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Distribution of K1and K2 serotypes of *Klebsiellapneumoniae* in water isolates in compare with clinical isolates

Elaf S. Mohammed[1] May T. Flayyih[2]
[1,2]Baghdad University, College of Sciences, Biology Department, Baghdad City, Iraq
Corresponding Author: Elaf S. Mohammed[1]

Abstract: *Serotypes of10 clinical isolates and 20 water isolates ofKlebsiellapneumoniaewere identified by detection of k2A and magA genes by using polymerase chain reaction (PCR) technique.k2Agene was detected in 30% (3/10) of clinical isolates and not found in water isolates, while magA gene was detected in 60% (6/10) of clinical isolates and 15% (3/20)in water isolates. 20% of clinical isolates found to be having both magA and k2Agene. The results in the current study showed that clinical isolates distributed according to their serotypes in to four groups K1(magA positive), K2(k2A positive), K1/K2(magA& k2A positive) and non K1/K2(magA& k2A negative) serotypes, but K1 serotype was more prevalence than other serotypes, while water isolates distributed in to K1(magA positive) and non K1/K2(magA& k2A negative) serotypes, but non K1/K2 serotype was more prevalence than K1 serotype.*

Key words*: Klebsiellapneumoniae, water, k2A, magA,serotype.*

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I. Introduction

Klebsiellapneumoniae is one of the most common Gram-negative bacteria, possess a prominent polysaccharide capsule [1]. It is composed of 63% capsular polysaccharide, 30% lipopolysaccharide and 7% protein. *K.pneumoniae* may colonize the skin, pharynx or gastrointestinal tract in humans. They form large moist colonies, which tend to coalesce into a mass with prolonged incubation, due to their large mucoid polysaccharide capsule (K antigen) that protects from phagocytosis and aids in adherence [2]. Typically, they express two types of antigens on their cell surface. The first is a lipopolysaccharide (O antigen); the other is a capsular polysaccharide (K antigen). Both of these antigens contribute to pathogenicity; about 77 K antigens and 9 O antigens exist. The structural variability of these antigens forms the basis for classification into various serotypes. The virulence of all serotypes appears to be similar [3, 4]. Capsular serotypes K1 and K2 are considered as predominant virulent strains of *K. pneumoniae*[5]. Several studies of bacterial pathogenesis have reported that serotype K1; *magA* is the possible virulence factor for *K. pneumoniae*liver abscess [6, 7]. Thus, PCR analysis of *magA* is a rapid and accurate method to detect capsular K1 strains [8].

This study aimed to detection of the distribution of K1and K2 serotypes of *Klebsiellapneumoniae* in water isolates in compare with clinical isolates.

II. Materials and Methods

2.1 Isolation and Identification of Bacteria

Thirty eight specimens (urine, blood, burns) were collected in sterilized containers and sixty five surface water samples were taken from different places. The collected clinical specimens were streaked directly on MacConkey agar[9] while water samples were isolated by pour plate method [10]. Clinical and water then cultured on Simmon Citrate agar[11]incubated at 37ºC for 24 hours, then the isolates were subcultured on MacConky agar to obtain pure single isolate. The isolates were identified depending on the morphological features, their response to stain by the Gram stain, and their ability to ferment lactose sugar. The identification was also achieved by using different biochemical tests [11]and by VITEK 2 compact System.

2.2 DNA extraction and estimation of concentration and purity of extracted DNA

DNA extracted from all clinical and water isolates by using Presto^{TM}Mini gDNA Bacteria kit and the concentration and purity of extracted DNA was tested using Nano- drop system

2.3 Detection of *magA*and*k2A* genes by Polymerase Chain reaction(PCR)

PCR assay was performed in a monoplex patterns in order to amplify different fragments of genes under study in a single tube for detecting of genes (*magA*and*k2A*).

The primers listed in table (1) were selected for this study; these primers were provided in a lyophilized form, dissolved in sterile distilled water to give a final concentration of 100 pmol/µL and stored in deep freezer until used in PCR amplification**.**

Table (1): The primers and their sequences used in conventional PCR for detection of*Klebsiellapneumoniae magA*and*k2A*genes.

