Antimicrobial susceptibility patterns of Klebsiella pneumoniae isolated from older smokers and non-smokers of inpatients in intensive care unit infected with chronic pneumonia in AL-Najaf hospital, Iraq

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Abstract

The main aim of this study is to detect the antimicrobial susceptibility patterns and the prevalence of two extended-spectrum beta-lactamase genes (bla-SHV and bla-TEM) in 80 K. pneumoniae isolates from older of inpatients (smokers and non-smokers) infected with chronic pneumonia in AL-Najaf hospital, Iraq. The antimicrobial susceptibility test of 80 isolates proved that K. pneumoniae were 100% resistance to amoxicillin and nitrofurantoin. While K. pneumoniae was resistance to Amoxiclav with percentage 88.75%, Cefotaxime 86.25%, Ceftaximzone 92.5% and Ceftazidime 85%. On the other hand, K. pneumoniae was resistance to Gentamicin, Amikacin, Tobramycin and Tetracycline with percentage 72.5%, 61.25%, 51.25% and 73.75% respectively. All K. pneumoniae isolates from smokers of inpatients were highly resistance to most antimicrobials as compare with those isolates from non-smokers. Also, the results proved that there were 73.75% isolates were multi-drug resistance, 15% isolates were extensive-drug resistance and 11.25% isolates were pan-drug resistance. The results of genotypic detection proved that there were 45 isolates (56.25%) were harbored bla-TEM, 31 isolates (38.75%) were harbored bla-SHV gene and 18 isolates (22.5%) were harbored both two genes. Most K. pneumoniae isolates from smokers of inpatients were harbored antimicrobials associated genes more that those isolates from non-smokers. In conclusions: K. pneumoniae isolates from older of inpatients infected with chronic pneumonia were more virulent than those isolates from non-smokers and we must be careful because these bacteria may be transferred to other healthy persons.

Keywords: Klebsiella pneumoniae, older inpatients, chronic pneumonia, antimicrobials, multi-drug resistance, bla-SHV, bla-TEM.

INTRODUCTION

Smoking is a most risk factor to cause respiratory infections and pneumonia worldwide. One of the best ways to prevent and control respiratory infections is to avoid smoking. The WHO reported that smoking is a cause of 1 in 4 deaths and the most common cause of 1 in 5 avoidable deaths. Smoking is the most risk factor to cause respiratory infections and pneumonia caused by viruses and opportunistic bacteria such as K. pneumoniae is a quittting smoking (Campagna et al., 2016). Klebsiella pneumoniae is an opportunistic pathogen, cause many infections, such as pneumonia and bloodstream infections. Colonization of the respiratory tract of healthy persons accounts for a significant proportion of healthcare-associated, or nosocomial, K. pneumoniae is gram negative bacteria, mannitol fermenter, capsulated and non-motile (Zhong et al., 2013; Aljanaby and Alhasnawi (2017). Many reports have been published worldwide about outbreaks of pneumonia caused by K. pneumoniae in intensive care units (Liu et al., 1992; Arpin et al., 2003) . The number of K. pneumoniae strains producing extended-spectrum beta-lactamase variants of the widespread plasmid-encoded beta-lactamases belonging to the enzyme families TEM, SHV, and CTX-M are constantly (Jacoby and Munoz-Price, 2005). Klebsiella pneumoniae is naturally resistant to penicillins, Therefore, the drug of choice for empirical treatment is often a cephalosporin. However, the use of cephalosporins is known to select for resistant K. pneumoniae strains (Bedenic, 2000). Klebsiella pneumoniae is inherently resistant to penicillins and early cephalosporins due to constitutive production of a chromosomally encoded class a beta-lactamase Petit, (1992). The most common belong to the enzyme families bla-TEM , bla-SHV and bla-CTX-M (Patel et al., 2009). Bacterial resistance is a frequent and significant problem in the hospital environment. Increased resistance among members of the Enterobacteriaceae family have culminated in the ever more frequent appearance of multi-resistant species, which represent an important public health problem that is in expansion, requiring multidisciplinary efforts for prevention and control, besides efficient laboratory detection (GISKE et al., 2011). Therefore, the main goal of this study was to investigate of antimicrobial resistance patterns and prevalence of bla-TEM and bla-SHV genes isolated from smokers and non-smokers older inpatients in intensive care units infected with chronic pneumonia in AL-Najaf hospital, Iraq.

