

Genotypic Characterization of *Klebsiella pneumoniae* Isolated from Human and Sheep in Al-Qadisiyah Province, Iraq

Abdulkareemsalman Alyassari¹ , Ahmed Jasim Neamah² , Majida M. Meteab Alshammari³  and Israa najm Abdullah Al-ibadi^{2*} 

¹College of Veterinary Medicine, University of Al-Qasim Green, Iraq. ²College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. ³Faculty of Medicine, Hayyan Medical University, Alnajaf, Iraq.

Abstract

The current study was performed to identify the genotypes related to *Klebsiella pneumoniae* isolated from urinary tract infection (UTI) of humans and pneumonia (PMN) of sheep in Al-Qadisiyah province, Iraq. 140 samples of (80 UTI and 60 post-mortems identified PMN) were collected and processed using traditional and molecular techniques. Following isolation of the microorganism, pure colonies were introduced into steps of a polymerase chain reaction (PCR) confirming method that targeted the 16S rRNA and the virulence-related capsular (magA and k2A) genes and partial gene sequencing (PGS) which focused on the 16S rRNA gene only. Primary results of the traditional techniques (TTs) highly revealed the presence of *K. pneumoniae* in 11 (13.75%) and 18 (30%) of the UTI and the PMN samples, respectively. However, rest samples showed the presence of different bacteria with no bacterial growth in others. This was confirmed by PCR results that strongly uncovered the identity of the *K. pneumoniae* in the positive samples. Using specific primers for Maga and k2A genes, PCR results revealed full percentage based presence of the k2A gene in the UTI and the PMN samples with less, 3/7 (43%) and 4 (100%) in the UTI and the PMN samples, respectively, presence of the magic gene. Moreover, PGS findings confirmed the accuracy of the TT and the PCR results plus recognized specific local strain identities that differently aligned with some of the worlds isolates on a phylogenetic tree. Current findings provide valuable information about virulence status of the *K. pneumoniae* isolates. in Al-Qadisiyah province, Iraq.

Keywords: Genotyping, *Klebsiella pneumoniae*, PCR, sequencing.

*Correspondence: Noormohammedheath@yahoo.com

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INTRODUCTION

Klebsiella pneumoniae is a bacterial microorganism that is considered as an opportunistic pathogen inducing influential human and animal related diseases¹. Although *K.pneumoniae* bacteria are present normally in/on the human and animal mucosal membranes of different body systems such as the urinary system or the respiratory system², the microorganism causes different infections in humans generated through various initiators such as nosocomial, direct or indirect contact with infected animals, and infected dairy products³, and some of these infections are infected wounds, UTIs, bronchus and lung infections, bacteremia, meningitis-related infections, differently-body-located abscesses^{3,4}. *K.pneumoniae* bacteria were isolated from mastitis in cows⁵ and upper respiratory tract infection (URTI) and pyogenic lung infections in sheep⁶. This wide range of bacterial presence and pathogenicity introduces important health problems accompanied by significantly increasing multi-antibacterial resistance (MAR) dilemmas⁷. One of the pathogenicity strength points of the bacterium is provided via the presence of the virulence factor; capsule, enhancing major self-protection against environmental and serum factor and the phagocytic killing activities generating variable degrees of infection severities according to the types of the capsular (K) antigens discovered that exceeded 80 components⁸⁻¹¹. For pyogenic related infections in Asian countries, K1 and K2 are recognized to be the major capsular antigenic types present¹². The well-known strains with low severity of *K. pneumoniae* have been evolved showing more virulent strains with increased mucoviscosity properties causing extremely severe infections such as abscesses of liver in immuno competent and immuno compromised young people inducing Gram positive pyogenic bacteria-like bodily dissemination with increasing worldwide infection rates that may alarm for a huge crisis especially with their elevating capabilities of acquiring new MAR properties^{11,13-15}. The current exploring study was performed to identify the genotypes, using the capsular virulence factors of *magA* and *k2A* genes, related to *Klebsiella pneumoniae* isolated from UTIs of humans and PMN of sheep in Al-Qadisiyah province, Iraq.

