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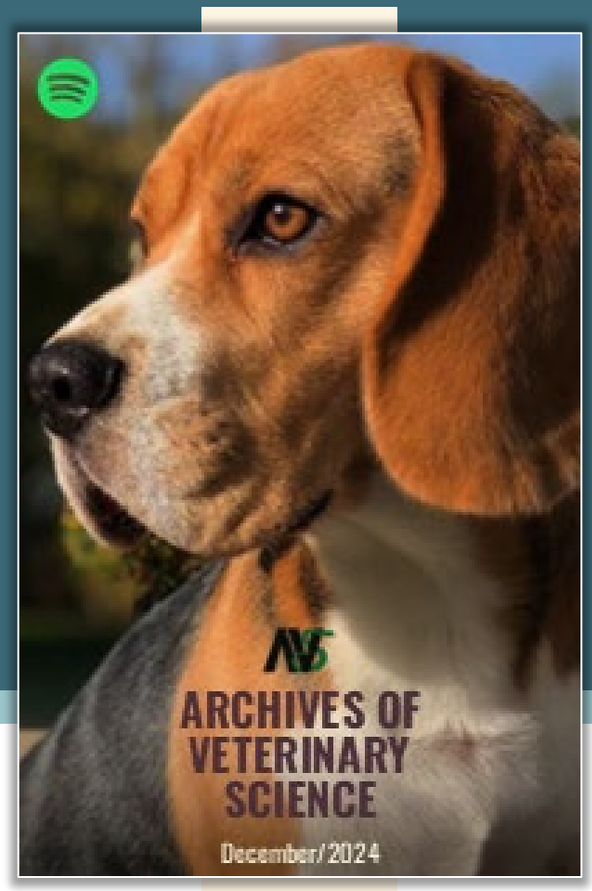

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Molecular detection of virulence genes of *Klebsiella pneumonia* from Camels

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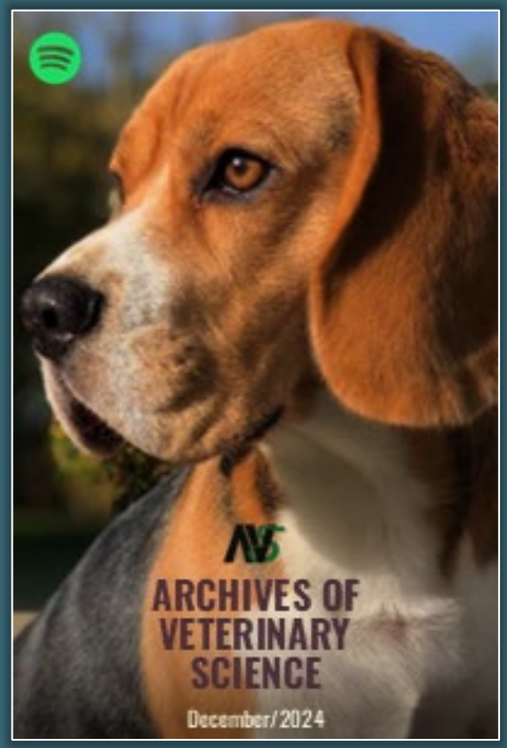

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ABSTRACT

Klebsiella pneumonia is recognized as one of the most important microorganisms of economic importance to the dairy industry worldwide, affecting almost all domestic animals. This study aimed to isolate K. pneumonia from Camels (60 nasal and fecal samples) appearing noticeable respiratory and GIT symptoms and clinical signs, antibiotics susceptibility investigation with frequency of some virulence factors genes.

ABSTRACT

The results on differential media showed primary identification of 55% Klebsiella spp., 21% E. coli, 15% Enterococcus spp., and 9% Pseudomonas spp. However, the quantitative PCR for the 16S rRNA gene confirmed that 27 out of 33 suspected isolates were K. pneumonia. Multi-sequence alignment of this sequenced isolate showed a high identical score to K. pneumonia strains from Hong Kong and Nigeria.

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The results also showed that 100% of K. pneumonia isolates were resistant to vancomycin and highly sensitive to levofloxacin, trimethoprim, and ceftriaxone. The molecular detection showed some virulence factors genes (ESBL).

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Differences in gene comparison with recently reported research on bovine (90.0 bla- ctx, 100.0 int1, 60.0 int2, 60.0 kpc, 10.0 hemo, and 0.0% of bla-shv) were found. In conclusion, K. pneumonia isolated from camels has a high sensitivity towards many antibiotics with a lower rate of some virulence genes in comparison with those isolated from cows.

