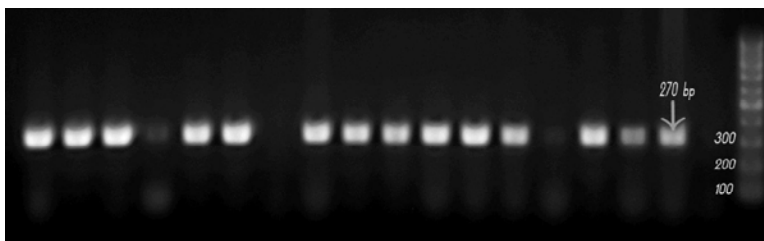


Fig. 1: Electropherotic profile of *Staph aureus* *nuc* gene at 270 bp size of different camel isolates, Marker 100 -1000 base pair DNA ladder.

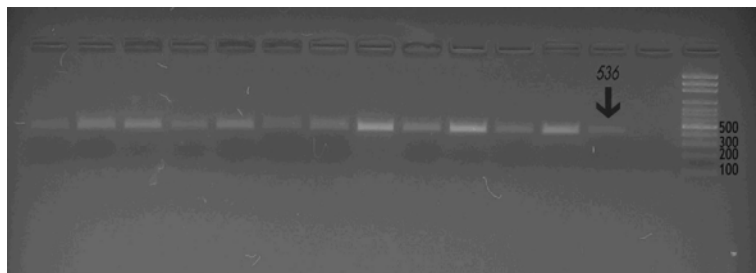


Fig. 2: Electropherotic profile of *Pseudomonas aeruginos* *groES* gene at 536 bp size of different camel isolates, Marker 100-3000 base pair DNA ladder

DISCUSSION

In the present study the total of bacterial isolates are seventy seven, Gram positive bacteria isolated by higher frequency (64.9 %) more than Gram negative bacteria (35 %) from apparently healthy animals. Most of the Gram positive bacteria isolated from healthy camels are common commensals on the mucous membranes of upper respiratory tract of healthy animals. Generally, it is accepted that Gram negative bacteria are commonly associated with systemic infections in human and animals [6].

Not surprisingly, the high rate of isolation of *staph aureus* (14.5 %) as Shighi, 1973 [10] reported a percent of 2.6 % of *S. aureus* isolation for 64 nasal swabs of healthy camels. Also, Chauhan *et al.* 1987 [11] confirmed the same point as he isolated *S. aureus* by percent of 10.5 % from 219 healthy camels. We attributed the little increase in our result to that, most of samples are collected from animals at regions of pre slaughtering, which the animals may subjected to various stress and predisposing factors could augment *S. aureus* growth and increase its recovery rate from respiratory passages. The percent of Gram negative bacteria varies between three genera, *Pseudomonas aeruginosa* (11.0 %), which considered as one of the most common opportunistic Gram negative bacteria [6]. In this point, there is a considerable reports for potential transfer of *P.aeruginosa* in between animal and human patients [12]. The very low rate of *E. coli*, *Klebsiella spp* and *Bacillus spp* is acceptable as environmental bacteria pass via apparently healthy camels respiratory passages. All of ruminants depend on eructation, which directly reflected on the microflora in the nasal passages and that explain the main reason for highest percent of bacterial isolation from nasal swabs table (2). This rate of isolation not an indication for a case of respiratory tract affection in camels. In this study, we used molecular for detection of two most isolated genera *S. aureus* and *P. aeruginosa* and confirmation of these isolates to be followed for further investigation and molecular analysis of normal bacterial flora of apparently healthy camels respiratory passages.

CONCLUSION

This study refers to normal microflora of apparently healthy camels respiratory passage normal microflora with focusing of the isolation of pathogenic *S. aureus* and *P. aeruginosa* without a subsequent a diseased condition in these camels which, needs further investigation for virulence potency of that types of microflora.

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