The Faecal Microbiota of Captive Gyr Falcons Falco rusticolus (Linnaeus 1758) and Hybrids in the Kingdom of Saudi Arabia

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The Faecal Microbiota of Captive Gyr Falcons *Falco rusticolus* (Linnaeus 1758) and Hybrids in the Kingdom of Saudi Arabia

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ABSTRACT

Falcons are traditionally used in falconry in the Kingdom of Saudi Arabia and many nations in the region. Many falcon species are kept captive and bred, selecting for their hunting capabilities in many of these countries. Of the several species of falcons, purebred Gyrfalcons (*Falco rusticolus*), the largest among falcons, is greatly appreciated by falconers for its hunting skill. Captive-bred Gyrfalcons can live, reproduce and hunt for long and are more stable. Aspergillosis is a non-contagious fungal disease of wild and domestic birds caused by fungus Aspergillus species. This fungal disease is economically important, being the main cause of mortality in captive birds, especially in falcons. This study assessed the effect of diet on the intestinal bacterial flora through faecal culturing of Aspergillosis-affected Gyrfalcons and discussed the potential of the isolated organisms as pathogens to spread to humans and avian species. An infected male Gyr and hybrid female (Gyr and Peregrine - *F. peregrinus*) were fed meat and a fresh chicken-rich diet for faecal samples, and the samples showed equal distribution of gram-positive and gram-negative bacteria relatively less harmful. The bacteria recovered here may be transient flora obtained through the diet and, under normal circumstances, may not actually be capable of colonizing and becoming permanent residents in the raptorial enteral tract. Thus, providing falcons with varied healthy diets is the best option in captivity, which could help falconers keep their birds free from infections.

Key words: Captive, Gyrfalcons, Hybrids, Peregrine, purebred etc.

INTRODUCTION

Falcons, a group of birds of prey belonging to the Falconidae family, are known for their incredible speed and agility, which they use to hunt other birds and small animals. There are about 40 species of falcons, including the Peregrines, the Gyrfalcon, hobbies and Kestrels. The Gyrfalcon (*Falco rusticolus*), widely known as the "Arctic Falcon", has a large range of distribution spread across the Arctic and sub-Arctic regions of North America, Europe, and Asia. It is considered the largest falcon in the world, with females being significantly larger than males. Adult males typically weigh 800-1300 gm and 48-61 cm long, while females weigh 1200-2200 gm and 51-65 cm long. The International Union for Conservation of Nature (IUCN) has categorised the Gyrfalcon as a species of "Least Concern" (IUCN). The Gyrfalcon was considered the bird of kings in traditional falconry, and throughout the Middle Ages, rulers would capture the bird and train it to be a hunting companion. Falconry, as it is known, involves training a falcon to hunt and return to its handler. The peregrine falcon (*Falco peregrinus*), or simply the "peregrine", is also a widely distributed species, well-known for its speed. Due to its excellent hunting abilities, great trainability, adaptability, and high success in captive breeding and consequent easy availability, the peregrine falcon is a highly valued bird in falconry across the world. For small to large game birds, peregrines are efficient hunters. Throughout centuries and regions of human civilisation, it has also been a religious, royal, or national symbol. Because they blend size and speed, hybrids of falcons are also common. Although several falcon species are hybridised, falconers prefer the Gyr-Peregrine crossbreeds. Such a hybrid blends a Gyr's speed and strength with the peregrine's friendliness and inclination to wait on flights on ambush. Gyr-peregrine hybrids are used to hunt a variety of prey, but falconers hunting sage-grouse particularly enjoy using them. Thus, this hybrid, blending the greatest traits of both species, is in high demand across the world.