Keywords: K. pneumonia, Camel, Antibiotics susceptibility, ESBL, 16S rRNA.

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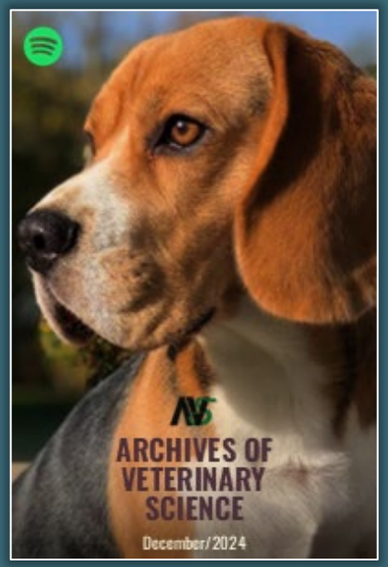
Gene		Primer sequence	Tm	Amplicon	REF
bla-shv	F	ATGCGTTATATTCGCCTGTG	55	730 bp	(Mirnejad et al., 2013)
	R	TGCTTTGTTATTCGGCCAA			
	F	CGCTTTGCGATGTGCAG	55	550 bp	(Dillon et al., 2005)
	R	ACCGCGATATCGTTGGT			
Into1	F	CAGTGGACATAAGCCTGTTC	55	160 bp	Hossain et al. 2004
	R	CCCGAGGCATAGACTGTA			
Into2	F	CAGGGATATGCGACAAAAAGG	54	788 bp	Esmaeel & Sadeq, 2018
	R	GTAGCAAACGAGTGACGAAATG			
bla-kpc	F	GCTACACCTAGCTCCACCTTC	55	989 bp	(Molana et al., 2011)
	R	ACAGTGGTTGGTAATCCATGC			
hemo	F	CCGGAGCGTTTTTCGATTGG	57	413 bp	(Eftekhar et al. 2012)
	R	AGCATCCGGGTAAAAAGGGG			

Table 1 – Primers for PCR amplification of virulence factors genes of *Klebsiella pneumoniae.*



Bacteria colony	MacConkey	Orientation Chrome agar	Number of Isolates	%
K. pneumonia	pink mucoid and lactose fermented	Metallic to dark blue color	33	55
E. coli	pink dry and lactose fermented	Dark rose to pink	13	21
Enterococcus	brown non lactose fermented	Turquoise to green	9	15
Pseudomonas	gray non lactose fermented	Creamy to transparent	5	9

Table 2 – Growth of bacteria colony on deferential mediums.

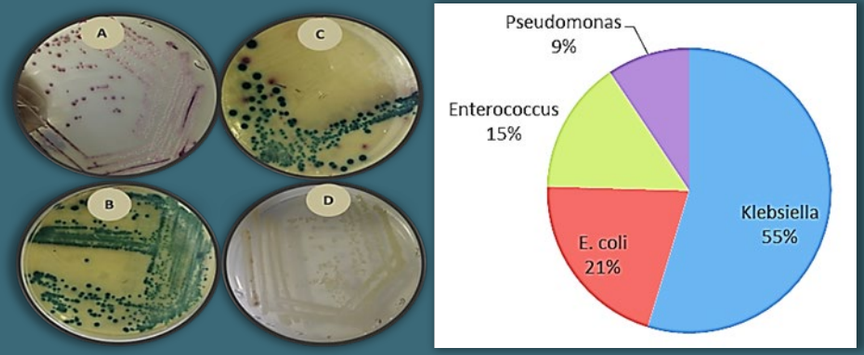


Figure 1 – Morphological examination of bacteria (left) and infection rate (right). The figure shows the growth of bacteria colonies on orientation Chrome agar. A- *E. Coli*, Pink colonies, B- *Enterococcus*, Green colonies, C- *Klebsiella*, Metallic Blue colonies, D- *Pseudomonas*, creamy color.

