MOLECULAR INVESTIGATION OF INTEGRONS IN KLEBSIELLA PNEUMONIAE ISOLATED FROM URINARY TRACT INFECTIONS IN THI-QAR PROVINCE, IRAQ

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ABSTRACT:
Due to the emergence of antibiotic resistance bacteria, treatment of urinary tract infection is becoming more problematic. Integrons are mobile genetic elements that lead to the spread and transfer of antibiotic resistance genes in bacteria. The aim of the present study was to determine the frequency of class 1 and 2 integrons as well as the antimicrobial resistance in Klebsiella spp strains isolated from urinary tract infections (UTIs). A total of 30 clinical isolates of uropathogenic K. pneumoniae were collected from women having urinary tract infection who visited Bint-AlHuda hospital in Nasiriyah City. All the isolated samples were confirmed by standard biochemical tests. The distribution of different integron classes was determined by polymerase chain reaction (PCR). The highest rate of antibiotic resistance in K. pneumoniae isolates was found in antibiotic ampicillin, amikacin, amoxicillin/clavulanic acid, tetracycline, (100%) and resistance meropenem (66.66%), Gentamicin(60%), tobraymicin(99.33%) respectively. K. pneumoniae isolated showed intermediate resistance to ipmenem(80%). The highest sensitivity was seen to ciprofloxacin(73.33%) and ceftraxone(40%). Ninety three percent of K. pneumoniae isolates carried class 1 integrons, whereas class 2 integrons were found in 100% respectively. Our data suggest that the antimicrobial resistance to some antibiotics as well as the frequency of class 1 and 2 integrons is very high in K. pneumoniae strains. Therefore, it is very important to monitor integron-induced drug resistance, especially class 1 integron, in order to control the urinary tract infections causing by MDRK pneumonia strains.

Keywords: Urinary tract infection, K. pneumoniae, Integrons

I. INTRODUCTION
Urinary tract infections (UTIs) are among the most common bacterial infections in humans and they primarily affect women. Indeed, it has been reported that 40–60% of adult women will experience at least one uncomplicated UTI episode during their lifetime (Flores et al., 2015). Klebsiella pneumoniae is a potential pathogen in the community, causing a variety of clinical manifestations such as septicemia, pneumonia, and urinary tract infection (UTI), meningitis, and purulent abscesses at various locations. K. pneumoniae is also the second most frequently isolated species from UTI, after Escherichia coli (Lin et al., 2010).

Klebsiella pneumoniae Gram-negative rods, non-motile with large polysaccharide capsule, lactose-fermenting colonies on MacConkey agar with mucoid appearance. It is found within the natural flora upper respiratory and enteric tract (Jawatz, 2016). Furthermore, K. pneumoniae is a major pathogen of nosocomial infections, including urinary tract infections, and is frequently associated with resistance to the most critical-priority antimicrobials (Magiorakos et al., 2017). The development of antibiotic resistance has led to discovery of many natural mobile analysis of these elements has finally led to discovery of integrons (Gangone et al., 2006).

The integrin consist of three genetic elements: the integrase gene (intI), which is in charge of site-specific recombination of mobile gene cassettes, the attachment site (attI), and the promoter (Pc) (Deng et al., 2015). Gene cassettes are unique genetic elements that consists of a single Open Reading Frame (ORF) and a recombination site attC site is a 59-bp element. The integrase is responsible for mediating the integration of circular gene cassettes between attl and attC sites (Gillings, 2014).

Integrons are gene exchange systems that have been shown to play an important role in the acquisition and dissemination of antimicrobial resistance genes as well as being selected by antimicrobial pressure. These
systems play a broad and important role in multidrug resistance (MDR) K. pneumoniae strains (Mazel, 2006).

There is a need for improved surveillance for drug resistance and its mechanisms of dissemination and persistence and resistance genes in the community and clinical. Considering the role of K. pneumoniae in urinary tract infections and increasing antibiotic resistance in this bacterium on the one hand and the importance of integrons in acquiring antibiotic-resistant gene cassettes on the other hand, the aim of this study was to investigate the antibiotic resistance and the frequency of class 1 and 2 integrons in K. pneumoniae strains isolated from urinary tract infections.

II. MATERIALS AND METHODS

Sample collection

Between September 2019 and March 2020, isolates of K. pneumoniae were collected from the urine samples of women patients with UTI who were referred to Bint Al-Huda hospital in Nasiriyah City.

Sample processing

For urine, mid-stream samples collected from patients using sterile container. The samples were cultured on MacConkey and blood agar plates by streaking without swab media.

Identification of bacterial isolates

First of all the bacterial isolates were identified according to cultural characteristics. Gamma-hemolysis of growing colonies on blood agar medium, lactose-fermenting colonies on MacConkey and EMB agar plates. After that, Gram stained smears of young bacterial isolates (18-hour) were examined under oil immersion lens to observe cell morphology, cell arrangement and reaction with Gram-stain. Finally, several biochemical tests were used to identify the K. Pneumoniae isolates including: catalase, oxidase, IMViC tests, urease, motility, glucose fermentation and H2S production.