MATERIALS AND METHODS

Isolation and identification of K. pneumoniae

A total of 80 K. pneumoniae strains were isolated from older inpatients in intensive care units (60-70 years old) 49 men and 31 women (41 smokers and 39 non-smokers) infected with chronic pneumonia (diagnosis by specialist physician) in AL-Najaf hospital, Iraq during period from January to July 2017. Sputum samples were collected by sterile cups (Hi-media, India). Immediately, Sputum incubated with brain Hart infusion broth (Oxoid, UK) at 37°C overnight and streaked (by sterile swab, Bioanalyse, Turkey) on blood agar (Oxoid,UK) and MacConkey agar (Oxoid,UK) surface and incubated aerobically in 37°C for 24h. All K. pneumoniae isolates were identified according standard biochemical tests (MacFaddin, 2000) and by using the Vitek®2 system (bioMe´rieux, France).

Antimicrobial susceptibility testing

Susceptibility testing was done for ten antimicrobials were performed by using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute 2014 (CLSI, 2014). These antimicrobials are; Amoxicillin 25 μg, Amoxiclav 30 μg, Cefotaxime 30 μg, Ceftriaxone 30 μg, Gentamicin 15 μg, Amikacin 30 μg, Tobramycin 10 μg, Tetracycline 30 μg. Any bacterial isolate resist to a minimum at least 3 different classes of antibiotics it is multi-drug resistance (MDR), any bacterial strain remain susceptible to only one or two class of antibiotics it is extensive-drug resistance (XDR) and any bacterial isolate resistance to all sub classes in all classes of antibiotics it is pan-drug resistance (PDR) (CLSI, 2014).
DNA extraction, PCR primers and thermo cycling conditions

Boiling method was used for total DNA extraction according to method by Aljanaby and Alhasnawi (2017). The primer sequences were as follows:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5' to 3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla-SHV forward</td>
<td>GGCCGCGTAGGCATGATAGA</td>
</tr>
<tr>
<td>bla-SHV reverse</td>
<td>CCCGGCGATTGCTGATTTC</td>
</tr>
<tr>
<td>bla-TEM forward</td>
<td>CAGCGGTAAGATCCTGATTTC</td>
</tr>
<tr>
<td>bla-TEM reverse</td>
<td>ACTCCCCGTCGTGTAGATA</td>
</tr>
</tbody>
</table>

with product size 714 and 643 bp respectively (Ensor et al., 2009).

The PCR program consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of DNA denaturation at 52 °C for 45s, primer annealing at 52 °C for 45 s (for bla-TEM gene) and 55 C for 60s (for bla-SHV gene) extension 72 °C, 45s . After the last cycle, a final extension step at 72°C for 7 min (Ensor et al., 2009). Fifteen-microliter aliquots of PCR product were analyzed by gel electrophoresis with 1.5% agarose. Gels were stained with ethidium bromide at 1.5 mg/ml and visualized by UV transillumination. A 100-bp DNA ladder.

Statistical analysis

Percentages were used for the comparison between samples of the study by using GraphPad Prism version 6 computer software.

