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Molecular Detection of *Klebsiella pneumoniae* serotype K2 Isolated Clinically

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Abstract

One hundred specimens (urine and sputum) were collected in sterilized containers from patients attending two hospitals in Baghdad province including: Educational Al-Karama Hospital and Educational Al-Yarmouk Hospital, in which, thirty-eight *K. pneumoniae* isolate were obtained out of one hundred specimens, all of which are of urine origin. The results obtained in this study showed that *Klebsiella pneumoniae* isolates were highly resistant to Augmentine (94.7%), also *Klebsiella* isolates were resistant to Ceftriaxone (86.8%), Cefotaxime (81.5%), and Ceftazidime (65.7%). Very low level of resistance was found toward Imipenem and Meropenem (18.4% and 5.2%) respectively. Only four isolates (10.52 %) were positive for *wzy* gene; K19, K20, K21, and K22.

Keywords: *Klebsiella pneumoniae*, serotype K2, antibiotics

الفحص الجزيئي للكليبسيلا الرئوية ذات النمط المصلي K2 المعزولة سريريا

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الخلاصة

مئة عينة (ادرار و قشع) جمعت في علب معقمة من المرضى الذين يزورون اثنين من المستشفيات في بغداد، مستشفى الكرامة و مستشفى اليرموك التعليمي، منها 38 عزلة للكليبسيلا الرئوية تم الحصول عليها من 100 عينة، جميعها كان مصدرها من الادرار. نتائج هذه الدراسة اثبتت ان عزلات الكليبسيلا الرئوية كانت شديدة المقاومة لمضاد الاوكمنتين (94.7%)، كذلك لمضاد السيفترياكسون (86,7%)، السيفوتاكسيم (81,5%)، السيفتازيديم (65,7%). بينما اظهرت العزلات قيد الدراسة مستوى قليل جدا للمقاومة لكل من مضادي الاميبينيم و الميروبينيم (18,4% و 5,2%) على التوالي. 4 عزلات (10,52%) فقط كانت موجبة للجين *wzy* و التي هي K19 و K20 و K21 و K22.

1. Introduction

Klebsiella has been known as human pathogen since it was first isolated in the late nineteenth century by Edwin klebs [1], and it's a genus belongs to the family *Enterobacteriaceae* which is Gram-negative straight rods bacteria, arranged singly, in pairs or short chains, and slightly shorter than other *Enterobacteriaceae* [2]. A wide repertoire among the virulence and resistance factors is present about *K. pneumoniae* genome allowing for the expression of capsule, siderophores, adhesins, and antimicrobial determinants [3]. Infections caused by *K. pneumoniae* can result in serious and life threating infections including pneumonia, urinary tract infections, intravascular line infections, soft tissue infections, intraabdominal infections and bacteremia [4].

Serotyping is one of the typing techniques used to identify microorganisms of same species that can vary in the antigenic determinants manifested on the cell surface [5]. Capsule is a major virulence

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factor of *K. pneumoniae*, and capsular types are related to the severity of infection. The prevalence of capsular types in each *K. pneumoniae*-related disease could be crucial for disease control and prevention [6].

This work aimed to detect the distribution of *K. pneumoniae* serotype K2 (*wzy* gene) among local isolates, using polymerase chain reaction (PCR) technique.

2. Materials and Methods

A. Specimens collection

One hundred specimens (urine and sputum) were collected in sterilized containers from patients attending two hospitals in Baghdad province including: Educational Al-Karama Hospital and Educational Al-Yarmouk Hospital.

B. Isolation and Identification of isolates

The collected specimens were streaked directly on MacConky agar(HiMedia, India), incubated at 37ºC for 24 hours, the large, pink, mucoid colonies were selected and subcultured on another MacConky agar to obtain discrete colonies.

The identification of the isolate included morphological characteristics and biochemical tests which carried out depending on Bergey's Manual of Systematic Bacteriology, 2nd edition [2] and [7], unless it is mentioned elsewhere.

B. Antimicrobial Susceptibility test

Susceptibility test was done for all *K. pneumoniae* isolates toward Augmentine 30µg/disk, Merpenem 10 µg/disk, Imipenem 10 µg/disk, Cefotaxime 30µg/disk, Ceftazideme 30µg/disk and Ceftriaxone 30µg/disk according to the method of Baur *et al*. [8]. All plates were incubated at 37°C for 24 hours, and the results recorded by measuring the inhibition zones according to the Clinical and Laboratory Standards Institute CLSI [9].

C. DNA extraction method

DNA was extracted from *K. pneumoniae* isolates according to Presto TM Mini gDNA bacteria kit protocol (Genaid / Thiland) according to instructions of the manufactural company.