Wild Gyrfalcons in captivity, due to stress, are prone to diseases such as aspergillosis. Captive-bred Gyrfalcons are steadier and can live, hunt and breed for many years. Captive breeding will provide a sustainable supply without impacting wild populations. However, the Gyrfalcon presents many challenges regarding diseases and managing the species in captivity, and a lot of work has been done in Saudi Arabia to improve their management. Aspergillosis, a fungal disease of wild and domestic birds caused by the fungus *Aspergillus* species, can occur as

a flock problem where husbandry conditions predispose birds to disease. A large number of spores of the fungus can be found in decomposing organic material, particularly hay, compost or wood. Poor ventilation and inadequate sanitation raise the risk of infection. Although other organs may also generally be affected by the fungus, it is distinguished by the predominant involvement of the respiratory tract, production of yellow cheesy plaques, and hard nodular masses in the lungs and air sacs. Combining flocks of birds, isolating social animals, or starting training are all potential stressors for consequent immuno-suppression that could lead to infections. Bringing injured or ill wild falcons into captivity is a known risk factor for aspergillosis for falconers engaged in rehabilitation. Aspergillosis is a major cause of bird mortality in captivity, with significant economic implications. The purpose of this study was to investigate the intestinal bacterial flora of these raptors through faecal culturing of Aspergillosis-affected gyrfalcons. In addition, the effect of diet on faecal bacteria was considered, and the potential of isolated organisms as pathogens in humans and avian species was discussed.

MATERIALS AND METHODS

Gyrfalcons and Gyr-Peregrine hybrid falcons kept as pets in a bird market in Riyadh, Saudi Arabia, were found afflicted by aspergillosis. An infected male Gyr and a hybrid female, fed on meat and fresh chicken-rich diets, were inspected, and their faecal samples were collected for further examination. The appearance and colour of the microbial colony served as the basis for the primary identification of bacterial strains. Gram staining was used on the samples, and they were then examined under a microscope to determine if they were gram-positive or gram-negative. By the spread plate method, the bacteria culture was kept alive on a nutrient agar medium at 40°C. Then, the test organism was isolated from colonies by the streak plating method and refrigerated for future use. Then, pure genomic DNA was removed from the cells. DNA extraction and isolation were done using Origin Genomic DNA Kit. About two Nano grams of genomic DNA was amplified using the PCR process. The PCR process consisted of an initial denaturation step of 5 min at 95°C, followed by 30 cycles of 10s at 95°C, 30s at 55°C and 45s at 72°C, ending with a final phase of 72°C for 3 min. The PCR products were resolved on a 2% TAE-agarose gel to confirm the target gene amplification. The PCR product was column

purified using Mo Bio UltraClean PCR Cleanup Kit (Mo Bio Laboratories, Inc. California). The purified PCR product was sequenced at SciGenom Labs Private Ltd., Cochin. The obtained sequence was checked for its quality by examining chromatograms, the forward and reverse sequences were assembled using Clustal W, and the consensus was taken for further analysis. Using NCBI's BLAST, the resulting sequence was examined for similarity (www.ncbi.nlm.nih.gov/). The phylogenetic tree was plotted using the neighbour-joining method using MEGA10 software.

The study was approved by the ethics committee of King Faisal University. All procedures performed in the study involving an the animals were in accordance with the ethical standards of King Faisal University, Deanship of Scientific Research. Reference no : KFU-REC-2023-DEC-ETHICS 1885.

RESULTS

The bacterial isolates obtained from the faecal samples of these falcons were sample 1- OR413817 (Image 1), sample 2 - OR413816 (Image 2), sample 3-OR413804 (Image 3) and sample 4- OR413813 (Image 4) given in table 1. The biochemical tests conducted on the samples are given in table 2. The 16SrRNA sequencing of the isolates is given in figures 1, 2, 3 & 4.