Genes	Primer type	Sequence 5' → 3'	Length	References
magA	*magA*-F	GGTGCTCTTTACATCATTGC	1280	[5]
	magA-R	GCAATGGCCATTTGCGTTAG		
k2A	*k2A*-F	CAACCATGGTGGTCGATTAG	543	[7]
	k2A-R	TGGTAGCCATATCCCTTTGG		

2.4 PCR Amplification

The extracted DNA, primers and PCR master mix (promega), were mixed together. PCR mixture was set up in a total volume of 20 µL of master mix kit included 2 µL of each primer, and 3µL of template DNA have been used, the rest volume was completed with sterile de-ionized distilled water, then vortexed. De-ionized water added first, then primers and DNA template added at last .Negative control contained all material except template DNA, so instead that distilled water was added. PCR reaction tubes were centrifuged briefly to mix and placed into thermocycler PCR instrument where DNA was amplified as indicating in the table (2) (3), these tables showed different programs that used for (*magA* and*k2A*) genes amplification.

Table (2): Program used to amplify the *magA* gene according to**:**

Stage	Temperature	Time	Cycle
Initial denaturation	95ºC	5min	35
Denaturation	94 ºC	1 Min	
Annealing	51ºC	1 Min	
Extension	72 ºC	1 min	
Final Extension	72 ºC	10min	

Table (3): Program used to amplify the *k2A* genes according to:

Stage	Temperature	Time	Cycle
Initial denaturation	95ºC	5min	30
Denaturation	94 ºC	1 Min	
Annealing	58ºC	1 Min	
Extension	72 ºC	1 min	
Final Extension	72 ºC	10min	

III. Results and Disscution

Bacterial isolates which had the ability to utilize citrate as a source of carbon and energy were tested by VITEK 2 system, 10/20 (50%) of clinical isolates and 20/26(77%) of water isolates were identified as *K. pneumoniae*.Great efforts have been made to understand the virulence determinants of *K. pneumoniae*especially the capsule serotypes, serotypes K1 and K2 considered the most virulent to humans [12]. Serotype-specific genes like a chromosomal gene *magA*(mucoviscosity associated gene A) is restricted to the gene cluster of *K. pneumoniae* capsule serotype K1 and the chromosomal K2 capsule associated gene (*k2A*) for the K2 serotype [13]. The concentration of DNA extracted from clinical isolates was (42.61 - 255.9) ng/ml, while DNA extracted from water isolates were (24.5 - 879). The purity of clinical isolates were (1.24- 2.08) ng/ml, while the purity of water isolates were (1.82- 2.09).Monoplex PCR technique was carried on to detect*k2A* and *magA* gene in water and clinical isolates of *K. pneumoniae*. In this assay, a specific primers were used, the results showed a band of PCR product with 543bp that represent *k2A*gene and a band of PCR product with 1280 bp that represent *magA*gene. The positive result of *k2A*and*magA*gene was confirmed by 1.5% agarose gel electrophoresis stained with red safe stain, electrophoresed in 70 volt for 1 hrs. and photographed under ultraviolet (UV) trans illuminator, figure (1) (2).

*k2A*gene was detected in 3/10 (30%) of clinical isolates and not found in water isolates, while*magA* gene was detected in 6/10 (60%) of clinical isolates and 3/20 (15%)of water isolates, table (3-5). 20% of clinical isolates found to be have both *magA*and *k2A*gene, 40% of clinical isolates and 85% of water isolates don't have *magA*or k2Agene.The results in the current study showed that clinical isolates distributed according to their serotypes in to four groups K1(*magA* positive), K2(*k2A*positive), K1/K2(*magA*&*k2A* positive) and non K1/K2(*magA*&*k2A* negative) serotypes, but K1 serotype was more prevalence than other serotypes, while water

isolates distributed in to K1(*magA* positive) and non K1/K2(*magA*&*k2A* negative) serotypes, but non K1/K2 serotype was more prevalence than K1 serotype.