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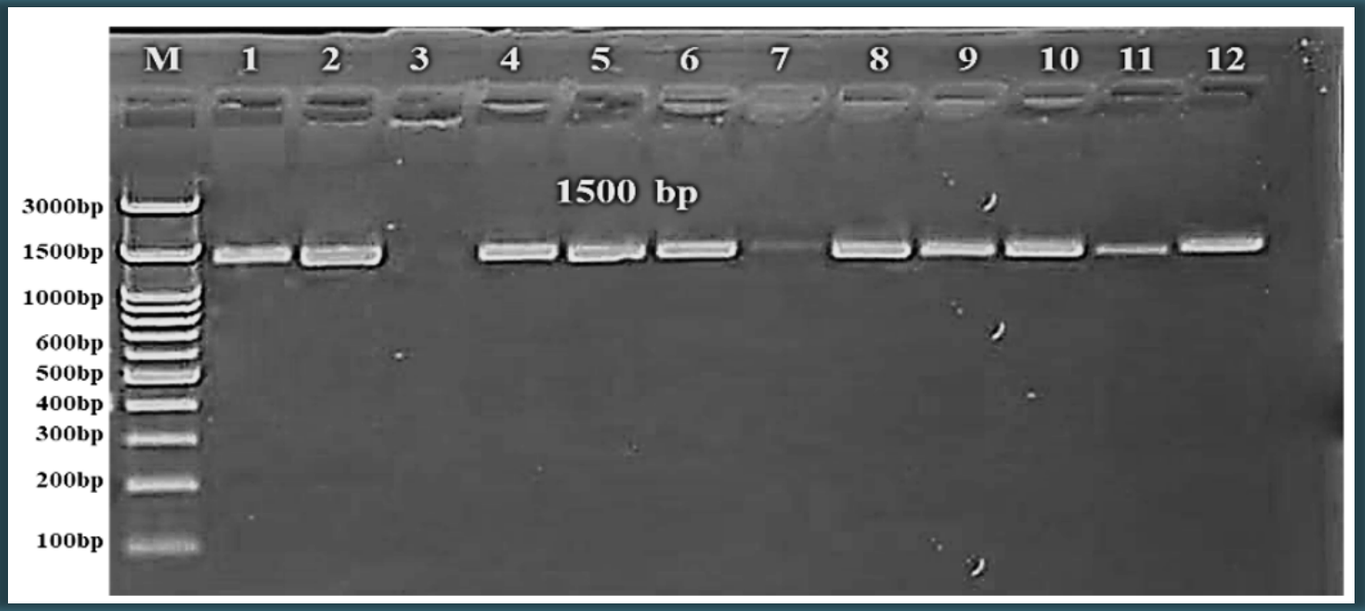


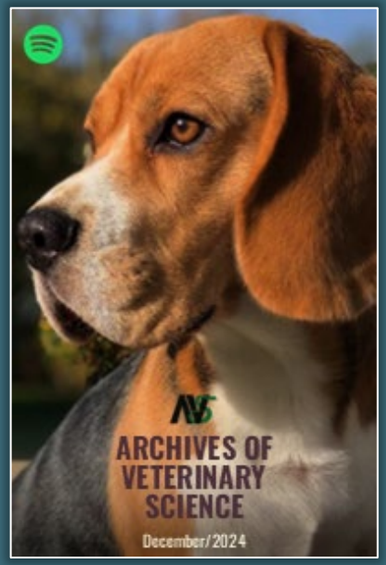
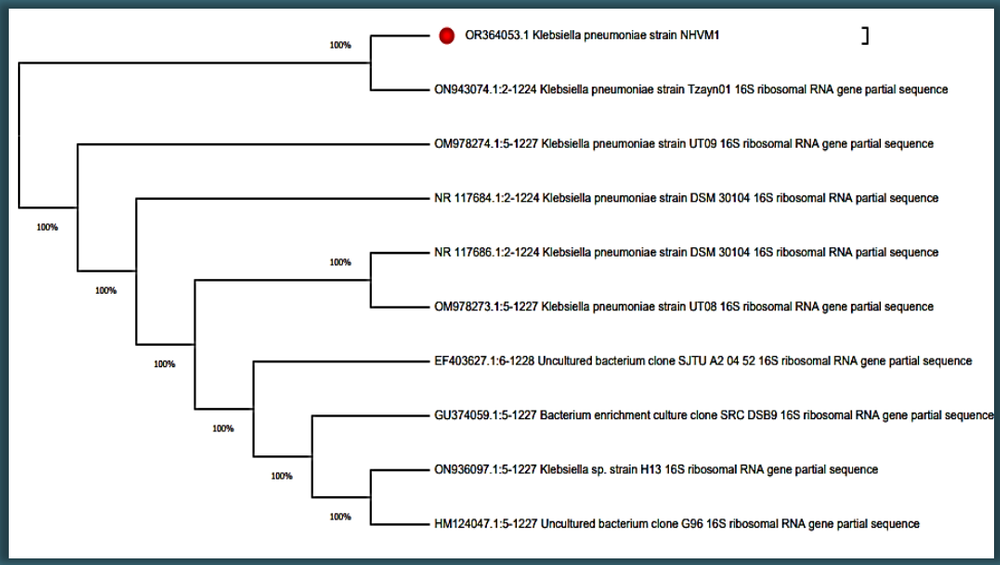


Figure 2 – PCR amplification of *16S rRNA gene.* Gel electrophoresis of PCR products of *16S rRNA* gene in suspected *K. pneumonia* isolates with particular molecular sizes (1500 bp). (M) 3 Kbp DNA marker. 1-12 represent isolates.