D. Primers preparation

wzy-F and wzy-R primers were provided in lyophilized form, dissolved in TE buffer to give a final concentration of 100 pmol/µl as recommended by provider (Promega/ USA) and stored in a freezer until use.

E. Gene amplification

Two microliters of each primer, 50 ng of DNA extracted from each *K. pneumoniae* isolates and deionized D.W. were added for 20 µl PCR premix tubes to reach 20µl as a final volume.

F. PCR technique

PCR was used for the detection of capsular K2 serotype for *K. pneumoniae* according to the procedure followed by Turton *et al*. [10].

Primers were wzy- forward, 5'-GACCCGATATTCATACTTGACAGAG-3' and wzy-reverse, 5'-CCTGAAGTAAAATCGTAAATAGATGGC-3' and the product size will be about 641 base pair. Nonetheless, thermocycler conditions of PCR was done as follows: 94°C for 1 min, followed by 30 cycles of 94°C for 30s, 59°C for 45s, 72°C for 1 min 30s and a final extension at 72°C for 6 min.

G. Agarose Gel (1% and 1.5 %)

These gels were prepared as described by Voytas [11] by dissolving 1 g and 1.5 g of agarose powder in 100 ml Tris- borate-EDTA buffer (TBE buffer), to prepare 1% and 1.5% solutions, respectively. 1% concentration was used for genomic DNA electrophoresis while the other concentration was employed for PCR product electrophoresis. The optical density OD 260 reading was considered as the concentration of the DNA, while the OD260/OD280 ratio was considered as the purity of DNA.

3. Results and Discussion

A. Isolation and Identification

Thirty-eight *K. pneumoniae* isolate were obtained out of one hundred specimens, all of which are of urine origin. Regarding the specimen type, all *K. pneumoniae* isolates were isolated from ninety-eight urine specimens, none of them were isolated from sputum specimens (two specimens).

Many *Klebsiella* were isolated from urine and other clinical specimens. *Klebsiella* spp. formed 54.16 % of total isolates from clinical specimens, 79.12% was identified as *K. pneumoniae* [12].

Al-Mathkhury and Assal [13] reported that *K. pneumoniae* was isolated with percentage 36.13%. it was isolated more frequently from urine (42.16%) followed by wound (31.25%) then sputum (28.57%). Recently, another local study done by Al-Jumaily and Al-Soundany [14] showed that 15 bacterial isolate from the total 50 sample were given characterization of *Klebsiella* spp., in which ten isolates was characterized as *K. pneumoniae.*

All these local studies indicated that *Klebsiella* was at prior of all nosocomial Gram-negative bacteria and an attention should be paid toward this nosocomial bacteria.

Pages *et al*., [15] showed that *K. pneumoniae* were obtained from urine with 41% as isolation percentage. The variations in isolation percentage of *Klebsiella* in recent and other studies may be attributed to differences in many factors such as sanitary practices for hospital staff, environmental conditions, isolation and identification techniques, social and cultural level of patients, differences in prevalence of infection from one country to another and different in patients state and other many factors which may inhibit or stimulate the growth and distribution of *Klebsiella* and other pathogenic bacteria in hospitals.

B. Antimicrobial susceptibility tests

From the results of the present study, various levels of susceptibilities to different antibiotics among isolates were observed. The results are depicted in Figure-1.