Antimicrobial susceptibility testing

The antibiotic susceptibility of K. pneumoniae to 10 antibiotics was determined using Kirby Bauer disk diffusion method according to CLSI, (2014). Antibiotics were used including ampicillin (25 mg/disc), amikacin (10 mg/disc), amoxicillin/clavulanic acid (20 mg/10 mg/disc), tetracycline (10 mg/disc), Gentamicin (10 mg/disc), tobramycin (10 mg/disc), imipenem (10 mg/disc), meropenem (10 mg/disc), ciprofloxacin (10 mg/disc), ceftriaxone (30 mg/disc).

DNA extraction

DNA of each K. pneumoniae isolate was extracted by boiling method. The overnight cultures of K. pneumoniae strains in nutrient broth were suspended in 250 μL of sterile deionized water and incubated at 100 °C for 10 min. After centrifugation at 10,000 g for 5 min, the supernatant were used as a template DNA and stored at −20 °C until use.

Detection and characterization of class 1, 2 integrons

The presence of class 1, 2 integrons in K. pneumoniae were investigated by amplification of integrons genes including intI1, intI2, specific primers (Table 1). The PCR reactions were prepared in a total volume of 25 μL and amplification was performed in a thermocycler (Eppendorf master cycler) as follows: 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 30 s at 72 °C; 10 min at 72 °C for detection of intI1 gene and 5 min at 94 °C; 32 cycles of 1 min at 94 °C, 1 min at 60 °C, 2 min at 72 °C; 10 min at 72 °C for detection of intI2 genes. Reaction mixtures without a DNA template used as negative control. The amplified products were electrophoresed on 1.5% agarose gel and after staining with ethidium bromide (0.5 mg/ml) visualized in gel document system.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Amplification Product (bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>

Table 1: Primers used for PCR

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According to the data available in this present study and during the 6 month study period 300 clinical isolation from urine from women with community acquired UTI from bnit-Alhuda hospital in Nasiriy Iraq. Klebsiella pneumoniae isolated from 300 patients, of which 30 (15%) belonged to k.pneumonia. The other isolates recovered from UTI patients were E.coli 67(33.5%), S.aureus 40(20%), P.mirabilis 25(12.5%), P.aeruginosa38(19%). Depending on the bases of cultural and morphological characteristics, microscopy and conventional biochemical testes, (10%) isolates were identified as K.Pneumoniae. All bacteria isolates, As shown in table 2.

Table (2): Cultural, Microscopical and Physiological characteristics of klebsiella spp

<table>
<thead>
<tr>
<th>Isolates</th>
<th>K. pneumoniae</th>
<th>K. oxytoca</th>
<th>K. ornitholytica</th>
<th>K. terrigena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Bacilli</td>
<td>Bacilli</td>
<td>Bacilli</td>
<td>Bacilli</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>LF</td>
<td>LF</td>
<td>LF</td>
<td>LF</td>
</tr>
<tr>
<td>Gram stain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>methyl red</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Simmon`s citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kliglar iron agar</td>
<td>A\A</td>
<td>A\A</td>
<td>A\A</td>
<td>A\A</td>
</tr>
</tbody>
</table>
Lactose fermenting (+) positive result (-) negative result V= variable result (LF) Lactose ferment (K) alkaline

Antimicrobial susceptibility test

The isolates showed high resistance toward ampicillin (AMP) (100%), amikacin (Ak) (100%), amoxicillin-clavulanic acid (AMC) (100%), tetracycline (TE) (100%), gentamicin (CN) (60%), tobramycin (TN) (93.33%), meropenem (MEM) (66.66%), imipenem (IPM) (20%), respectively. The highest sensitive was seen to ciprofloxacin (CIP) (73.33%), and ceftrilaxone (CRO) (40%). According to the antibiotic susceptibility results, most strains were considered as multidrug resistance (MDR).

Table (3): Antimicrobial resistance pattern of K. pneumoniae isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
<th>R</th>
<th>%</th>
<th>I</th>
<th>%</th>
<th>S</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>25</td>
<td>15</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
<tr>
<td>AK</td>
<td>10</td>
<td>15</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
<tr>
<td>AMC</td>
<td>30</td>
<td>15</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
<tr>
<td>TE</td>
<td>10</td>
<td>15</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
<tr>
<td>CIP</td>
<td>30</td>
<td>4</td>
<td>26.66%</td>
<td>1</td>
<td>6.66%</td>
<td>11</td>
<td>73.33%</td>
<td>15</td>
</tr>
<tr>
<td>CRO</td>
<td>10</td>
<td>5</td>
<td>33.33%</td>
<td>4</td>
<td>26.66%</td>
<td>6</td>
<td>40%</td>
<td>15</td>
</tr>
<tr>
<td>MEM</td>
<td>10</td>
<td>10</td>
<td>66.66%</td>
<td>5</td>
<td>33.33%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
<tr>
<td>IPM</td>
<td>10</td>
<td>3</td>
<td>20%</td>
<td>12</td>
<td>80%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
<tr>
<td>GN</td>
<td>10</td>
<td>9</td>
<td>60%</td>
<td>3</td>
<td>20%</td>
<td>3</td>
<td>20%</td>
<td>15</td>
</tr>
<tr>
<td>TN</td>
<td>10</td>
<td>14</td>
<td>93.33%</td>
<td>1</td>
<td>6.66%</td>
<td>0</td>
<td>0%</td>
<td>15</td>
</tr>
</tbody>
</table>

