Investigation of Some Antibiotic Susceptibilities, Plasmid Profiles and ESBL Characteristic of Klebsiella pneumoniae Isolated from Urinary System Infections

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Abstract: The aim of this study was to determine the resistance of Klebsiella pneumoniae causing urinary tract infections to antibiotics, the ESBL (Extended Spectrum Beta Lactamase) rates and plasmid size, the correlation between the plasmid size, the antibiotic resistance of strains and ESBL production. 125 K. pneumoniae were performed biochemical test for the conforming species diagnosis. All samples strains of susceptibility of antibiotics were determined by using disc diffusion method. Determination of ESBL producing of all strains was used by double disc synergy test. The isolation of their plasmids was performed the method by which Kado and Liu were stated. It was determined that 36% of all strains showed ESBL producing activity and majority of these strains contained plasmids (65.6%). The size ranges of the plasmids detected to 8 different plasmid profiles were 1.6 to 30.1 kb. Multiple resistance K. pneumoniae strains carrying plasmid were conjugated with recipient Salmonella spp. strains, after conjugation 19.3 kb plasmids transferred from donor strains (K. pneumoniae) were determined to Salmonella spp. No relationship was found between plasmid size of K. pneumoniae strains and their antibiotic resistance, but it was concluded that plasmids in larger size are more effective in ESBL production than smaller size plasmids.

Key words: Klebsiella pneumoniae • Plasmid • Antibiotic resistance • Conjugation • ESBL

INTRODUCTION

Klebsiella pneumoniae strains are opportunistic pathogen and have been associated with various ailments such as urinary tract infection, nosocomial infection respiratory tract infection, wound infection and diarrhea [1-4]. Antimicrobial resistance among the family of Enterobacteriaceae especially in Klebsiella spp and Escherichia coli responds major problem in nosocomial infections, including urinary tract infection and bacteremia particularly in elderly or debilitated patients [5, 6]. As a cause of nosocomial gram negative bacteremia Klebsiella is secondly only to E. coli [7-9].

K. pneumoniae is resistant to a number of antibiotics mainly extended-spectrum cephalosporin’s and penicillin’s, due to acquisition of plasmid that encode for the production of extended-spectrum β-lactamases (ESBL) especially TEM and SHV enzymes have been described worldwide [10-14, 21]. ESBL producing Klebsiella spp. was first reported in 1983 from Germany [15]. Since, these enzymes have been described in isolates of E. coli and more recently, Salmonella species [16]. ESBL’s are more prevalent in Klebsiella spp. than any other enterobacterial species and outbreaks of infection caused by ESBL producing Klebsiella spp. have been widely reported [17]. These enzymes confer variable depress of protection against expanded-spectrum cephalosporin’s such as cefotaxime, ceftazidime cefoxitin and the monobactam aztreonam [18, 19]. Resistance plasmids are the major source of ESBL’s, which appear to have evolved in recent years by the mutation of β-lactamases that previously had poor activity against newer cephalosporin’s such ceftazidime [20].

The main aim of this study is to determine the resistance of K. pneumoniae - causing urinary tract infections- to antibiotics, the ESBL rates and plasmid size and the correlation between the plasmid size and the antibiotic resistance of strains and ESBL production.

MATERIAL AND METHOD

K. pneumoniae strains were 125 clinical isolates obtained from patients infected urinary system in Ankara Numune Hospital, Ankara Hospital, Gazi Hospital, Ankara Ibni Sina Hospital and Konya Numune Hospital from January 2004 until February 2005.
All isolates identified by the microbiological laboratory as carried out biochemical tests [TSI (Triple Sugar Iron test) and IMVIC (Indol, Methyl Red, Voges Proskauer, Citrate) test].

Bacterial susceptibility to all antimicrobial agents was determined according to criteria of the National Committee for Clinical Laboratory Standards by means of Kirby Bauer disk diffusion method. Disks containing penicillin (10 U), ampicillin (10 μg), chloramphenicol (30 μg), amikacin (30 μg), gentamycin (10 μg), streptomycin (10 μg), cefotaxime (30 μg), ceftizidime (30 μg), ceftriaxone (30 μg), sulbactam/cefoperazone (30+75 μg) and ciprofloxacin (5 μg) were obtained from Oxoid (Oxoid Ltd. England). Disk diffusion susceptibility testing was performed from Mueller Hinton II agar plates (Oxoid). Test strains were preincubated in brain heart infusion broth (Oxoid) at 37°C to an optical density equal to that of 0.5 McFarland turbidity Standard. This suspension was then used to inoculate Muller Hinton II agar plates by swabbing them with a cotton swab. The results were interpreted by using the instructions of the disk manufacturer.

