Molecular Detection Of Some Capsulargenes Of Klebsiella Pneumoniae Isolated From Clinical Samples

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Molecular Detection of Some Capsulargenes of Klebsiella pneumoniae Isolated from Clinical Samples

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**ABSTRACT**
*Klebsiella pneumonia* is an opportunistic pathogen that has been implicated as one of the commonest causes of hospital and community-acquired infections. The capsule of *Klebsiella pneumonia* is an important virulence factor, involved in pathogenic mechanisms. So, this study aimed to isolate *Klebsiella pneumonia* from different clinical specimens from patients in Al-Diwaniyah teaching hospital and determine some virulence factors (capsular genes) that used for serotyping of isolates. The study extended from August to November 2020. A total of 31 isolates from 80 different clinical specimens identified as *Klebsiella pneumonia* by traditional biochemical tests, Vitek system, and 16SrRNA gene. The existence of three genes from 7 tested capsular genes was detected by Polymerase chain reaction. The most common serotype were k2, k54, k57 where K2 detected in 4 (12.9 %), K54 in 12 (38.7 %), k57 in 4 (12.9). But, the other capsular polysaccharide genes k1, k3, k5, k20 not detected in all isolates of this study.

**1. Introduction**

*Klebsiella pneumonia* is an important opportunistic pathogen that causes a variety of infectious diseases in humans, including septicemia, liver abscesses, diarrhea, and pneumonia [9]. It is one of the known pathogens acquired in hospital and is associated with increased patient morbidity and mortality [2].

*K. pneumonia* can express a variety of virulence factors including capsules, endotoxins, siderophores, iron scanning systems, and adhesins, which have been shown to play important roles in their etiology. The capsule is an important virulence factor, involved in at least two pathogenic mechanisms: (1) protecting bacteria from phagocytosis, and (2) inhibition of the host's immune response [12]. Some types of capsules (K), especially K1, K2, K54, K57, K20 and K5, are often associated with community-acquired invasive purulent liver abscess syndrome, septicemia, and pneumonia [11]. K2 and K5 are associated with community-acquired pneumonia [1]. K3 is generally associated with rhinoscleroma [11]. Capsules typing is currently the most used technique for typing *K. pneumonia*
isolates and shows good reproduction in distinguishing clinical isolates [17]. Several PCRs targeting the *wzy* genes have been developed for capsule typing of *K. pneumonia* [4]. The aim of this study was to detect the existence of some capsular serotypes genes in *K. pneumonia* isolated from different clinical samples (sputum, urine, burn, wound) because the important role of these genes in evade bacteria from phagocytosis.

2. Methodology

2.1. Bacterial isolation and biochemical identification

A total of (80) different clinical specimens including burns swabs, wounds swabs, urine, sputum were collected from patients in Al-Diwanian teaching hospital in Al-Diwanian city during a period from August to November 2020. The samples were placed in separate sterile plastic bags and then immediately transported to the laboratory in a cooler with ice packs (below 4°C) and processed within 2–4 h.

By sterile swab, the sample was streaked onto MacConkey agar, followed by incubation at 37°C for 24 h. From MacConkey agar, three pink, mucoid colonies were picked up and subcultured onto nutrient agar at 37°C for 24 h, followed by identification by traditional biochemical tests, Vitek system (Biomerieux, France). Confirmed cultures were preserved in Brain-heart infusion broth containing 20% glycerol and stored at −20°C for further study.

2.2. Molecular Identification of *Klebsiella pneumonia* by 16S rRNA gene:

2.2.1. DNA extraction

A DNA extraction kit (Geneaid, Taiwan) was used to perform the process of extracting the DNA from fresh growth of *K. pneumonia*. The procedure was generated according to the manufacturer’s protocol. The DNA was measured for quality and quantity using a NanoDrop (Nabi-Korea).

2.2.2. Amplification of 16SrRNA gene by PCR

The primers (Forward primer: AGCGTCAGTCTTTGTCCAGG and Reverse primer: GATGACCAGCCACACTGGAA) used to amplify regions in the 16S rRNA gene have been designed by using program of Primer 3 Plus. The reaction of the PCR employed by using Mastermix (Bioneer, Korea) with total volume (20 μl) of reaction mixture; including 5μl DNA template, 2μl (10pmol) for each primer, 11 μl of nuclease free water. The conditions of the thermocycler were at 95°C for 2 min of initial denaturation, 35 cycles (denaturation at 95°C for 30s, annealing at 58°C for 30sec, and extension at 72°C for 1 min), and final extension at 72°C for 5 min. Using 1.5 %–agarose gel including 0.5μg/ml Red Safe dye, DNA bands were separated via electrophoresis and visualized via a Gel documentation system (Wisd-Korea).