RESULTS

Antimicrobial susceptibility testing

The results demonstrated that all 80 K. pneumoniae isolates (100%) were resistance to Amoxicillin and Nitrofurantoin. On the other hand, K. pneumoniae isolates were resist to Amoxiclav 88.75%, Cefotaxime 86.25%, Ceftriaxone 92.5%, Ceftazidime 85% and Tetracycline 73.75%. Also, K. pneumoniae showed moderate resistance to Gentamicin 72.5, Amikacin 61.25% And Tobramycin 51.25% (Table 1) and (Figure 1). All K. pneumoniae isolates from smokers patients were high resistance to antimicrobials other than those isolates from nonsmokers patients (Table 2) and (Figure 2). The results proved that there were 59 isolates (73.75%) were multi-drug resistance (33 isolates from smokers patients and 26 isolates from nonsmokers patients), 12 isolates (15%) were extensive-drug resistance (8 isolates from smokers patients and 4 isolates from nonsmokers patients) and 9 isolates were (11.25%) pan-drug resistance (7 isolates from smokers patients and 7 isolates from nonsmokers patients) (Figure 3) and (Table 3).

Table 1: Antimicrobial susceptibility test of 80 K. pneumoniae isolates from older inpatients infected with chronic pneumonia.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Resistance (100%)</th>
<th>Intermediate (100%)</th>
<th>Sensitive (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin 25 μg</td>
<td>80(100)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Amoxiclav 30 μg</td>
<td>71(88.75)</td>
<td>0(0.0)</td>
<td>9(11.25)</td>
</tr>
<tr>
<td>Cefotaxime 30 μg</td>
<td>69(86.25)</td>
<td>2(2.5)</td>
<td>9(11.25)</td>
</tr>
<tr>
<td>Ceftriaxone 30 μg</td>
<td>74(92.5)</td>
<td>0(0.0)</td>
<td>6(7.5)</td>
</tr>
<tr>
<td>Ceftazidime 30 μg</td>
<td>68(85)</td>
<td>2(2.5)</td>
<td>10(12.5)</td>
</tr>
<tr>
<td>Gentamicin 15 μg</td>
<td>58(72.5)</td>
<td>3(3.75)</td>
<td>19(23.75)</td>
</tr>
<tr>
<td>Amikacin 30 μg</td>
<td>49(61.25)</td>
<td>1(1.25)</td>
<td>30(37.5)</td>
</tr>
<tr>
<td>Tobramycin 10 μg</td>
<td>41(51.25)</td>
<td>9(11.25)</td>
<td>30(37.5)</td>
</tr>
<tr>
<td>Tetracycline 30 UI</td>
<td>59(73.75)</td>
<td>3(3.75)</td>
<td>18(22.5)</td>
</tr>
<tr>
<td>Nitrofurantoin 30 μg</td>
<td>80(100)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
</tbody>
</table>

Table 2: Total numbers and percentages of K. pneumoniae isolates that were resistance to 10 antimicrobials isolated from older inpatients infected with chronic pneumonia according to gender and. (N= 80 isolates).

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Smokers (49)</th>
<th>Non-smokers (31)</th>
<th>Total resistance 80 (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin 25 μg</td>
<td>29(100)</td>
<td>20(100)</td>
<td>80(100)</td>
</tr>
<tr>
<td>Amoxiclav 30 μg</td>
<td>29(100)</td>
<td>20(100)</td>
<td>71(88.75)</td>
</tr>
<tr>
<td>Cefotaxime 30 μg</td>
<td>29(100)</td>
<td>20(100)</td>
<td>69(86.25)</td>
</tr>
<tr>
<td>Ceftriaxone 30 μg</td>
<td>29(100)</td>
<td>20(100)</td>
<td>74(92.5)</td>
</tr>
<tr>
<td>Ceftazidime 30 μg</td>
<td>29(100)</td>
<td>20(100)</td>
<td>70(90.9)</td>
</tr>
<tr>
<td>Gentamicin 15 μg</td>
<td>27(91.3)</td>
<td>13(42.2)</td>
<td>50(62.5)</td>
</tr>
<tr>
<td>Amikacin 30 μg</td>
<td>20(68.9)</td>
<td>12(38.7)</td>
<td>32(40)</td>
</tr>
<tr>
<td>Tobramycin 10 μg</td>
<td>14(48.2)</td>
<td>11(35.5)</td>
<td>25(31.25)</td>
</tr>
<tr>
<td>Tetracycline 30 UI</td>
<td>24(82.7)</td>
<td>15(47.5)</td>
<td>39(48.75)</td>
</tr>
<tr>
<td>Nitrofurantoin 30 μg</td>
<td>29(100)</td>
<td>20(100)</td>
<td>80(100)</td>
</tr>
</tbody>
</table>