All samples were screened for the production of an ESBL by the double disk synergy test (DDST) as described by Jalier et al. [22]. An enhanced zone between either ceftazidime or ceftriaxone or cefotaxime or aztreonam and the clavulanic acid source (amoxicillin/clavulanic acid disk) represented a positive result.

The isolation of plasmids was performed isolation method described by Kado and Liu [23] and Birnboim and Doly [24]. The plasmid DNA's were displayed using agarose gel by means of UV and photographed (DS34 Polaroid Direct Sceen Instant Camera 100X81 mm).

In conjugation test Salmonella spp. (lactose negative) was used as a receptive strain. The conjugation was executed between K. pneumoniae strains fermenting lactose isolated from urinary tract infections and Salmonella spp. non-fermenting lactose in conjugation.

RESULTS

During the 13-month study period, a total 125 of K. pneumoniae were isolated from urinary tract infection specimens; antibiograms and production of ESBL's were recorded. While number of 45 strains (36%) produced ESBL, number of 80 strains (64%) didn't produce ESBL. ESBL producing and ESBL non-producing strains were presented in the Table 1.

Table 1: The resistance and sensitivity numbers and rates of ESBL producing and non-producing K. pneumoniae strains to the antibiotics used

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ESBL (+) strains n: 45</th>
<th>ESBL (-) strains n: 80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance (%)</td>
<td>Sensitivity (%)</td>
</tr>
<tr>
<td>Penicillin (P)</td>
<td>45 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin (AM)</td>
<td>43 (95.5)</td>
<td>2 (4.5)</td>
</tr>
<tr>
<td>Chloramphenicol (C)</td>
<td>8 (17.8)</td>
<td>37 (82.2)</td>
</tr>
<tr>
<td>Amikacin (AK)</td>
<td>1 (2.3)</td>
<td>44 (77.8)</td>
</tr>
<tr>
<td>Gentamycin (CN)</td>
<td>16 (35.6)</td>
<td>29 (64.4)</td>
</tr>
<tr>
<td>Streptomycin (S)</td>
<td>8 (11.8)</td>
<td>37 (82.2)</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>7 (15.6)</td>
<td>38 (84.4)</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>5 (11.2)</td>
<td>40 (88.8)</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>7 (15.6)</td>
<td>38 (84.4)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>8 (11.8)</td>
<td>37 (82.2)</td>
</tr>
<tr>
<td>Sulbactam/cefoperazone (SCF)</td>
<td>1 (2.3)</td>
<td>44 (77.8)</td>
</tr>
</tbody>
</table>

Table 2: The number and percentage of plasmids of ESBL producing and non-producing K. pneumoniae

<table>
<thead>
<tr>
<th>Number of plasmids</th>
<th>ESBL strains (%)</th>
<th>Non-ESBL strains (%)</th>
<th>Total strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13 (10.4)</td>
<td>30 (24)</td>
<td>43 (34.4)</td>
</tr>
<tr>
<td>1</td>
<td>15 (12)</td>
<td>26 (20.8)</td>
<td>41 (32.8)</td>
</tr>
<tr>
<td>2</td>
<td>8 (6.4)</td>
<td>17 (13.6)</td>
<td>25 (20)</td>
</tr>
<tr>
<td>3</td>
<td>4 (3.2)</td>
<td>5 (4)</td>
<td>9 (7.2)</td>
</tr>
<tr>
<td>4</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>4 (3.2)</td>
</tr>
<tr>
<td>5</td>
<td>1 (0.8)</td>
<td>-</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

%: calculated out of total number of strains. #: Strains with plasmids; \#: strains without plasmids.
In the study, the in vitro susceptibility of ESBL producing \textit{K. pneumoniae} strains - isolated from urinary tract infections - to antibiotics was identified. The highest susceptibility rates were found to sulfactam/cefoperazone and amikacin with 97.7%. Among the cephalosporin antibiotics used, the highest susceptibility were to cefazidime with 88.8%. The ESBL producing \textit{K. pneumoniae} isolates were determined to be 100% resistant to penicillin and 95.5% to ampicillin. ESBL producing \textit{K. pneumoniae} isolates were found to be mostly susceptible to sulfactam/cefoperazone and gentamycin. It was determined highest susceptible against ceftriaxone from the cephalosporin (Table 1).