Image 1

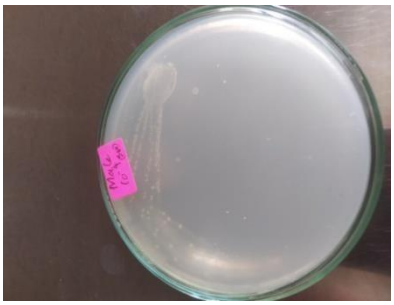

Image 2

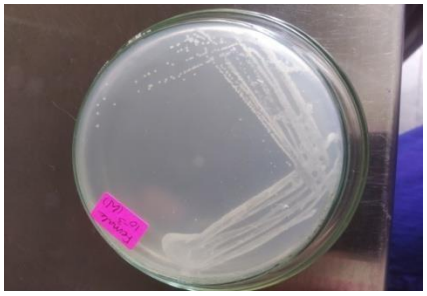

Image 3

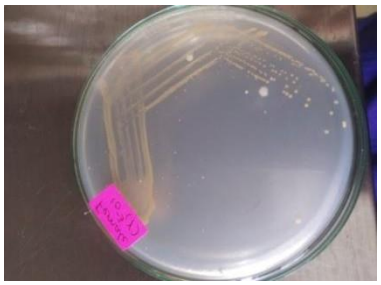

Image 4

Table 1- The bacterial isolates identified.

Species Gyrfalcon	Bacteria isolated	Bacteria identified	Nature of the bacteria
Male	Gram-positive	*Enterococcus faecalis* (sample 1- OR413817)	Opportunistic bacteria used as probiotic
	Gram-negative	*Enterobacter sichuanensis* (sample2- OR413816)	Potential pathogen in humans
Hybrid Female	Gram-positive	*Bacillus tropicus* (sample 3- OR413804)	Feather-degrading bacteria, a potential pathogen in humans

	Gram-negative	*Serratia marcescens* (sample 4- OR413813)	Opportunistic pathogen causing nosocomial infections

Table 2- The biochemical tests conducted

Biochemical test	Bacteria isolated			
	Enterococcus faecalis	*Enterobacter sichuanensis*	*Bacillus tropicus*	*Serratia marcescens*
Citrate	_	+	+	+
Carbohydrate	+	+	_	+
Catalase	_	+	_	+
Indole	_	_	_	_
Oxidase	_	_	+	_
Methyl-red	_	_	_	_
Starch	+	_	+	+
Urease	_	_	_	_
Voges-Proskauer test	+	+	_	+

Figure 1. Showing OR413817 ***Enterococcus faecalis*** **strain ATCC 19433 16S ribosomal RNA, partial sequence**

Sequence ID: NR_115765.1, Length: 1483Number of Matches: 1				
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Query  61     GACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACGTTGGCAAACA   120
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Query  121    GGTGCTAATACCGCTATAACAGCTTTATGCCGCATGGCATAAGAGTGAAAGGCGCTTTCG   180
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Query  361    AGCAACGCCGCGTGAGTGAAGAAAGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAA   420
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Sbjct  384   AGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAA   443

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Sbjct  444   CAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACT   503

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Phylogenetic tree

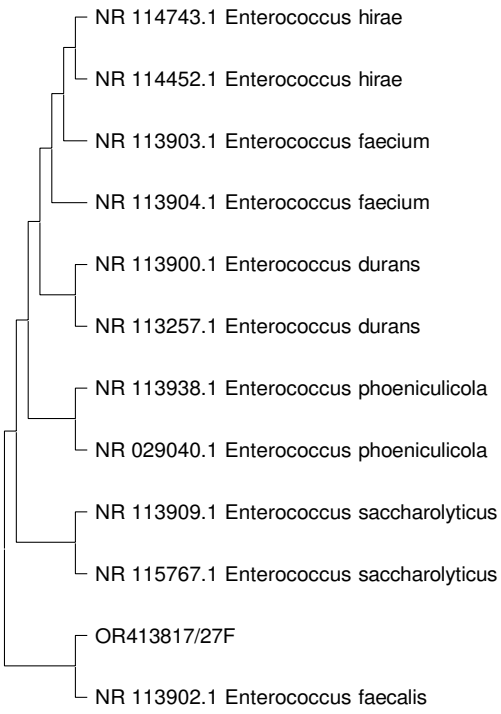

Figure 2. Showing OR413816 ***Enterobacter sichuanensis*** **strain WCHECL1597 16S ribosomal RNA, partial sequence**

