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Molecular Detection of Virulence Gene (*mag A*) in the Clinical Isolates of Klebsiella Pneumonia

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Abstract

This study sought to examine the mucoviscosity-associated gene A in Klebsiella pneumoniae isolates, by using polymerase chain reaction. From November 2020 to February 2021, 40 *Klebsiella. pneumoniae* isolates were gathered from several hospitals in AL-Kut City. the isolates were collected from medical samples in healthcare settings. Based on API20E and the Vitek-2 compact system, all *K. pneumoniae* isolates were identified. The disc diffusion method was used to test the sensitivity of 12 antibiotics. Ampicillin and Amoxicillin + Clavulanic acid had a 100% level of antibiotic resistance, while Imipenem had a 5% degree of resistance. To find the mucoviscosity-associated gene A (mag A), polymerase chain reaction was used. Results from the PCR method revealed that 25% of samples were positive.

Keywords: *Klebsiella pneumoniae* , *mag A* gene, PCR

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الكشف الجزيئي عن جين الضراوة *mag-A* في عزلات سريرية لبكتريا Klebsiella pneumonia

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المستخلص

هدفت هذه الدراسة إلى التعرف على عزلات *Klebsiella pneumoniae* عن طريق تفاعل البلمرة المتسلسل للجين المرتبط باللزوجة المخاطية A. تم جمع أربعين عزلة من هذه البكتريا من بعض المستشفيات في مدينة الكوت من تشرين الثاني2020 إلى شباط 2021 من بيئة المستشفى. تم التعرف على جميع عزلات *Klebsiella pneumoniae*. الرئوية بناءً على نظام API 20 E و Vitek2 . تم إجراء اختبار حساسية المضادات الحيوية نحو 12 مضاد حيوي باستخدام طريقة الانتشار القرصي. كان مستوى مقاومة المضادات الحيوية 100٪ للأمبيسيلين والأموكسيسيلين + حمض الكلافولانيك ، بينما كان المستوى المنخفض لمقاومة المضادات الحيوية 5٪ للإيميبينيم. تم إجراء تفاعل البلمرة المتسلسل (PCR) للكشف عن الجين المرتبط باللزوجة المخاطية (mag A). أظهرت نتائج تفاعل البلمرة المتسلسل وجود 25٪ إيجابية في تقنية تفاعل البلمرة المتسلسل

الكلمات المفتاحية : *Klebsiella pneumoniae* , *mag A* gene, PCR

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Introduction:

Klebsiella pneumoniae are colonies skin, oropharynx or gastrointestinal tract. It causes a number of diseases, such as meningitis, osteomyelitis, cholangitis, pneumonia, and urinary tract infections (UTI). particularly within the immunocompromised or in those enduring from basic illness conditions such as diabetes mellitus [1]. Varieties within the clinical range of diseases can be somewhat credited to the nearness or expression of the harmfulness variables in expansion to the antimicrobial defenselessness profile [2]. The virulence factor that has been well characterized to date in K. pneumoniae comprise of capsule, lipopolysaccharides (LPS) siderophores and fimbriae. These are critical in adherence colonization, attack and improvement of disease [3 ,4]. K1 and K2 are two of the 77 capsular serotypes associated with significant human contaminations [3]. Capsule-associated gene A (K2A) is only found in serotype K2, whereas mucoviscosity-associated quality A (MagA) is only found in serotype K1 [5]. Liver abscess is usually linked with both serotypes [6, 7].

However, information on infections brought on by the K1 or K2 serotypes at non-hepatic locations is scarce [8]. The pathogenesis of hypervirulent K. pneumoniae (hvKP) infections is thought to be influenced by MagA and K2A [9,10]. K. pneumoniae produces the siderophores enterobactin (entB), yersiniabactin (YbtS), and aerobactin. The bacterium can avoid being neutralized by the host thanks to the creation of numerous siderophores. The entB siderophore is the most prevalent one [3]. YbtS is markedly overexpressed in respiratory tract strains when compared to entB, but not in blood, urine, or stool. The start, invasion, dissemination, severity, and outcome of infection with K. pneumoniae are all influenced by the aforementioned virulence genes individually or in diverse combinations [11,12]. The goal of this work was to identify some of the mag A virulence gene

Materials and Method:

Bacterial isolates:

This study was carried out in the Iraqi province of Wasit's Al-Kut City. 325 samples from various clinical sources, including feces, burns infections, lung infections, urinary tract infections, and samples from hospital surroundings, were gathered between November 2020 and February 2021. The urine, stool, sputum, Burns, and hospital environment samples were streaked on the surface of MacConkey agar and blood agar (Oxoid, UK). After that, the plates were incubated for 24 hours at 37°C.