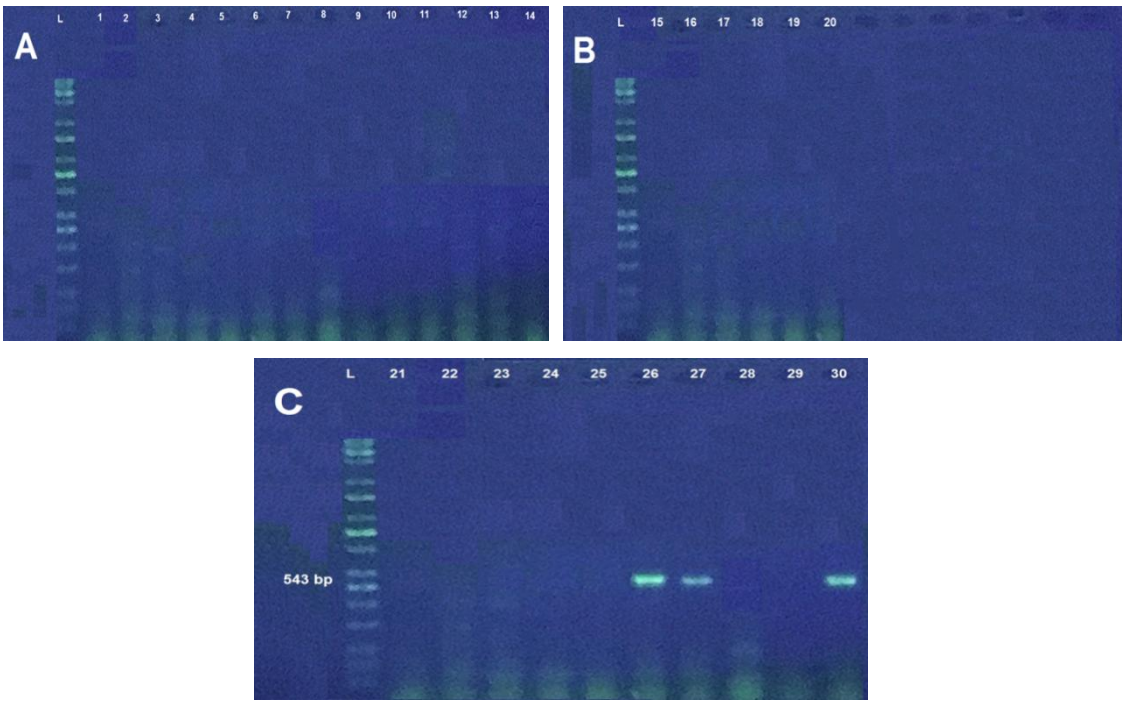

Figure (1): Gel electrophoresis of amplified PCR product of *k2A*gene (543bp) in monoplex pattern, agarose (1.5%). (A, B) represent water isolates, (C) represent clinical isolates, all water isolates were negative to *k2A* gene while three clinical isolates were positive to it. Lanes (26,27,30) were *k2A* gene positive,TBE buffer (1x), 70 volt for 1 hrs. Stained with red safe stain.DNA ladder (100 bp).