Figure 3 – phylogenetic tree analysis of *K. pneumoniae.* It shows the similarity approach between the new *K. pneumoniae* strain (NHVM1, red highlight) and other closely related global strains of *K. pneumoniae* according to the sequence of the 16S rRNA gene. The tree was constructed based on the neighbor-joining method. Numbers at nodes represent levels of bootstrap support (%) based on analysis of 1000 replications.



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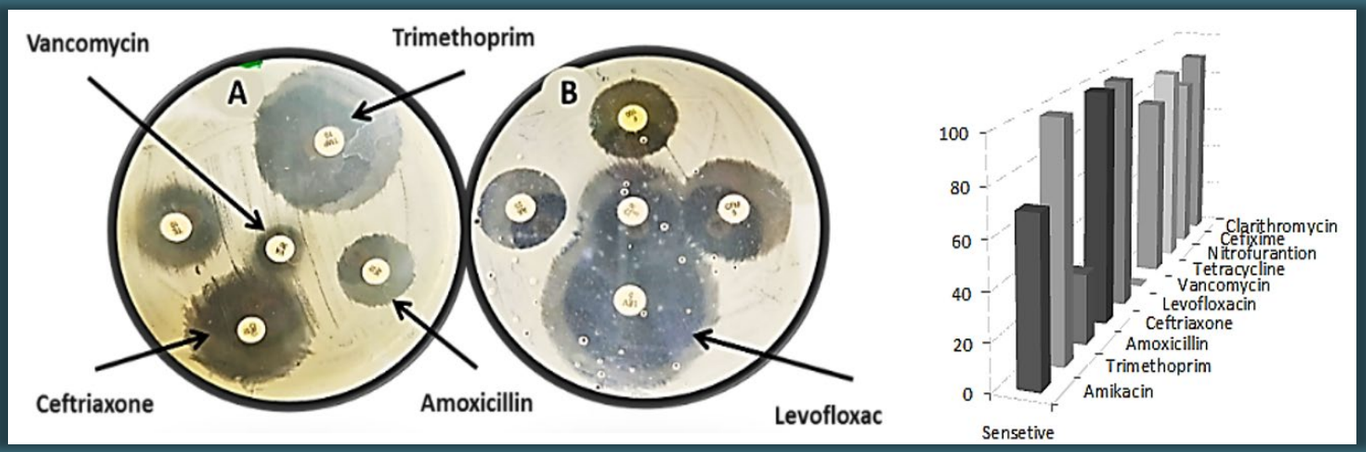

Figure 4 – Disc deffusion test and Antibiotics resistance patterns of *K. pneumoniae* isolates. It shows the resistance ratio of Klebsiella to Vancomycin and Amoxicillin. Although its sensitivity towards the rest of the applied antibiotics. The efficiency of Levofloxacin, Trimethoprim, and Ceftriaxone on this bacteria was very obvious as a zone of inhibition showed.