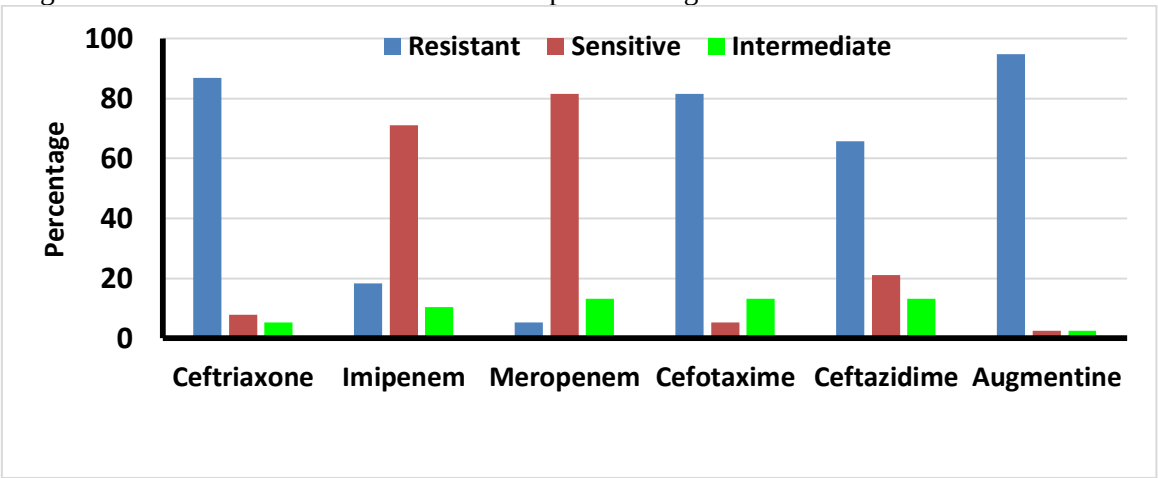

Figure 1- Antibiotic susceptibility of *Klebsiella pneumoniae* isolates (n=38).

The results in recent study showed that *K. pneumoniae* isolates were highly resistant to Augmentine (94.7 %), to Ceftriaxone (86.8 %), Cefotaxime (81.5 %), Ciftazidime (65.7 %), hence *Klebsiella* considered as a multidrug resistant organism. Very low level of resistance was found toward Imipenem and Meropenem (18.4 % and 5.2 %, respectively), which are the most effective drugs.

Interestingly, the results of the present study proved that all 38 isolates of *K. pneumoniae* were highly resistant to the third generation of Cephalosporins and other antibiotics. All Gram-negative bacilli harbor series of antibiotic resistant genes which could be transferred to other bacteria horizontally and can cause different nosocomial infections in hospitals [16].

A study done by Al-charakh *et al*. [17] showed that 50 % of *Klebsiella* isolates were resistant to Cefotaxime. Very low levels (18.4%) of resistance was found in isolates to Ceftazidime and 34.2% to Ceftriaxone.

Aljanaby and Alhasani, [18] stated that all twelve isolates of *K. pneumoniae* were highly resistant to the 3rd generation of Cephalosporins, in which 83.33% was resistant to Cefotaxime. What's more, 66.66% and 91.66% were resistant to Ceftriaxone and Ceftazidime, respectively.

Eighteen *K. pneumoniae* isolates were tested against 19 different antimicrobial drugs by disc diffusion method, all isolates were resistant against Ceftazidime and sensitive to Imipenem [19].

K. pneumoniae of clinical isolates are evolving toward increasing levels of antimicrobial drug resistance, placing this species among the infectious bacterial pathogens that are most challenging to control [20].

C. DNA extraction

Results showed that the recorded range of DNA concentration was (59-127.6) ng/ml and the DNA purity was (1.6-1.87). The obtained quantities and purity of DNA are fair enough for amplification by PCR.A ratio of ~1.8 is generally accepted as "pure" for DNA, if the ratio is appreciably lower than the indicated ratio, it may specify the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm [21]. Gel electrophoresis was done to confirm the purity of extracted DNA. Figure-2 depicted a single band; hence the extracted DNA was pure and ready to use in the PCR reaction.