2.3. Detection of Capsular serotype genes in *K. pneumonia*

All confirmed *K. pneumonia* isolates were grown overnight in nutrient broth at 37°C. Genomic DNA was extracted using a commercial universal DNA Extraction Kit (Geneaid-Taiwan) according to the manufacturer's instructions. Seven individual PCRs were performed to detect the presence of Capsule serotype genes (*k1,k2,k3,k5,k20,k54,k57*) in *K. pneumonia* isolates as in previous studies [1,20]. Primer sequences, and amplicon sizes are shown in Table1. Amplified PCR products were analyzed by gel
electrophoresis on 1.5% agarose gels containing Safe Red(0.005% v/v) in 1 × TAE buffer, and imaged using Gel documentation system (Wisd-Korea).

**Table (1):** Primer sequences and reference of Capsule serotype genes used in this study

<table>
<thead>
<tr>
<th>Target virulence genes</th>
<th>Primer Sequence(5′-3′)</th>
<th>Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>wzyK1 (magA)</td>
<td>F: GGTGCTCTTTACATCATTGC</td>
<td>1283</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>R: GCAATGGCCATTITTCGTTAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wzyK2</td>
<td>F: GACCCGATATTTCTACCTTGACAGAG</td>
<td>641</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>R: CCTGAAGTAAAATCGTAATAGTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zxK5</td>
<td>F: TGGTAGTGATGCTCGCGA</td>
<td>280</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>R: CCTGAACCCACCCCCAATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wzyK20</td>
<td>F: CGGTGCTACAGTGATCGTTAC</td>
<td>741</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>R: GTTATACGATGCTCGTCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wzxK54</td>
<td>F: CATTAGCTCAGTGTTGGCTAG</td>
<td>881</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>R: GCTTGACAAACACCATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wzyK57</td>
<td>F: CTCAGGGCTAGAAGTGTCAT</td>
<td>1037</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>R: CACTAACCACAAAAAGTCGAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wzyK3</td>
<td>F: TAGGCAATTGACTTTAGGTG</td>
<td>549</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>R: AGTGAATCAGCCTCTCCT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (2):** PCR Thermocycling conditions for all genes that used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial Denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final Extension</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>magA(k1)</td>
<td>95°C 4 min</td>
<td>95°C 45 sec</td>
<td>50 °C 30 s</td>
<td>72°C 1 min</td>
<td>72°C 10 min</td>
<td>30 cycles</td>
</tr>
<tr>
<td>WzyK2</td>
<td>95°C 4 min</td>
<td>95°C 45 sec</td>
<td>52 °C 30 s</td>
<td>72°C 1 min</td>
<td>72°C 10 min</td>
<td>35 cycles</td>
</tr>
<tr>
<td>WzyK3</td>
<td>95°C 4 min</td>
<td>95°C 2 min</td>
<td>53 °C 30s</td>
<td>72°C 1 min</td>
<td>72°C 5 min</td>
<td>35 cycles</td>
</tr>
<tr>
<td>zxK5</td>
<td>95°C 4 min</td>
<td>95°C 30 sec</td>
<td>50 °C 30 s</td>
<td>72°C 1 min</td>
<td>72°C 7 min</td>
<td>30 cycles</td>
</tr>
<tr>
<td>wzyK20</td>
<td>95°C 4 min</td>
<td>95°C 30 s</td>
<td>50 °C 30 s</td>
<td>72°C 1 min</td>
<td>72°C 10 min</td>
<td>30 cycles</td>
</tr>
<tr>
<td>wzxK54</td>
<td>95°C 4 min</td>
<td>96°C 30 s</td>
<td>50 °C 30 s</td>
<td>72°C 1 min</td>
<td>72°C 10 min</td>
<td>30 cycles</td>
</tr>
</tbody>
</table>
3. Results & Discussion

3.1. Prevalence of *K. pneumonia* in different clinical specimens

A total of 31 isolates were identified as *K. pneumonia* from (80) clinical specimens that collected from different clinical cases from Al-Diwaniyah teaching hospital in Al-Diwaniyah city/Iraq, by traditional biochemical tests and Vitek system; distributed as shown in Table (3). In the study of Ssekatawa et al.[18], a variety of clinical specimens such as wound, burn, sputum and urine were used to study the prevalence of *K. pneumonia* in a hospital based study and they were able to isolate the microorganisms from all these samples supporting our finding that *K. pneumonia* has the ability to cause a wide spectrum of clinical illness and can be isolated from a variety of clinical samples.