In our study, whilst 125 clinical strains of \textit{K. pneumoniae} isolated from urinary tract infection were found plasmid in the 82 strains (65.6%), it weren't determined plasmid in the 43 strains (34.4%). The molecular sizes of plasmids were described between

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{The plasmid profiles of 125 \textit{K. pneumoniae} strains determined by Agarose Gel Electrophoresis. \textbf{M: Marker:} Lambda-pUC Mix marker 4 (Fermentas) ø: The samples isolated out of urinary system. (these sample were later omitted from the study).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image2.png}
\caption{The plasmid profiles of receptive strains and standard receptive strains identified after conjugation in Agarose Gel Electrophoresis
\textbf{M: Marker:} Lambda-pUC Mix marker 4 (Fermentas) ø: The samples isolated out of urinary system. (these sample were later omitted from the study.) Trc 1,2,3,4,5: \textit{Salmonella} spp. of transconjugant S: \textit{Salmonella} spp. (Standart strain)}
\end{figure}
1.6 and 30.1 kb. Some of the strains were detected to have more than one plasmid. The plasmid with 19.3 kb size was detected to be the most common plasmid in strains and found in 28 strains. Among the strains, 8 different plasmid profiles were determined. Some profiles detected by comparing the fragment size of marker and the plasm id size of samples (Fig.1). It is indicated that number of 30<sup>th</sup> strains than 43 producing ESBL K. pneumoniae had between 1 to 5 plasmid but 13<sup>th</sup> strains didn’t have any plasmid (Table 2).

In conjugation test, while 125 clinical strains of K. pneumoniae with resistance to at least three antibiotics and with at least one plasmid were chosen as donor, Salmonella spp. was chosen as a receptive strain. Salmonella spp. strains were tested antibiotic resistances (ciprofloxacin, chloramphenicol, amikacin, gentamycin, streptomycin, cefotaxime, ceftazidime, ceftriaxone, sulphatam/cefoxoperazone, ampicillin) and it was found that becoming susceptible all antibiotics. Both donor and receptive isolates are tested plasmid isolation and the plasmids sized 19.3 kb are founded at Salmonella spp. which is not determined any plasmid before conjugation test and is used as donor. Thus the plasmids in donors were transmitted to receptive strains. It was showed that the plasmids in donors were transmitted to receptive strains.

**DISCUSSION**

ESBLs were identified many hospital at worldwide. Even though ESBL’s are determined a lot of pathogen gram negative bacteria, Klebsiella strains are dominant. ESBL producing isolates were firstly reported in 1992 in Turkey. In many recent studies, the ESBL productions of Klebsiella spp. strains isolated from various clinical materials were between 18.4%, 44%, 63% and 49.3% in Turkey [25-28], 25.6%, 40%, 48.1% in India [29-31], 13.5% in Taiwan [32], 19.8% in Brazil [33], 22.4% in Korea [34], 30.6% in Portugal [35], 30.7% in China [30], 33.3% in Somalia [36], 83.4% in the USA [37] and 80.2 % in Scotland [38].

In our study, the ESBL producing in K. pneumoniae strains were determined to be 36%. The ESBL rate determined was lower than ESBL rate of the nosocomial infected patients. The important of ESBL is to increase more and more in the nosocomial infections.

In some studies, the ESBL producing Klebsiella spp. strains isolated from urinary system infections were determined to be 21% resistant to chloramphenicol [39], 28%, 68%, 86% resistant to ceftazidime [37, 30, 40], 13.3%, 18%, 23% resistant to cefotaxime [37, 41, 42], 88% resistant to ceftriaxone [30], 20%, 23%, 63.9% resistant to ciprofloxacin [40, 43, 31] and 17.5% resistant to sulphatam/cefoxoperazone [44]. The distinction of our results than the results of the other research come from our study group isn’t composed of nosocomial infected patients.

Many researchers detected plasmids of 210 kb to 3.4 kb size in ESBL producing K. pneumoniae strains. It was verified that the strains resisted to various antibiotics carried most of the plasmids and that these plasmids can be transferred among members of Enterobacteriaceae by conjugation [45-53]. In our study, It was not shown any correlation between the size and number of plasmids and antibiotics resistance, such as other similar studies and determined correlation between the base size of plasmids and ESBL production. Besides, multi-resistant K. pneumoniae strains have transferred their plasmids sized 19.3 kb to Salmonella spp.

Consequently, in studies preventing infection, after microorganisms caused infection are detected phenotypes and genotypes by microbiologic, biochemical and molecular tests, we suggest testing the antibiotic susceptibility tests. It will also be significant to performed more detailed studies, detecting plasmid sizes, ESBL types and whether transferring resistance cases. it was concluded that plasmids in larger size are more effective in ESBL production than smaller size plasmids.

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