Table 1. The sequences of the primers and the conditions of the thermocycler followed in this study

Gene	Sequences 5' → 3'	Denaturation (°C-min)		Annealing (°C-s) (°C-min)	Extension (°C-min)	Final extension	Cycle No.	Amplicon size (bp)	Reference
		Initial	Main						
16S rRNA	F AGAGTTTGATCCTGGCTCAG	95-4	94-1	60-40	72-1	72-10	30	1500	(17)
	R GGTACCTTGTACGACTT								
<i>magA</i>	F GGTGCTCTTACATCATTC	95-5	94-1	51-60	72-1	72-10	35	1282	(18)
	R GCAATGGCCATTGGCGTTAG								
<i>k2A</i>	F CAACCATGGTGGTCGATTAG	95-5	94-1	58-60	72-1	72-10	30	523	(19)
	R TGGTAGCCATATCCCTTTGG								

MATERIALS AND METHODS

Sampling

The work was initiated by collecting of 140 samples (80 UTI from patients attended Al-Diwaniyah Teaching Hospital and 60 post-mortem identified PMN from sheep in a slaughterhouse, Al-Qadisiyah Provonce, Iraq) samples. These samples were processed using traditional and molecular based techniques. MaConkey’s agar was used to cultivate all the samples overnight incubation at 37°C that was followed by overnight eosin methylene blue (EMB) agar and chrom agar based sub-cultivating of all the lactose

fermenting colonies at 37°C. Using morphological and biochemical tests accompanied by the API20E System, a primary diagnosis of the *K. pneumoniae* was performed following standards from¹⁶.

After isolation of the microorganism had done, pure colonies were introduced into steps of a polymerase chain reaction (PCR) confirming method that targeted the 16S rRNA and the virulence-related capsular (*magA* and *k2A*) genes and partial gene sequencing (PGS) which focused on the 16S rRNA gene only.

Extraction of DNA and PCR method

Extraction of the DNA was enhanced recruiting Bosphore® Bacterial DNA Extraction spin kit (Bosphore®, Germany) with depending on the protocol accompanied the kit. The obtained DNA was tested for its quality and quantity using a NanoDrop.

The PCR amplification method was launched using 20µl as a total volume for the PCR reaction. The primers shown in table (1) were used for this purpose. A DNA template at 5µl was added to a mixture of 1.5µl (10 pmol/ µl) of each of the forward and the reverse primers, 250µM of dNTP, 30mM of KCl, 1.5mM of MgCl₂, and Taq DNA polymerase at 1 unit. The total volume was

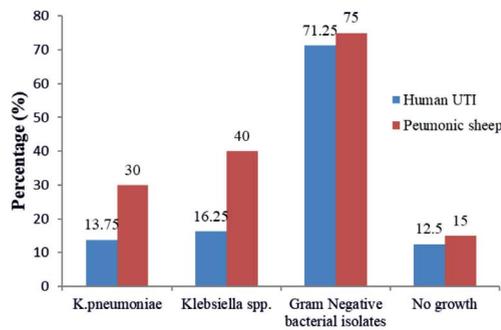


Fig. 1. Numbers of bacterial isolates obtained from UTI and PMN samples.

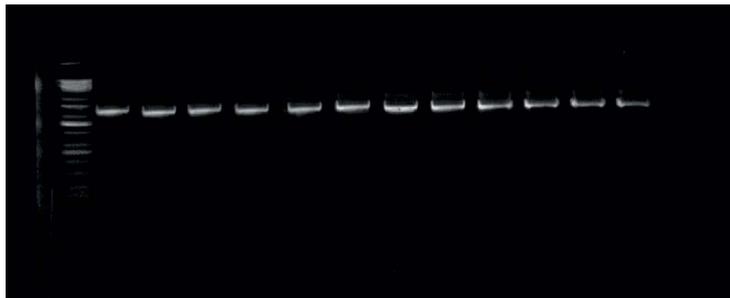


Fig. 2. Image of the electrophoresed agarose gel. The PCR products of *K.pneumoniae* targeting the 16S rRNA gene at 1500bp. The size of the ladder is 10Kbp.

Table 2. Numbers of bacterial isolates obtained from UTI and PMN samples

Source of samples	N	Bacterial isolates (No. (%))			No bacterial growth (No. (%))	X ² (Pvalue)
		<i>K.pneumoniae</i>	<i>Klebsiella spp.</i>	Gram –ve		
Human	80	11(13.75)	13(16.25)	57(71.25)	10(12.5)	96.35(0)S
Sheep	60	18(30)	24(40)	45(75)	15(25)	37.44(0)S
Total	140	29(20.71)	37(26.42)	102(72.85)	25(17.85)	124.18(0)S
X ² (pvalue)		3.379(0.066)NS	9.94(0.002)S	0.244(0.621)NS	3.65(0.056)NS	

S: Significant alterations ($p < 0.05$); NS: Non-significant changes ($p > 0.05$).

then reached by adding water made for molecular biology use. The conditions for the athermocycler (TECHNE, USA) used are shown in table (1). Then, the products of the PCR at 10µl/each were electrophoresed through a 1.5%-agarose gel pre-treated with 0.5µl/ml ethidium bromide. After the process was done, the gel containing the amplicons was screened and photographed using a UV imager and a specific camera, respectively. For the sequencing, 11 positive samples were sent out to do PGS.

