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Original Research Article

Molecular Detection of Some Virulence Genes in *Klebsiella pneumoniae* Isolates from Patients with UTI Infection.

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Abstract
Klebsiella pneumoniae causes a wide range of bacterial diseases like pneumonia, UTI and sepsis. Therefore, this study was done to assess the prevalence and molecular characteristics of *K. pneumoniae* in 24 samples of men and women isolated from Iraqi patients which was suffering from urinary tract infection (UTI) compared with (10) of healthy individuals. All samples were collected from Educational laboratories of Medical city/Iraqi Health Ministry. The samples were screened for the presence of kfu and k2A genes through PCR. The results of this study represented by (7 out of 24) isolates percent with (29.17%) positive for kfu gene and (9 out of 24) isolates (37.5%) were positive for k2A gene while the other (8) isolates were none of these genes. The two genes revealed significant differences between them ($p< 0.5$), so as with healthy group ($p< 0.01$). The study concluded that two genes kfu and k2A may affect on pathological of *K. pneumoniae*. The aim of this study represented by detection of kfu (iron uptake system gene) and k2A genes in patients with UTI which have a role in the pathogenicity of *K. pneumoniae* isolates.

Key Words: *K. pneumoniae*, kfu and k2A genes, UTI, Molecular detection, PCR.

التحري الجزيئي عن بعض جينات الضراوة في عزلات الكليبسيلا الرئوية من المرضى الذين يعانون التهاب المسالك البولية

الخلاصة
تسبب الكليبسيلا الرئوية مجموعة واسعة من الأمراض البكتيرية مثل الالتهاب الرئوي، التهاب المسالك البولية وخمج الدم. لذلك، فان هذه الدراسة قد بحثت الانتشار والخواص الجزيئية لـ (٢٤) عينة من النساء والذكور تم عزلها من المرضى العراقيين اللذين يعانون من إصابات المسالك البولية (UTI) مقارنة بـ (١٠) أفراد أصحاء. جميع العينات المرضية قد تم جمعها من المختبرات التعليمية لدائرة مدينة الطب / وزارة الصحة العراقية. تم فحص العينات عن وجود جينات (kfu) و(K2A) من خلال تقنية تفاعل البلمرة المتسلسل PCR تمثلت نتائج هذه الدراسة بوجود (٧ من ٢٤) عزلة موجبة من جين (kfu) بنسبة (١٧،٢٩ %) و (٩ من ٢٤) عزلة موجبة من جين (k2A) بنسبة (٥،٣٧ %) بينما تمثلت بقية العزلات الـ (٨) بعدم وجود هذه الجينات. اظهرت نتائج الدراسة عن وجود فروق معنوية بين وجود الجينين بنسبة ($p<0.5$) وكذلك بين وجود هذين الجينين في العزلات مقارنة مع الاصحاء. ($p<0.01$)
الهدف من هذه الدراسة يتمثل في الكشف عن وجود جين (kfu) و جين (k2A) في المرضى المصابين بالمجاري البولية (UTI) والتي لها علاقة بامراضية بكتيريا الكلبسيلا الرئوية.

الكلمات المفتاحية: الكليبسيلا الرئوية، جينات kfu و k2A، التهاب المسالك البولية، التحري الجزيئي، تفاعل البلمرة المتسلسل.

Introduction
Klebsiella is the oldest genus among their family, the normal habitat of this bacteria is the intestinal tract of human and animal, but may be transferred to another site causing a wide range of infectious diseases like in burn, wound, respiratory and urinary tract so as bacteremia. Klebsiella have a capsule that oppose host defenses which depend mainly on impair immune defenses and the bactericidal of effect serum mediated in large part by complement proteins [1].
They are facultative anaerobic, fermentative, They produce different virulence factors like adhesins, large capsule that are antiphagocytic, siderophores, and various endotoxins [2]. They are motile by peritrichous flagella, lactose fermenters, oxidase negative, and catalase positive. A common opportunistic pathogen of

community-acquired and nosocomial infections [1-3].