Sequence ID: NR_179946.1, Length: 1528Number of Matches: 1

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
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Sbjct  244    AGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCC   303

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Query  241    AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAACGCCTTCGGGTTGTAAA   300
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Phylogenetic tree

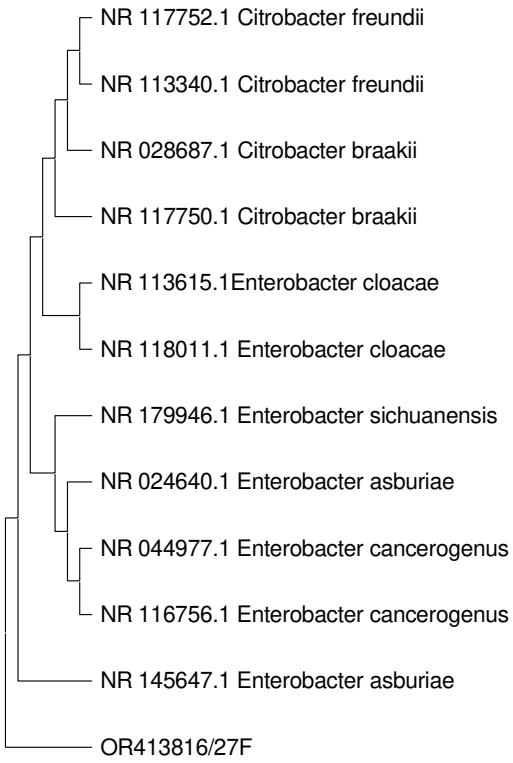

Figure 3. Showing OR413804 ***Bacillus tropicus*** **strain MCCC 1A01406 16S ribosomal RNA, partial sequence**

Sequence ID: NR_157736.1, Length: 1509Number of Matches: 1

Alignment statistics for match #1				
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Query  423   CTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG   482
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Query  723   GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAG   782
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Query  903   TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTTCTGACAACC   962
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Query  1023   TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC  1082
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Query  1083   ATCATTAAGTTGGGCACTTTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG  1142
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Sbjct  1253  AAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGT   1312

Query  1263  AGGCTGCAACTCGCCTACATGAAGCTGGAATCGTTAGTAATCGCGGATCACCATGCCGCG   1322
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Phylogenetic tree

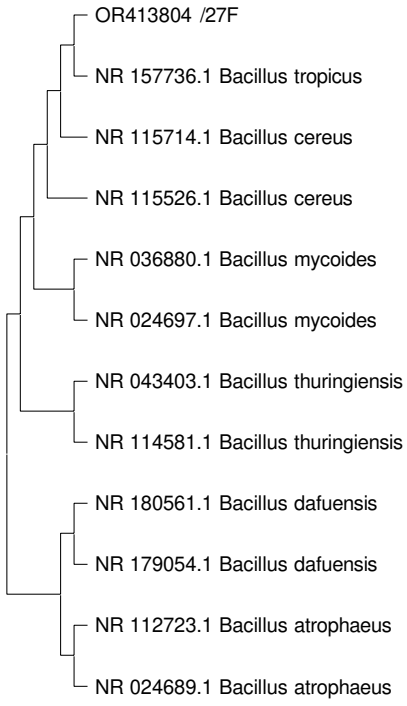

Figure 4. Showing OR413813 ***Serratia marcescens*** **strain KRED 16S ribosomal RNA, partial sequence**

Sequence ID: NR_036886.1, Length: 1532Number of Matches: 1

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
2538 bits(1374)	0.0	1379/1381(99%)	1/1381(0%)	Plus/Plus