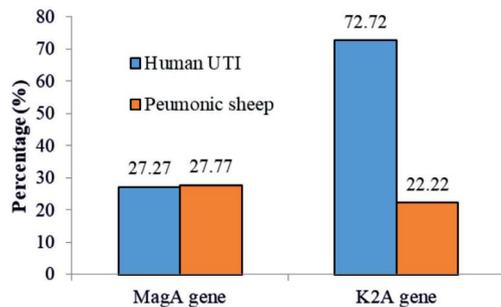


Fig. 3. PCR results regarding the *magA* and the *k2A* genes in the positive *K. pneumoniae* samples from UTI and PMN.

RESULTS

Traditional techniques

The primary results of the traditional techniques (TTs) highly revealed the presence of *K.pneumoniae* in 11/80(13.75%) and 18/60(30%) of the UTI and the PMN samples, respectively. However, the rest of the samples showed the presence different bacteria with no bacterial growth in 25 (17.9%), 10 UTI and 15 PMN, samples, table 2 and Fig. 1.

Result of PCR

The results of the PCR method strongly uncovered the identity of the *K.pneumoniae* in the positive samples according to the amplified piece of the 16S rRNA gene at size 1500bp Fig. 2.

Using the specific primers mentioned above for the *magA* and *k2A* genes, the PCR results revealed full percentage based presence of the *k2A* gene, 523bp, in the UTI and the PMN samples with less, 3/7 (43%) and 4 (100%) in the UTI and the PMN samples, respectively, presence of the *magA* gene, 1282bp, table 3 and Fig. 3, 4, and 5.

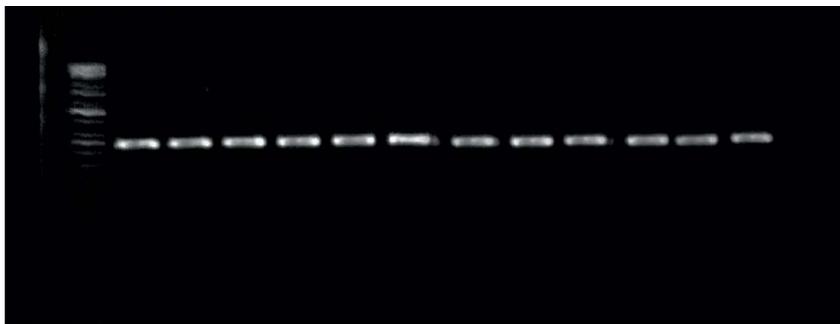


Fig. 4. Image of the electrophoresed agarose gel. The PCR products of *K.pneumoniae* targeting the *k2A* gene, 523bp. The size of the ladder is 10Kbp

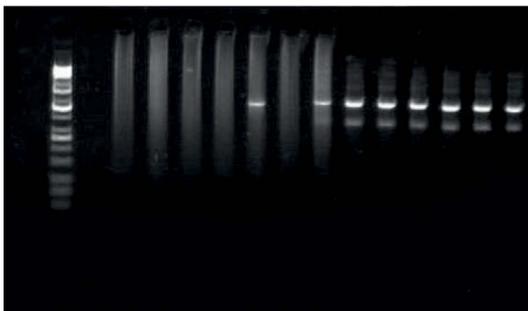


Fig. 5. Image of the electrophoresed agarose gel. The PCR products of *K.pneumoniae* targeting the *magA* gene, 1282bp. The size of the ladder is 10Kbp

DISCUSSION

Klebsiella pneumoniae is a bacterial microorganism that is considered as an opportunistic pathogen inducing influential human and animal related diseases¹. There is a big increase in the emerging of hyper virulent strains with increased mucoviscosity properties causing extremely severe infections such as abscesses of liver in immunocompetent and immunocompromised young people with elevating capabilities of acquiring new MAR properties that increase the complexity of the problems facing these strains^{11,13-15}.

