Investigation of Imipenem and Meropenem Susceptibilities, Plasmid Profiles and ESBL Characteristic of Klebsiella pneumoniae Isolated from Urinary Tract Infections

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Abstract: Klebsiella pneumoniae bacteria are a significant agent of urinary system infections, upper respiratory tract infections and nosocomial infections. Carbapenems, because of the fact that they are stable against the hydrolyzation of the beta-lactamases, have been a suitable choice at the infections caused by gram negative bacteria. In our study, totally 87 K. pneumoniae strains were isolated from the urinary samples provided from suspected patients with urinary system infections and their resistant characteristics were investigated against imipenem and meropenem. The numbers of the strains producing Extended Spectrum Beta Lactamase (ESBL) and plasmid profiles of the strains including plasmids were investigated. It was determined that 44 % of all strains producing ESBL, 76 % of strains producing ESBL contained plasmids, the size of these plasmids ranges 1.6 to 30.1 kb and these plasmids from eight different plasmid profiles. ESBL (+) and ESBL (-) strains were determined to be sensitive against imipenem and meropenem. No relationship was found between plasmid size of K. pneumoniae strains and imipenem and meropenem resistances, it was concluded that larger plasmids are more effective than smaller plasmids in ESBL producing.

Key words: Klebsiella pneumoniae · Urinary system infection · Imipenem · Meropenem

INTRODUCION

Carbapenems have been considered the pharmacotherapy of last resort for managing multi-drug-resistant infections caused by Enterobacteriaceae bacteria. Over the past decade, however, resistance to carbapenems has emerged and appears to be increasing among these pathogens, particularly Klebsiella pneumoniae [1]. Resistance to the most widely used carbapenems i.e., imipenem and meropenem can be mediated by a variety of mechanisms, including β-lactamases porin changes and changes in penicillin-binding proteins [2]. ESBL producing organisms is the carbapenem class (imipenem and meropenem), since they are more stable to hydrolysis by ESBLs. Clinical studies also report a significantly higher success rate for carbapenems over other antimicrobials [3].

MATERIALS AND METHODS

In this study, 87 Klebsiella pneumoniae obtained from patients infected urinary tract are investigated. All isolates identified by the microbiological laboratory as carried out biochemical tests. Bacterial susceptibility to imipenem (10µg) (Oxoid) and meropenem (10µg) (Oxoid) was determined according to criteria of the National Committee for Clinical Laboratory Standards by means of Kirby Bauer disk diffusion method. Disk diffusion susceptibility testing was performed from Mueller Hinton II agar plates (Oxoid). Test strains were preinoculated 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.

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Table 1: Non-producing and producing ESBL, numbers of plasmid, sizes of plasmid of Klebsiella pneumoniae

<table>
<thead>
<tr>
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<th>Number of strain (%)</th>
<th>Number of plasmid (%)</th>
<th>Sizes of plasmid</th>
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<tr>
<td>ESBL (+)</td>
<td>38 (44%)</td>
<td>66 (76%)</td>
<td>21.1, 3.0, 22.4, 23.0, 22.7, 21.7, 21.4, 20.3, 19.3, 17.4, 17.0, 15.5, 9.7, 7.5, 6.1, 5.5, 5.0, 5.2, 4.9, 4.8, 4.1, 3.2, 2.9, 2.6, 2.3, 2.1, 1.8, 1.9, 1.8, 1.6</td>
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<tr>
<td>ESBL (-)</td>
<td>49 (56%)</td>
<td>21 (24%)</td>
<td>26.6, 25.3, 23.6, 21.7, 21.6, 21.4, 20.3, 19.3, 17.4, 17.0, 11.6, 6.5, 5.1, 2.9, 4.6, 4.4, 4.3, 3.7, 3.1, 2.5, 2.0, 2.5, 2.2, 2.1, 1.9, 1.7, 1.6</td>
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Fig. 1: The plasmid profiles of 87 K. pneumoniae strains determined by Agarose Gel Electrophoresis

All samples were screened for the production of an ESBL by the double disk synergy test (DDST) as described by Jalier et al. [4, 5]. 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 [6]. The plasmid DNA's were displayed using agarose gel by means of UV and photographed (DS34 Polaroid Direct Scene Instant Camera 100X81 mm).

RESULTS

87 Klebsiella pneumoniae isolated from urinary tract infection were fulfilled antibiotic tests and ESBL tests. While 38 (44%) strains produced ESBL, 49 (56%) strains did not produce ESBL (Table 1). It was found that all strains, producing ESBL and non-producing ESBL, are susceptible to imipenem and meropenem (100%).

In our study, It were determined that 66 (76%) producing ESBL K. pneumoniae were found plasmid. The molecular sizes of plasmids were described between 1.6 and 30.1 kb. While it were detected that producing ESBL K. pneumoniae were found 4 different plasmid profiles, non-producing ESBL K. pneumoniae were found 3 different plasmid profiles (Table 1) (Fig. 1).

DISCUSSION

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 [7-10], 25.6%, 40%, 48.1% in India [11-13], 13.5% in Taiwan [14], 19.8% in Brazil [15], 22.4% in Korea [16], 30.6% in Portugal [17], 30.7% in China [14], 33.3% in Somalia [18], 83.4% in the USA [19] and 80.2% in Scotland [20].

In our study, the ESBL producing in K. pneumoniae strains were determined to be 44%. We found ESBL producing rates as 36% in K. pneumoniae at our previous study but now it shown that ESBL producing rates increase expeditiously. The important of ESBL is to increase more and more in the nosocomial infections.

In many studies, Klebsiella pneumoniae strains isolated urinary tract infections are highly susceptible to imipenem and meropenem [21-23]. Our results shown that correlated the other studies performed in the many countries.

In some study, it were detected that plasmids of 210 kb to 3.4 kb size in ESBL producing K. pneumoniae strains [24-32]. It was not shown any correlation between the size and number of plasmids and imipenem and meropenem resistance, such as other similar studies and determined correlation between the base size of plasmids and ESBL production.

Consequently, it is important that detected phenotypes and genotypes by microbiologic, biochemical and molecular tests in preventing infection studies. It will also be significant to performed more detailed studies, detecting plasmid sizes, ESBL types. It was concluded that larger plasmids are more effective than smaller plasmids in ESBL producing.
REFERENCES