Table (3): The clinical specimens and rates (%) of each bacterial species from these specimens

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Burn</th>
<th>Sputum</th>
<th>Urine</th>
<th>Wound</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>3</td>
<td>9.4</td>
<td>10</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td><em>Shigella spp.</em></td>
<td>0</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>1</td>
<td>14.3</td>
<td>2</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>3</td>
<td>42.9</td>
<td>16</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7</td>
<td>100.0</td>
<td>32</td>
<td>100.0</td>
<td>80</td>
</tr>
</tbody>
</table>

The samples of *K. pneumonia* obtained in the current study were taken from four main clinical patterns: burn, urine, wound and sputum as shown in figure (1). It should be mentioned that clinical samples yielded 31 isolates.

Figure (1). The clinical samples obtained in the current study from which *K. pneumonia* were isolated.
3.2. Molecular identification of *K. pneumoniae* by 16srRNA

It has been confirmed the identification of isolates by molecular detection of 16SrRNA gene as shown if figure (2). The 16SrRNA gene is used as a diagnostic tool for bacteria because it is found in all bacteria, even mutants. 16SrRNA is composed of highly conserved nucleotide sequences interspersed with variable regions of bacterial species and species. PCRs target protected areas of rRNA [3].

![Figure 2: Agarose gel electrophoresis image showed PCR product size at 457 bp for identification gene(16SrRNA) of *K. pneumonia* isolates. Marker ladder (100-1000bp), lane (1-20): Isolates numbers.](image)

3.3. Detection of Capsular Serotype genes in clinical specimens

The serotype k1,k3, k5 and k20 genes were not detected in *K. pneumonia* isolates . Regarding serotype K2 gene, it was identified in 12.9 % isolates of our study but was not detected in study of Hamam et al. [10] and Shakib et al. [14] . However, it was reported in 6.7 % in the study of Chen et al.[5]  Serotype K54 detected in 37.8 % isolates of our study and 2.1 % in the isolates of study by Chen et al. [5].Capsule serotype k57 in 25.8 % isolates of our study, while in the study of Ssekatawa et al. [18] there was no detection of K57 serotype gene. The K genes are capsular virulence factors involved in highly virulent *K. pneumonia* [5].

![Figure 3: Image of Agarose gel electrophoresis showed PCR product of k2 serotype gene in *K. pneumonia* Clinical isolates with product size 641bp. M:Marker ladder (1500-100bp), lane (1-20): Isolates number.](image)
Figure (4): Image of Agarose gel electrophoresis showed PCR product of k57 serotype gene in *K. pneumonia* Clinical isolates with product size 1037bp. M: Marker ladder (1500-100bp), lane (1-20): Isolates number.

Figure (5): Image of Agarose gel electrophoresis showed PCR product of K54 serotype gene in *K. pneumonia* Clinical isolates with product size 881bp. M: Marker ladder (1500-100bp), lane (1-20): Isolates number.

*K. pneumonia* strains typically express both smooth lipopolysaccharide (LPS) with O antigen molecules and capsule polysaccharide (K antigen with different types as K1, K2, K3, K5, K20, K54, K57) confers resistance against the bactericidal activity of antimicrobial peptides, complement, and phagocytes. In addition, the capsule averts fulminate activation of the immune response[12].

Several capsular (K) types, K2, K54, K3, K57, are frequently linked to community-acquired invasive septicemia, pyogenic liver abscess syndrome and pneumonia[7,8,15]. Pathogen survival requires the acquisition of virulent factor such as capsule genes [6] and the acquired traits have been postulated to play an important part in the pathogenesis of *K. pneumonia* infections.

**Conclusion:** The high prevalence of virulent *K. pneumonia* among clinical isolates that get from the hospital (patient) as revealed by this study pose a great threat to healthcare. Our findings underline the epidemiological and public health risks and implications of this pathogen.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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