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Query  1     ATGC-AGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCTGGGTGACGAGCGGCGGACGGGT  59
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Sbjct  49     ATGCAAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCTGGGTGACGAGCGGCGGACGGGT  108

Query  60     GAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA  119
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Sbjct  109    GAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA  168

Query  120    CCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCC  179
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Sbjct  169    CCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCC  228

Query  180    CAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGT  239
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Sbjct  229    CAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGT  288

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Sbjct  289    CTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG  348

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Sbjct  409    AGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGTGGTGAACTTAATACGCTCA  468

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Sbjct  469    TCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT  528

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Sbjct  529    ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTT  588

Query  540    AAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAAGCTAG  599
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Sbjct  589    AAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAAGCTAG  648

Query  600    AGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGG  659
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Sbjct  649    AGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGG  708

Query  660    AATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGG  719
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Sbjct  769    GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGT  828

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Sbjct  829   TGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACG   888

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Query  1020  TTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGG   1079
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Sbjct  1069  TTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGG   1128

Query  1080  TTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGT   1139
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Sbjct  1129  TTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGT   1188

Query  1140  CAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGTATACAAAGA   1199
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Query  1200  GAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTACGTCGTAGTCCGGATTGGAGTC   1259
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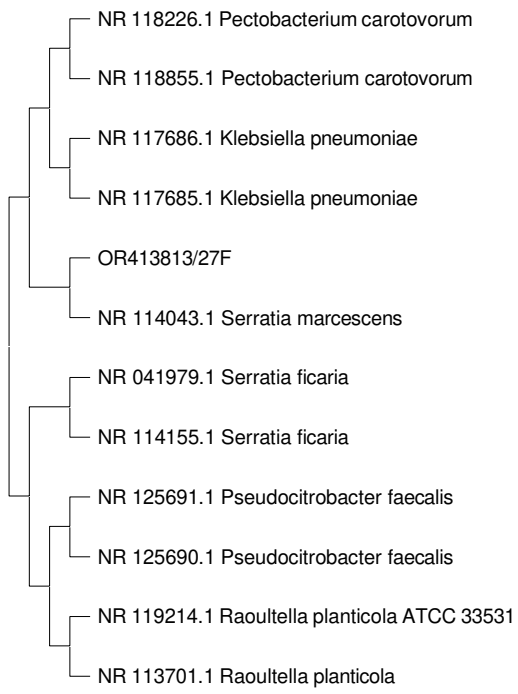
Phylogenetic tree



All the research data supporting this publication are available from NCBI repository with Accession numbers OR413817, OR413816, OR413804 and OR413813.

DISCUSSION

The composition of faecal microbiota is influenced by a range of intrinsic and extrinsic factors; host genetics, diet, environmental factors, behavioural habits, social contacts, age and sex. Thus, a range of elements, including nutrition, environmental exposure, and geographic location, determine the organisms in these birds' faeces. It makes sense that a range of elements, including nutrition, environmental exposure, and geographic location, would affect the organisms in these birds' faeces. In the present investigation, focussing on diet, food being a significant factor in microbiome composition, the faecal samples collected from the unhealthy captive gyrfalcons on a chicken-rich diet showed equal distribution of gram-positive and gram-negative bacteria. However, in another study (Bangert *et al*., 1988) on raptors fed on commercially prepared chicken, gram-negative bacteria were the most common in the faeces of 47 healthy raptors in captivity. Both the studies showed similar results where the bacterial isolates identified were varied and were potential pathogens; among them, the two gram-positive bacteria, *Enterococcus faecalis,* is used as probiotic, and *Bacillus tropicus* is a feather-degrading bacteria, whereas the other two gram-negative bacteria can be a cause of nosocomial infections, if not controlled appropriately in captive areas. Since diet plays a crucial role in determining the spectrum of intestinal microflora, an attempt was made to correlate faecal bacteria with the diet, so it is possible that by maintaining the quality of the diet close in composition to that of a natural diet to maintain a normal intestinal microflora in captive specimens. The bacteria recovered in this investigation represent transient flora obtained through the diet and, under normal circumstances, may not actually be capable of colonizing and becoming a permanent

resident in the raptorial intestinal tract. Providing falcons with varied healthy diets, including frozen or freshly killed healthy quails, day-old chicks, chicken liver, and mice, is the best option in captivity. It could help falconers keep their birds free from infections (Zubair, 2016).