All isolates were identified by using morphological and biochemical assays, including the Gram stain, Growth on MacConkey Agar, Catalase and Oxidase, Urease production, Motility, Indole production, Methyl Red, Voges-Proskauer, Simmons Citrate, and Triple Sugar Iron [13]. Last but not least, the API 20E and Vitek2® system (BioMerieux®) were used to identify all probable isolates of K. pneumoniae. (BioMerieux® -France)

Mucoviscosity of the Isolates:

All K. pneumoniae isolates were grown in blood agar medium, which contains 5% sheep blood, for 24 hours at 37°C. The mucoviscosity of the sample is then determined by using the string test, which entails running a loop through a bacterial colony and measuring viscous threads in millimeters. HV

is defined as samples with viscous strings longer than 5 mm[14].

Antibiotic Sensitivity Test:

Kirby-Bauer technique antimicrobial susceptibility testing was done [15]. Antibiotic discs were chosen in accordance with the Clinical and Laboratory Standards Institute (CLSI)[16].

DNA Extraction:

DNA was extracted by using the boiling process described by [17], with the addition of suspending 24 hrs old bacterial growth by loopfulls on BHA in 1 ml of sterile (1X) TE buffer (pH8.0) rather than sterile D.W. The cell suspension was boiling for 10 min in a waterbath at (95°C). Before use, the solution was kept at (-20°C) and centrifuged for 5 minutes at 10,000 rpm.

Primers Selection and Preparation

The sequence of primers were used to detect mag A gene: F-GGTGCTCTTTACAT CATTGC, R- GCAATGGCCATTTGCGTTAG and the product size which are given, are 1283bp [18].These primers were supplied by (Macrogen/ Korea Republic) lyophilized form were dissolved in nuclease-free water to create a stock solution (100 pmol/ml), which was then diluted to a final concentration of (10 pmol/ml) as the work solution.

PCR Mastermix and Reaction Program:

Twenty µl of volume were used for the PCR reactions. In all amplification studies, a negative control blank containing all PCR product except target DNA was employed. Final volume including 1 µl of bacterial DNA, 10 pmol of the GoTaq Green Master Mix PCR Kit (Promega/USA) and 10 pmoles of each primer. The PCR reactions were carried out by using a PCR thermal cycler (Techne/USA). The PCR program was set to denaturation at 95 °C, annealing at 56 °C, and extension at 72 °C. There were 35 cycles of the reaction. The PCR products were separated by using gel electrophoresis on a 2% agarose gel with 0.5 g/ml Ethidium bromide...

Statistical Analysis:

Chi square was employed to look for variations in the analyzed determinants' distributions. [19] Statistical significance was defined as a P value less than 0.05.

Results:

***Klebsiella Pneumoniae* Isolate:**

Sixty-two gram-positive bacteria, 223 gram-negative bacteria, and 40 growth-free bacteria have been identified out of the 325 total specimens. In total, 285 bacterial isolates were found in hospital surroundings, burns, and other clinical sources (79 from burns, 80 from sputum, and 100 from urine). Six isolates from the hospital environment, 22 isolates from urine, four isolates from sputum, and eight isolates from burns made up the distribution of K. pneumonia in clinical samples..

Mucoviscosity Test:

The blood agar medium, which contains 5% blood, was streaked (plated) with each K. pneumoniae isolate. The creation of a string measuring about 7 cm in length was used to determine the hypermucoviscosity positive phenotype in 10 (or 25%) of the 40 K. pneumoniae isolates, while other isolates produced moderate and infrequent mucoviscosity, as indicated in Table 1.

Table 1. Mucoviscosity Test of *Klebsiella Pneumoniae*

Mucoviscosity character	Total of Isolates	Percentage
Hypermucoviscous (HvKP)	10	25%
Moderate	9	22.5%
Few	21	52.5%

Virulence Factor Gene Detection:

The virulence factor magA gene was found in 40 isolates of K. pneumoniae by using the monoplex PCR technique. 10 of the 40 isolates (about 25%), as indicated in Figure 1, were magA gene positive.