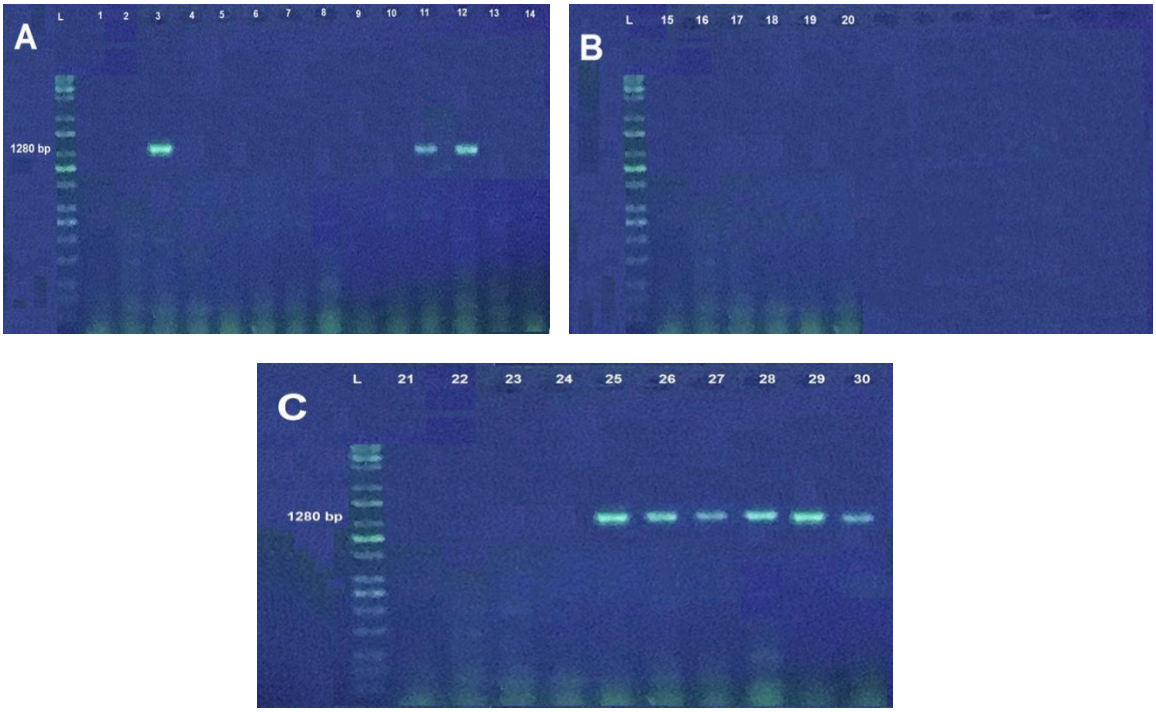

Figure (2): Gel electrophoresis of amplified PCR product of *magA*gene (1280bp) in monoplex pattern, agarose (1.5%). (A,B) water isolates, (C) clinical isolates, lanes(3,11,12) of water isolates were positive to *magA* gene ,lanes (25,26,27,28,29,30) of clinical isolates were positive to it,TBE buffer (1x), 70 volt for 1 hrs. Stained with red safe stain.DNA ladder (100 bp).

Local study by [14] recorded K1, K2, K1/K2 serotypes in clinical isolates of *K.pneumoniae* but not recorded non K1/K2 serotype, their results recorded depending on genotype detection of magA, rmpA genes. They also found that the prevalence of K2serotype were higher than K1 serotype and these results were not in agreement with the results of the current study which recorded that K1 serotype was more prevalence than other serotypes, but the results of the current study were in agreement with [15, 16] who found that K1 serotype was significantly higher. Also study by [17] found that K1 most common serotype in community acquired and nosocomial *K. pneumoniae* infections. On the other study, 21 isolates where classed as non K1/K2 .These isolates may belong to other serotypes such as K5, K14 , K20 [18].

Results of the current study were not agreed with study by [19], they reported that *magA* virulence gene was not detected in all of the aquatic-borne *K. pneumoniae*isolates, also thePCR assay for the K1 and K2 serotypes illustrated the K2 serotype for six of water isolates. K1serotype is a major cause of primary liver abscesses and has greater potential for causing metastasis, while K2 is a major cause of secondary liver abscesses [20]. Also K1 serotype was primarily responsible for community-onset bacteremia in patients with less severe underlying illness [21]. The results in the current study showed that clinical isolates have multi serotypes of *K. pneumoniae* than water isolates, so clinical isolates considered more virulent than water isolates.

Local study by [22] revealed the k2A fragment of 543 bp was detected in 11 (27.5 %) of clinical *K. pneumoniae*isolates and not found in environmental isolates, these results were corresponding with the results of the current study. Their results also referred that these (pathogenic) isolates have a K2 serotype, and 23 isolate (57.5%) was positive for *magA* gene (gave a band 1283 bp in size).Their results demonstrated that these pathogenic (23 isolates) have a K1 serotype. Al- Jailawialso pointed that *K. pneumoniae*serotype K1 was the most common found in clinical and environmental samples than K2 and Non-K1/K2 serotype. Based on their study, Molecular diagnosis of *K. pneumoniae* serotype K1 using magA gene is rapid and accurate while using k2A is a rapid and accurate method to molecular diagnosis of *K. pneumoniae* serotype K2.

The k2A gene of *K. pneumoniae*could be used as a highly specific diagnostic method to identify the *cps*of *K. pneumoniae*capsule K2 serotype, which corresponds to the *magA*region in the *cps*gene clusters of K1 isolate [23].

IV. Conclusion

k2A gene was absent in water isolates while its detected in clinical isolates. The results of the present study revealed that*K. pneumoniae*clinical isolateshad K1, K2, K1/K2 and non K1/K2serotypes while water isolates hadK1and non K1/K2, which indicated that clinical isolates had various serotypes more than water isolates.

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