CONCLUSION

Falcons frequently exhibit a wide range of viral, bacterial, and fungal infections. Nutritional deficits and metabolic diseases are intimately correlated with the environment and food quality in confined falcons. If intensive farming of the birds is carried out, various factors such as hygiene, food storage and preparation, cage placement, stress factors and other variables could cause the birds to be infected by Aspergillosis and such common infections. To an extent, almost all diseases can be prevented via proper management, improved hygiene, a balanced diet, and regular check-ups. The diseased falcon's intestinal biota, through the faecal media, can cause infections of consequence if it enters the human cycle.

Acknowledgement

We acknowledge the falcon hospitals, falcon clinics and falcon shops in the Kingdom of Saudi Arabia for permitting us to collect samples.

REFERENCES

1. Ahmad, A., Ridgeway, S., Shibl, A. A., Idaghdour, Y., &Jha, A. (2022). Falcon gut microbiome is shaped by diet and enriched in Salmonella. BioRxiv, 2022-11.
2. Bangert, R. L., Ward, A. C. S., Stauber, E. H., Cho, B. R., &Widders, P. R. (1988). A survey of the aerobic bacteria in the feces of captive raptors. Avian Diseases, 53-62.
3. Hejazi, A., &Falkiner, F. R. (1997). Serratia marcescens. Journal of Medical Microbiology, 46(11), 903-912.
4. Holt, J. G., ed. (1986). Bergey's manual of systematic bacteriology, Vol. 2. Williams and Wilkins, Baltimore, Md.
5. Khanna, A., Khanna, M., &Aggarwal, A. (2013). *Serratia marcescens* - a rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients. Journal of clinical and diagnostic research: JCDR, 7(2), 243.

6. Needham, J. R., J. K. Kirkwood, and J. E. Cooper. (1979). A survey of the aerobic bacteria in the droppings of captive birds of prey. Research. Veterinary. Science. 27:125-126.
7. Oyedele, O. A., Kuzamani, K. Y., Adetunji, M. C., Osopale, B. A., Makinde, O. M., Onyebuenyi, O. E., & Ezekiel, C. N. (2020). Bacteriological assessment of tropical retail fresh-cut, ready-to-eat fruits in south-western Nigeria. Scientific African, 9, e00505.
8. Quesenberry, K. E., and B. G. Short. (1983). Serratia marcescens infection in a blue and gold macaw. J. Am. Veterinary Medical Association 183:1302-1303.
9. Rahim, M. A., Bakhiet, A. O., & Hussein, M. F. (2013). Aspergillosis in a gyrfalcon (Falco rusticolus) in Saudi Arabia. Comparative Clinical Pathology, 22, 131-135.
10. Rocas, I. N., SiqueiraJr, J. F., & Santos, K. R. (2004). Association of *Enterococcus faecalis* with different forms of periradicular diseases. Journal of Endodontics, 30(5), 315-320.
11. Winsor, D. K., A. P. Bloebaum, and J. J. Mathewson. (1981). Gram-negative, aerobic, enteric pathogens among intestinal microflora of wild turkey vultures (Cathartes aura) in west central Texas Applied Environment Microbiology 42:1123-1124.
12. Zubair, M. (2016). Investigation on Prevalence and Management of Health Impediments in Captive Falcons of United Arab Emirates. foot, 44(8), 49.