It is also linked with a distinguishing clinical syndrome characterized by community-acquired bacteremia with liver abscesses, and metastatic meningitis [4]. Serotype-specific genes like a chromosomal gene magA (mucoviscosity associated gene A) is restricted to gene cluster of *K. pneumoniae* capsule serotype K1 and the chromosomal K2 capsule associated gene A (k2A) for the K2 serotype [5-7] which isolates with capsule serotypes K1 and K2 are more resistant to phagocytosis than Non-K1/K2 strains [8, 9]. The k2A gene of *K. pneumoniae* could be used as a specific diagnostic technique to identify the cps of *K. pneumoniae* capsule K2 serotype, which matches to the magA region in the capsules gene clusters of K1 isolate [10]. While the kfu gene which codes for an iron uptake is a virulence gene, related with hyper-mucoviscosity phenotype and it is also linked to purulent infections of tissue caused by this potent pathogenic bacteria species [11].

The aim of this study was to detect the virulence genes kfu and k2A genes of *K. pneumoniae* in patients with UTIs that may have a role in the pathogenicity of this bacteria.

Materials and Methods:

Samples collection

A total of (24) UTI samples from apparently sick patients were collected from established private laboratories and transported to the laboratory on ice. All specimens were managed for isolation of suspected bacterial isolates. Isolation and identification of bacterial isolates.

The isolation of bacteria was achieved using different standard techniques [1, 2] by culturing of urine specimens on blood agar and incubated for 24 hrs at 37ºC. After that, the bacterial isolates were identified to the level of species using cultural and morphological characteristics of colonies grown on MacConkey agar. In addition to Gram's staining, and finally by biochemical tests according to the Cowan and Steels' manual for identification of medical bacteria [12]. The bacterial identification of isolates was confirmed using API 20E system strips.

DNA Extraction

Template DNA from the colony was prepared with minor modifications. Genomic DNA extracted directly as leaflet kit (Geneaid company/Korea) for blood/culture. The assay was carried out following the instructions in the kit's leaflet from bacterial colonies grown on agar plates.

Amplification of virulence genes by PCR technique:

PCR was achieved using template DNA (3µl), primers (2µl) for two genes kfu F: (5-AGAACCTTCCTCGCTGAACA-3), R: (5 -ATAGTAGGCGAGCACCGAGA-3) and k2A gene F: (5-CAACCATGGTGGTCGATTAG-3), R: (5 -TGGTAGCCATATCCCTTTGG-3) and completed by 13µl DNase free water (Promega/ USA) in a total volume of 20 µl. The DNA for two genes were amplified using the modified cycling conditions (Applied–Biosystem PCR/USA) as in: Initial denaturation 95c° for 5 min. followed by 40 cycles consisting of 30 s of denaturing at 94°C, 30 Sec of annealing at 54°C, and 1 min of elongation at 72°C, followed by a final extension step at 72°C for 10 min. [13].

Results:

In the present study, the hyper mucoviscosity signs were positive in most isolates of all UTI patients and revealed the two genes kfu and k2A detected by conventional PCR technique (using specific primer sequences) yielded product sizes of 520 bp and 532 bp respectively Figure (1).

The study showed that out of a total of 24 isolates, nine (37.5%) were positive for k2A gene and (7) isolates (29.17%) were positive for kfu gene. The two genes revealed significant differences compared between them so as with negative control of healthy group represented by ($p< 0.5$) and ($p< 0.01$) respectively. While the other (No.= 8) isolates (33.3%) were negative for both genes and no significant differences were obtained compared with healthy negative of control group which had no bacterial growth (Tables 1 and 2).