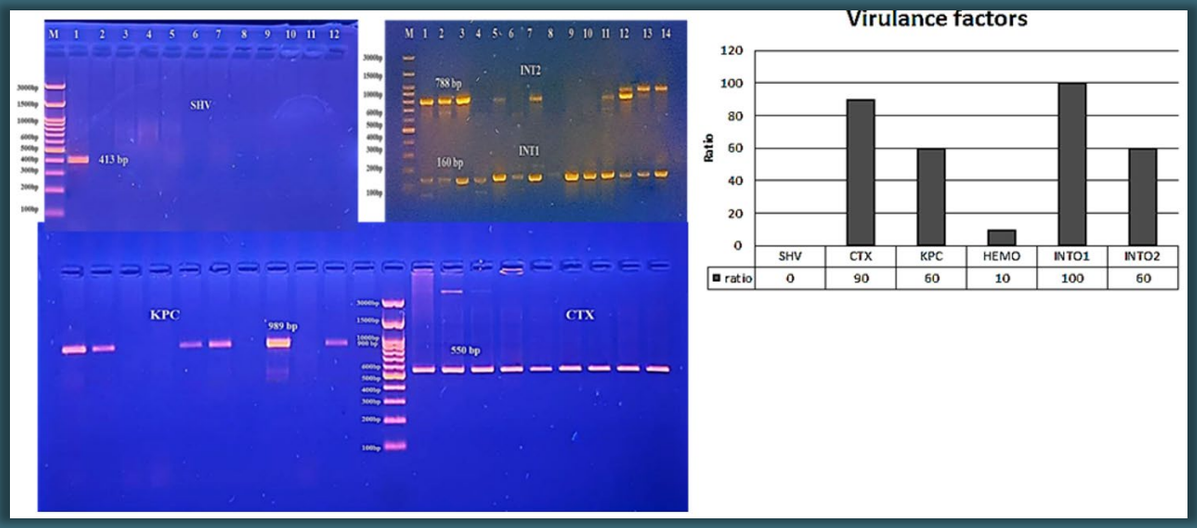

Figure 5 – PCR amplifectoin of virulence genes in *K. pneumoniae.* It shows PCR products of the subjected genes with particular sizes on agarose gel using two types of DNA marker (3kb) according to the size of amplicon. The diagram shows the ratio of detected genes in *K. pneumoniae* isolates

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CONCLUSION

This study illustrated that K. pneumoniae was responsible for the majority of respiratory and JIT infection in camels. In addition, this pathogen appeared to have low resistance toward the tested antibiotics with variable frequencies of virulence factor genes.

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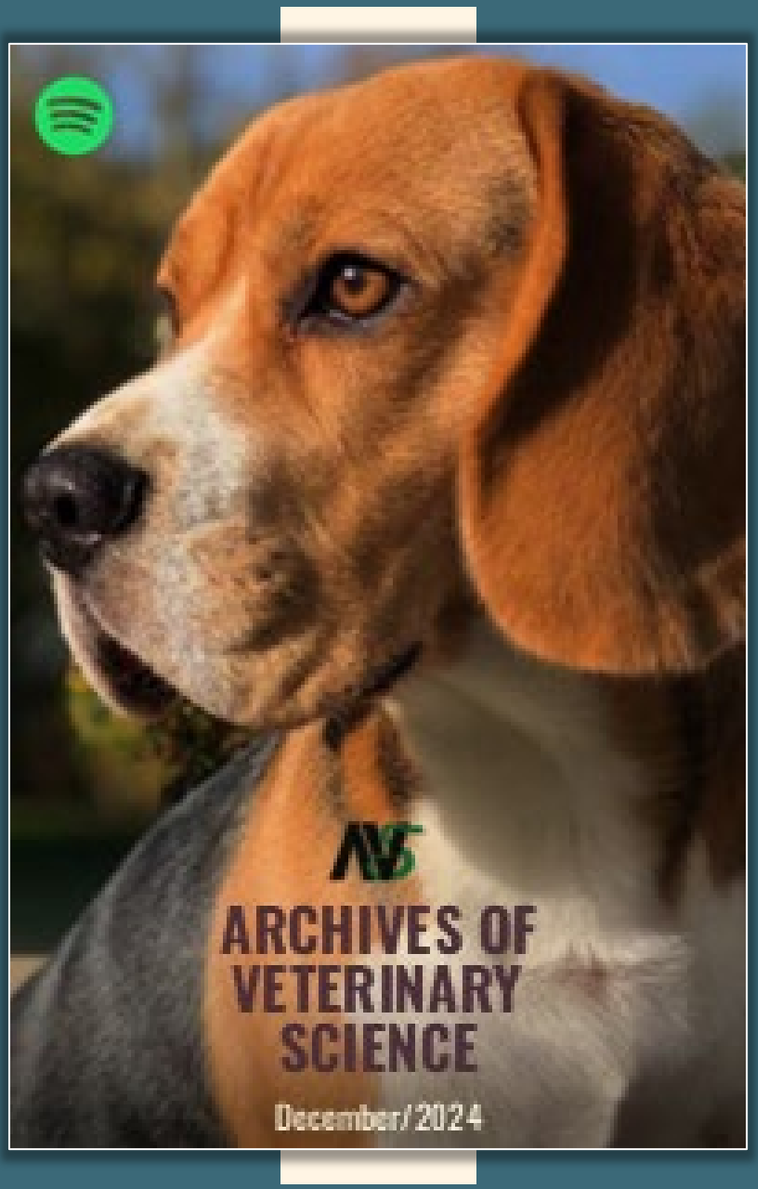

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