AM: Aoxcillin; AK: Amikicin; AMC: Amoxicillin-clavulanic acid; TE: Tetracycline; CIP: Ciprofloxacin; CRO: Ceftrilaxone; MEM: Meropenem; IPM: Imepenem; GN: Gentamicin; TN: Tobramycin; Note done R: Resistance; S: Sensitivity; I: Intermediate

Detection of class I and II integron genes

Evaluation of two class of integrons by PCR method showed that 15 isolates carried class I (93.33%) and class II integrons (100%), respectively (Fig. 1 and Fig. 2). More than 96.66% of isolates that carried class I, 2 integrons were isolated from urine samples women patients. There was significant association between resistance to ampicillin, amikacin, amoxicillin-clavulanic acid, tetracycline, gentamycin, tobramycin, imipenem, and meropenem with the presence of class I and class II integron among K. pneumonia isolates in this study.

Fgi-(1) Electrophoresis of PCR product on 1.5% agarose gel for Int1 gene in K. pneumonia. Lane 1: 15 positive except Lane 6-7 negative for Int1, ladder as the molecular size marker; 100- 3000bp, Lane Int1 gene at 788bp
IV. DISCUSSION

This paper describes a study undertaken and evaluated to investigate the antibiotics susceptibility pattern and the prevalence of integron and its relation to antimicrobial resistance in K. pneumoniae strains isolated from women having UTI. The level of antibiotic resistance among hospital and community-acquired isolates has steadily increased and become a major global health problem. Antibiotic resistance pattern of isolates from UTI, which is one of the most frequent infectious diseases and most common infection in hospital and care institution, is compatible with (Kahlmeter, 2003; Heiat et al., 2014). A total of 300 isolates (10% K. pneumoniae) associated with UTI were analyzed. These isolates were isolated and identified according to the standard biochemical method (Levinson, 2016 and Jwetaz, 2016). In the present study, which was conducted on K. pneumoniae strains causing urinary tract infections, the most susceptibility was observed to ampicillin, amikacin, Tetracycline, Amoxicillin-clavulanic acid (100%), Gentamicin (60%), Tobramycin (93.33%), Meropenem (66.66%), respectively. This is in accordance with (Sexdjavadi et al., 2013) who showed high incidence of antibiotic resistance among K. pneumoniae strains in Iran. While the isolates were sensitive to the antibiotics Imipenem (80%), Ciprofloxacin (73.33%), Ceftriaxone (40%), this result of the present study was different with (Lina et al., 2007 and Mirkalantari et al., 2017).

In our study, we identified class 1, 2 integrons in clinical MDR K. pneumoniae isolates from Iraq. According to the findings of this study, all strains of K. pneumoniae with multidrug resistance are positive for class 1, 2 integron, which is consistent with the findings of Firoozeh et al., 2017 and Xu et al., 2017; and Wu et al, 2013). High prevalence of integron in our K. pneumoniae isolates (96.66%) presume that the role integrons in the spread of resistance may be more prominent than other mobile genetic elements such as plasmids and transposons. Furthermore, the presence of integrons at high levels in K. pneumoniae strains confirms K. pneumoniae proclivity to carry and transfer MDR genes between enteric pathogens. Integrons from K. pneumoniae strains were found to be significantly associated with resistance to ampicillin, amikacin, amoxicillin-clavulanic acid, tetracycline, tobramycin, gentamicin. According to resistance observed among our isolated, in conforms to earlier studies, emphasizes that acquisition of resistance determinants may not be random process (Leverstein et al., 2003). Acquisition of one or two important resistance genes may thus serve as a platform for acquiring more resistance determinants for these are frequently carried by integrons, and mobilization of resistance determinants by plasmids or transposons would be alternative approaches. In summary, our study found that K. pneumoniae is a major cause of urinary tract infection in women. Ciprofloxacin and Ceftriaxone were the most effective antibiotics against strains isolated from women with community acquired UTI in Iraq. The high level of resistance to antibiotics between K. pneumoniae strains under the study and its association with integrons suggested that integrons facilitate the spread of antibiotic drug resistance.

REFERENCES