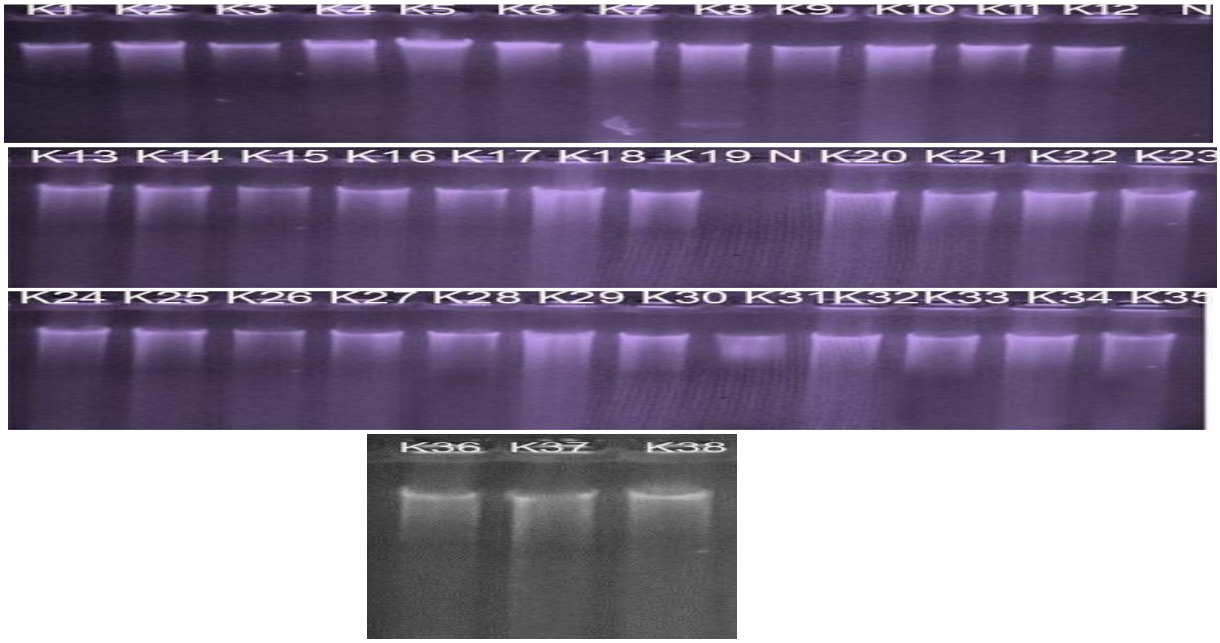

Figure 2- Analysis of genomic DNA of *K. pneumoniae* isolates on agarose gel (1%) at 5 V/cm for 1 hr, stained with ethidium bromide and visualized on a UV transiluminator documentation system. K1-K38 represent isolates (1-38), N represents negative control.

D. Detecting of *K. pneumoniae* serotype K2 by PCR technique

Only four isolates (10.52 %) were positive for *wzy* gene; K19, K20, K21, and K22 all of which was isolated from urine specimens at Al-yarmouk hospital. Which emphasized the fact that "pathogenic" isolates have a K2 serotype can infect tissues other than lung. The rest of isolates (34 isolates) were negative to amplified *wzy* gene thus considered as a Non K2 serotype, these isolates may belong to another serotypes. The molecular technique for K2 gene (*wzy* gene) among isolates of *K. pneumoniae* was confirmed by gel-agarose electrophoresis as shown in Figure-3.

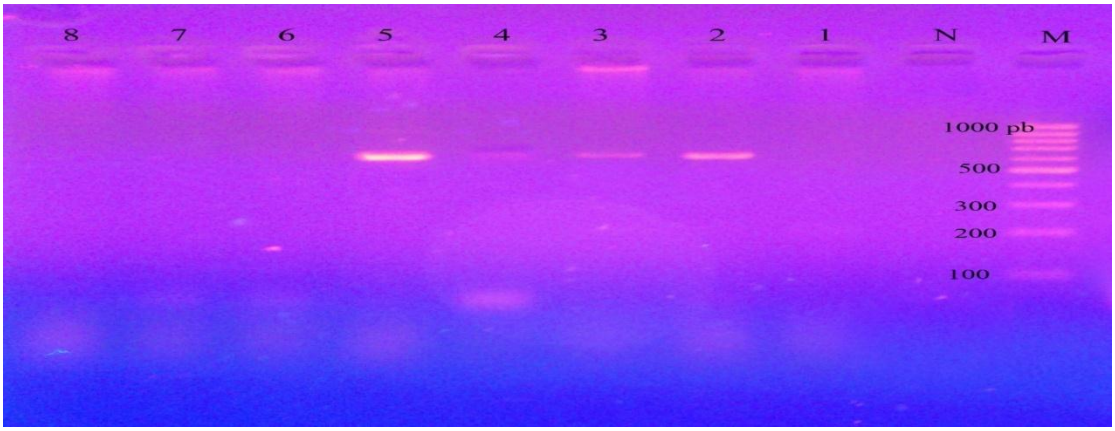

Figure 3- Gel electrophoresis for amplification of *wzy* gene (641 bp) specific for K2 serotype of *K. pneumoniae*. Lanes 1 – 8 represented isolates (18, 19, 20, 21, 22, 23, 24, and 25, respectively). M and N lanes represented 100 pb ladder and negative sample, respectively.

The results of the present study was in partial agreement with Abd Al-Rhman and Al-aubaydi, [19] who found that out of 18 isolates of *K. pneumoniae* only 4 isolates was positive for K2 serotype and all isolates were negative for K1 serotype. Abdul-Razzaq *et al*. [22] found that among 43 isolates of *K. pneumoniae* only 14 isolates gave positive with K2 serotype. Feizabadi *et al*. [23] stated that of 89 isolates of *K. pneumoniae*, only 13 (14.6 %) and 10 (11.2 %) isolates belonged to K2 and K1 serotype, respectively. All these results indicated that K1 serotype of *K. pneumoniae* is the most abundant among other serotypes including K2 serotypes. K1 and K2 capsular antigens with the mucoid phenotype are considered the main virulence factors in *K. pneumoniae* [24].

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