Figure 1: Total numbers of K. pneumoniae isolates from older inpatients infected with chronic pneumonia that were resistance to 10 antimicrobials. (N= 80 isolates).
Figure 2: Total numbers of *K. pneumoniae* isolates from older smokers and non-smokers of inpatients infected with chronic pneumonia. (N= 80 isolates).


Table 3: Numbers and percentages of multi-drug resistance, extensive-drug resistance and pan-drug resistance *K. pneumoniae* isolates from older inpatients (smokers and non-smokers) infected with chronic pneumonia.

<table>
<thead>
<tr>
<th>Older patients</th>
<th>MDR (100%)</th>
<th>XDR (100%)</th>
<th>PDR (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>26(32.5)</td>
<td>4(5)</td>
<td>2(2.5)</td>
</tr>
<tr>
<td>Smokers</td>
<td>33(41.25)</td>
<td>8(10)</td>
<td>7(8.75)</td>
</tr>
<tr>
<td>Total</td>
<td>59(73.75)</td>
<td>12(15)</td>
<td>9(11.25)</td>
</tr>
</tbody>
</table>


**Genotype detection of antimicrobials associated genes**

The result of the current study proved that out of 80 *K. pneumoniae* isolates there were 45 isolates (56.25%) were harbored *blaTEM* (Figure 4) 35 isolates from smokers patients and 10 isolates from nonsmokers patients , 31 isolates (38.75%) were harbored *blaSHV* gene (Figure 5) 24 isolates from smokers patients and 7 isolates from nonsmokers patients , 18 isolates (22.5%) were harbored both genes (Figure 6) 12 isolates from smokers patients and 6 isolates from nonsmokers patients (Table 4).

**DISCUSSION**

There are many factors effect on lung and heart and lead to different complications. Smoking injures lung and alveolar epithelial cells resulting in diffuse infiltrates and parenchymal fibrosis (Caminati et al., 2012; Kumar et al., 2017). *Klebsiella pneumoniae* is one of the most important gram negative bacteria cause several diseases such as, pneumonia , wounds , blood infections and liver abscess (Aljanaby and Alhasnawi, 2017). *Klebsiella pneumoniae* harbored *bla-TEM bla-SHV* and *bla-CTX-M* genes, therefore remain the most important extended spectrum beta-lactamase producing bacteria isolated from different clinical courses from hospital’s patients worldwide (Chang et al., 2015). In the present study proved that *K. pneumoniae* was high resistance to different antimicrobials such as beta lactam and 3rd generation cephalosporins (Table 1 and Figure 1). All *K. pneumoniae* isolates from smokers older of inpatients were highly resistance to most antimicrobials as a compare with those isolates from non-smokers (Table 2 and Figure 2). Also, the results of current study proved that there were 73.75% isolates were multi-drug resistance, 15% isolates were extensive-drug resistance and 11.25% isolates were pan-drug resistance (Figure 3 and Table 3) and most *K. pneumoniae* isolates from smokers older of inpatients were multi-drug resistance as a compare with those isolates from non-smokers.