In the current work, the findings revealed more frequent presence (100%) of the *k2A* gene in the UTI and the PMN samples than that for the case of the *magA* gene. These results agree with²⁰ who identified that the *k2A* genotype was more dominant than that for the case of the *magA* genotype in samples of human urine, exudate, bronchial wash, endo-tracheal aspirates, fluids of the pleura, and blood. However, these findings showed dissimilarities with results obtained from human samples in different countries such as K1 appeared to take over the occurrence rate (68.9%)

results in east China with less appearance rate (20%) of the K2 based serotype from abscesses in the liver²¹ with showing the same results in a study from Germany²². In a study performed in Taiwan, the serotype K1 was revealed in a higher occurrence rate, 63.4%, that that for the case of K2, 14.2%, of *K. pneumoniae* isolates from liver abscess²³. In Japan, it has been detected that the rate for the K1 serotype occurrence was lower than that regarding the K2 serotype of *K. pneumoniae* isolates from liver abscesses²⁴ which shows similar to the current study results. These serotypes were

Table 3. Numbers of positive bacterial isolates to *K. pneumoniae magA* and *k2A* genes obtained from UTI and PMN samples

Gene name	No. of isolates and percentage (%) X2 (p value)				
	UTI of humans (N=11)		Pneumonic sheep (N=18)		
	Positive	%	Positive	%	
<i>MagA</i> (K1serotype)	3	27.27	5	27.77	0.001(0.976)NS
<i>k2A</i> (k2 serotype)	8	72.72	4	22.22	7.18(0.007)S
X2 (P value)	4.545(0.033)S		0.148(0.700)NS		

S: Significant alterations ($p < 0.05$); NS: Non-significant changes ($p > 0.05$).

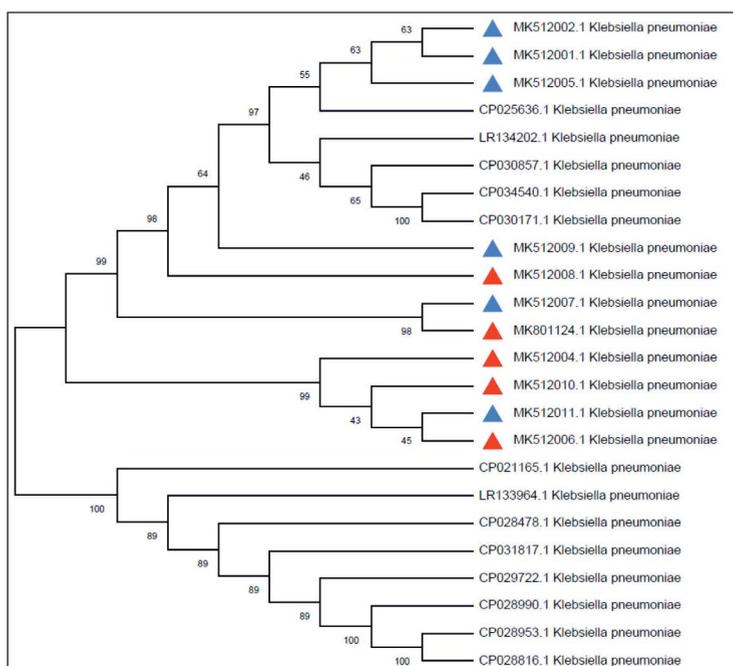


Fig. 6. The phylogenetic tree based on the *16S rRNA* gene partial sequencing. The tree was built up using the Maximum Composite Likelihood method that was applied to MEGA X software.

also identified from the globe different regions such as from Europe, Africa, Australia, North America, and the Middle East²⁵. The results from the respiratory tracheal swabs showed matching to the findings observed by²⁶ who identified the presence of lung invasion signs after performing the PM investigations such as lung hemorrhage and congestions detected in dead minks and mice with higher occurrence rates of the K2 serotype than that regarding the K1 serotype.

For the case of the phylogenetic study, the results recognized specific local strain identities that were differently aligned with some of the world isolates on the phylogenetic tree, Fig. 6.

For those strains aligned together, these results might indicate similar ancestors as they may have evolved and transmitted from similar regions of the world; however, some strains have aligned in distinguished branches of the tree suggesting a complete evolving process resulting in new line of strains that are not similar to other strains from the selected world regions²⁷. The current findings provide valuable information about the current virulence status of the *K.pneumoniae* isolates in Al-Qadisiyah province, Iraq.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial contribution to the work and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All data generated during the study are included in the manuscript.

ETHICS STATEMENT

The study was undertaken after obtaining approval from the Institutional Research and Ethics Committee.

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