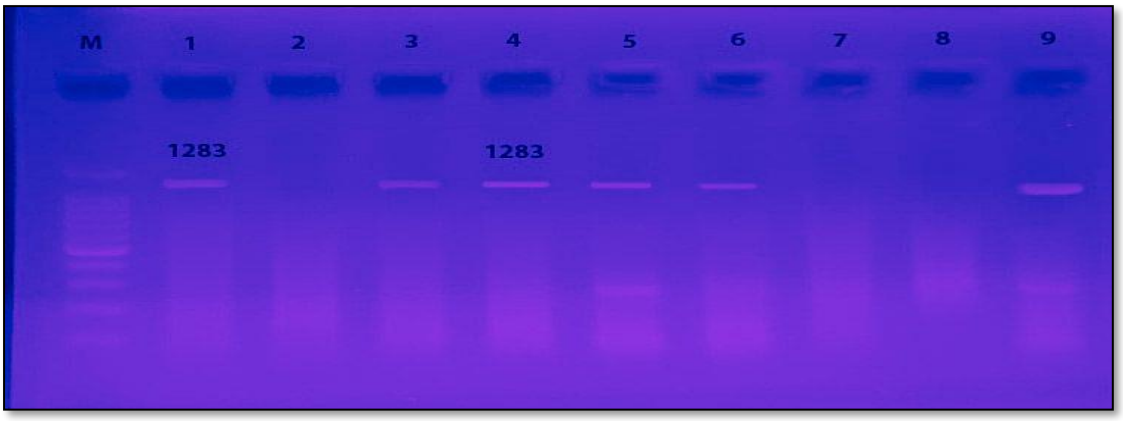

Figure 1: MagA Gene Amplification in K. Pneumoniae by using gel Electrophoresis. On a 1% agarose gel, electrophoresis was carried out by using a 100 volt/35 mAmp current for one an hour. Lines 1, 3,4,5,6, and 9 are on a (100 bp) ladder in lane M. Test and positive control strains of magA Negative controls are Lane 2 and Lane 7. DNA-free negative control in lane 8

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing revealed isolates were sensitive to I Gentamicin ,(72%), Imipenem (80%) , Meropenem (77.5%) , Tobramycin (62.5%) and Trimethoprim\sulfamethoxazole (60%) were highly resistant to Ampicillin, Amoxicillin + Clavulanic acid (100%), Piperacillin (80%), Cefotaxime (67.5%) and Ceftriaxone (52.5%).Table(2).

Table 2: Antimicrobial Susceptibility Testing of K. Pneumoniae

Antibiotics sensitivity pattern	Resistant	Sensitive
Trimethoprim\sulfamethoxazole	37.5%	60%
Gentamicin	17.5%	72%
Cefotaxime	67.5%	5%
Ceftriaxone	52.5%	12.5%

Imipenem	5%	80%
Meropenem	25%	77.5%
Piperacillin	80%	5%
Amoxicillin + Clavulanic acid	100%	0%
Ampicillin	100%	0%
Tobramycin	20%	62.5%
Amikacin	25%	17.5%
Tetracyclin	40%	37%

Discussion:

A significant pathogen that can cause nosocomial and community acquired illnesses is Klebsiella pneumoniae. Numerous virulence factors present in this pathogen enable it to induce infections. The majority of the K. pneumoniae isolates examined demonstrated high levels of resistance to most antibiotic substances. This might be because antibiotics are being misused in clinical settings. This is further impacted by the absence of a defined national antibiotic policy and the accessibility of over-the-counter antibiotics in this nation. [20,21,22] Furthermore, percentages of MDR K. pneumoniae isolates as high as 84% were found in other research.[23] The relative ease with which mobile genetic elements, such as plasmids, transposons, and integrons, can transfer resistance determinants across and across several bacterial species is a significant factor that can contribute to the rise in multi-resistant bacteria[24].

The capsule, endotoxins, siderophores, iron-scavenging mechanisms, adhesins, and other virulence factors all contribute to the pathogenicity of K. pneumoniae. These components support the bacterium's capacity to subvert the immune system and cause various illnesses. [25].

Hypervirulent *K. pneumoniae* produce more aggressive disseminated infections than cKp strains. In the present study, 25% of isolates were classified as hvKp phenotypically. This finding is in agreement with Shakib et al. [32] and El Fertas et al .[33] who noticed hypervirulent strains in 14.3% and 9.2% respectively. In disagreement with this study, high percentages were reported by Tan et al.[34] and Aljanaby et al.[33], who stated that 42.6%, 62.5% of their strains respectively were hypermucoviscous.

In this study, magA (specific to K1 serotype) gene was detected in 25% of the isolates. The results of the K1 serotype were consistent with Abdul-Razzaq et al. [35], who reported a prevalence of 18.6% for serotype K1, and with Sahoo et al. [36], who found a frequency of 25% for serotype K1. This is in contrast to what has been observed in other nations. Bilal et al. [37] found one K1 and one K2 serotype in liver abscess patients in Germany.

K1 and K2 serotypes coexisted with additional virulence factors like siderophores, fimbriae, and LPS, when compared to other strains, the enhanced virulence associated with the K1 and K2 serotypes has been proposed to be the result of the co-carriage of numerous virulence genes [3]. The rmpA gene was found in all isolates (34 of the serotype K1 isolates), all isolates (15 of the serotype K2 isolates), and 66.7% (16 of the 24) of

the non-K1/K2 isolates, according to Yeh et al. [41]. As a result, serotypes other than K2 were found to have rmpA.

Conclusion:

Urine from females more often than males, particularly, contains a higher number of K. pneumoniae isolates, followed by burn. In tests for antibiotic susceptibility, the majority of K. pneumoniae clinical isolates—especially those from the penicillin family, which includes amoxicillin + clavulanic acid, ampicillin, and piperacillin—showed multidrug resistance. The majority of isolates had considerable mucoviscosity, and the most prevalent capsular typing was K2A, whereas the least number of isolates had the non-K1/K2 serotype and the least amount of mucoviscosity.

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