Figure 4: Ethidium bromide-stained agarose gel electrophoresis of PCR amplified products from extracted total DNA of *K. pneumoniae*. Lane: (1 to 10 isolates) amplified with diagnostic *blaTEM* gene, show positive results at 643 bp. The electrophoresis was performed at 70 volt for 80 Minutes. (L): DNA molecular size marker (100bp ladder).
Cephalosporins (92%), cephalosporins are used as first-line agents. Many strains have a high degree of resistance to third-generation cephalosporins. For example, in this study, 38.75% of isolates were harbored blaTEM and blaSHV genes which yield product sizes of 643 bp and 714 bp, respectively. The results of the present study are in agreement with many reporters such as Coyle et al. (2005), Abdulsalaam et al. (2013) and Aljanaby and Alhasnawi (2017) when they reported that K. pneumoniae was resistance to Amoxicillin, Ceftazidime, and Cefotaxime with high percentage. But in another study by Mariya and Sunil (2015) K. pneumoniae was resistance to amikacin, nitrofurantoin, ceftriaxone, cefotaxim and ceftazidime with moderate percentage. Klebsiella pneumoniae strains have a high degree of resistance to third-generation cephalosporins (92%), cephalosporins are used as first-line therapy for burns infections and septicemia (Khosravi et al., 2013; Cruz-Córdova et al., 2014). K. pneumoniae is capable of resistance to different antibiotics, this bacterium has series of antibiotic resistance genes which can be transferred horizontally to other gram negative bacteria (Piddock, 2006) and associated with series of nosocomial infections in hospitals (Lewis et al., 2007; Chikere et al., 2008). Multi drug resistance K. pneumoniae caused many problems worldwide; the increasing prevalence of clinical MDR isolates has been associated with higher morbidity and mortality rates (Cao et al., 2014). In the present study some genes responsible for production of extended spectrum beta-lactams (blaTEM and blaSHV) in K. pneumoniae have been detected by PCR using specific primer sequences which yield product sizes of 643 bp and 714 bp, respectively. The result of the 38.75% isolates were harbored blaSHV gene (Figures 5) and 22.5% isolates were harbored both genes (Figure 6) and most K. pneumoniae isolates from smokers older of inpatients were harbored blaTEM gene, blaSHV gene and both genes as a compare with those isolates from non-smokers (Table 4).

The results of the present study are in agreement with Khosravi et al., (2013) who reported that the prevalence of blaSHV gene in K pneumoniae isolates were 88%, on the other hand, in the same study, the prevalence of blaTEM gene was 34.61% of total K. pneumoniae isolates. About 80-90 % of K. pneumoniae strains are now considered to carry a blaTEM and blaSHV enzyme, transfer of plasmid between different bacterial species has been important way to transmission of drug resistance between bacterial species.

In middle east, the prevalence of extended spectrum beta-lactams producers among K. pneumonia, a commonest nosocomial associated bacteria is reported to be more than 65 % while for Escherichia coli it ranges from 45%-65% (Newire et al., 2013; Aljanaby and Alhasnawi, 2017; Aljanaby and Medhat, 2017). Plasmids resistance are the important source of extended spectrum beta lactamase transmission, transferable elements conferring resistance to antibiotics other than beta-lactams travel on or alongside the extended spectrum beta lactamase containing plasmids, lead to multidrug resistance bacteria. It is also that mechanism other than, addition to, plasmid mediated assist transfer of many kinds of resistance factors account for the phenomenon of co-resistance observed (Vaidya, 2011). These pathogens account for an increased demands of beta-lactam drugs which lead to mutation of the bacteria resistance genes, this mutation causes production of the most feared beta-lactamase enzymes like blaTEM and blaSHV genes which have ability to hydrolyze all beta- lactam drugs including carbapenems and 3rd generation of cephalosporins antibiotics (Aljanaby and Medhat, 2017).

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**CONCLUSION**

K. pneumoniae isolates from older smokers of inpatients infected with chronic pneumonia were more virulent than those isolates from non-smokers and we must be careful because these bacteria may be transferred to other healthy persons.

**REFERENCES**