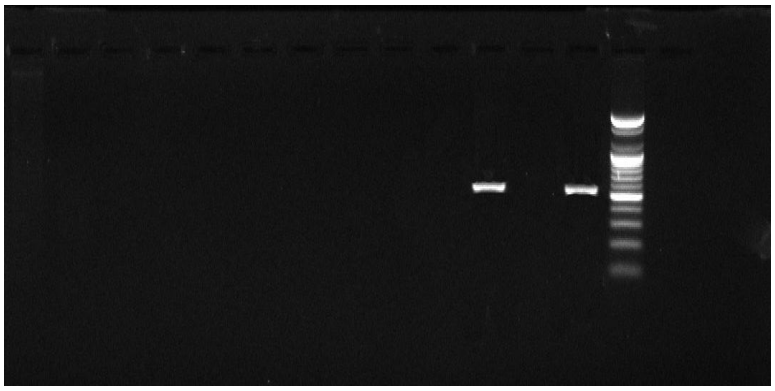

Figure (1) Ethidium bromide-PCR products separated in agarose gel (1.2%, Bio-Basic/Canada) at 75V, for 90 minutes, detected by UV transillumination. Amplified genes *kfu* and *k2A* with M.W. 520 and 532 respectively identified in *K. pneumoniae*, isolated from UTI (Lane M: 25bp/Bioneer ladder).

Table (1): Comparison between negative and positive of pathogenesis of *K. pneumoniae* growth in patients with UTI infections.

Test	***K. pneumonia* growth**	
	No.	**%**
+	16	66.67
–	8	33.33
Total	24	100%
P value	-----	0.0001**

**(P<0.01)

Table (2): Comparison between two genes *kfu* and *k2A* affected on *K. pneumoniae* pathogenesis

Group	Total No.	Gene *k2A*		Gene *kfu*		P value
		+	-	+	-	
Patients	24	9 (37.5)	15 (62.50)	7 (29.17)	17 (70.83)	0.041*
Control	10	0 (0.00)	10 (100)	0 (0.00)	10 (100)	
P value	----	0.0001**	----	0.0001**	----	----

(P<0.5)*, (P<0.01)**

Discussion:

The majority of gene encoding capsule has been carried on bacterial plasmids, which the studies revealed that the rmpA gene responsible for produce of capsule protect bacteria from macrophage [14-16].

In the present study, the hyper mucoviscosity signs were positive in most isolates of all UTI patients. These results were in agreement with a study reported by Turton and his colleagues [17]. An extensive range of *K. pneumoniae* infections had been described worldwide,

including pneumonia, UTIs, meningitis, in addition to different types abscess at different sites.

The mechanism of infections in *Klebsiella* species involve production of various virulence factors such as hyper-mucoviscosity capsular serotype rmpA gene, particularly K1 or K2, and virulence related genes, like kfu and k2A [9, 18]. The study revealed the two genes kfu and k2A yielded product sizes of 520 bp and 532 bp respectively.

As a later of previous studies for, magA and k2A specific to K2 capsule serotype were reported to be specific to capsule gene clusters of K1 and K2 serotype, respectively [19, 20]. A specific to the capsule gene clusters of K1 and K2 of both kfu and k2A serotype were found in this study [13], which known to be related to virulence factors of *K. pneumonia*e [20].

The study done a huge number of virulence gene dissemination and clinical conditions caused by *K. pneumoniae*, which found that there was a statistically significant difference in the incidence of phenotype, *kfu* and *k2A* as explained above which corresponded with previous studies [18-21].

Nevertheless, comprehensive evidence about the spreading of capsular K serotypes of causative organisms and the clinical features of subjects with *K. pneumoniae* in UTI and their interrelationships were reported. The investigation of the laboratory data, disease outcomes and clinical relevance of patients acquired purulent *K. pneumoniae* infections was done, and the prevalence of virulence associated of *kfu* genes were found at the rate of 29%, which is in extremely contrast with a study of Yu and his colleagues who detected the prevalence at the rate of 35% in UTI disease from these defect of genes. Detection of these genes may specify the virulence impending of the isolates [5, 13].

However, until now, little data are available about the pathogenicity of this bacterium. Certain current clonal analyses of *K. pneumoniae* isolates show that there are diverse clonal groups, certain of which may be linked with definite disease conditions. Nevertheless, what render one clonal cluster more virulent and what change the disease outline are not so far clear and continue as a vital question for the upcoming days [22-24].

Conclusion:

The genetic analysis of these genes kfu and k2A offers an opportunity for prompt diagnosis of the infection from predisposed patient.

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