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ORIGINAL ARTICLE

Characterization of CTX and TEM types of ß-lactamases in clinical isolates of *Klebsiella pneumoniae* producing Extended Spectrum ß-lactamases in sari, Iran, 2014

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ABSTRACT
Extended spectrum ß-lactamases (ESBLs) enzyme are major sources of resistance to ß-lactam antibiotics especially in Enterobacteriaceae such as Klebsiella pneumoniae. Increasing frequency of the ß-lactamases in bacteria is a serious threat for treating bacterial infections.The aim of this study was to determine the presence of TEM and CTX types of ß-lactamases in clinical isolates of K. pneumoniae producing ESBLs. Resistance to different antibiotics was determined using the standard disk diffusion method. ESBLs ß-lactamases were detected by the combination double disk test (CDDT) method and polymerase chain reaction (PCR) was used to determine blaCTX and bla TEM genes in the ESBLs positive isolates.The prevalence of ESBLs producer isolates was 45 (30%). The prevalence of blaCTX and TEM among isolates was 30(66.66%) and 15 (33.33%) respectively. Outbreak of isolates ESBLs can cause serious problems in the future, regarding the treatment of infections caused by this common pneumonia pathogen.
Keywords: Antibiotic Resistance, ESBLs; blaCTX, bla TEM, Klebsiella pneumoniae, Sari

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INTRODUCTION

Klebsiella pneumoniae are important causes of different bacterial infections, including, bacteremia, urinary tract infections (UTI), neonatal meningitis and pneumonia (1, 2). The ß-lactams antibiotics are one of the treatment choices for these bacterial infections (3). One of the main mechanisms of resistance to ß-lactams antibiotics is via the actions of ß-lactamase enzymes (1). Extended spectrum ß-lactamases (ESBLs) are ß-lactamases that hydrolyze extended-spectrum cephalosporins such as cefotaxime and ceftazidime(4). According to Ambler classification, AmpC-ß-lactamases are an important group of class C ß-lactamases that hydrolizepenicillins, extended-spectrum cephalosporins, cephamycins and aztreonam, however they can't be inhibited by ß-lactamase inhibitors such as clavulanate, sulbactam and tazobactam, but are inhibited by phenylboronic acid and cloxacillin (6, 7). CTX and TEM type of the ß-lactamase enzymes is a ESBLs type that is widely reported in Enterobacteriaceae such as K. pneumoniae. This enzyme is the predominant ESBLs type in some countries (8). The CTX enzymes are usually encoded by genes that are carried on the plasmid and have greater activity against cefotaxime than other ceftazidime (9).

MATERIALS AND MTHODS

Bacterial Strains

In total, 150 K. pneumoniae were isolated from clinical specimens including, urine ,sputum, tracheal tube, wound and blood of patients admitted to educational hospitals of sari city (Immam Khomeini, Boali, Zare) located in three different regions of sari , The isolates were identified by their cultural characteristics and reactions to standard biochemical tests.

Antimicrobial Susceptibility Testing

The antibiotic resistance pattern of isolates was determined by using the disk diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (10). The antibiotics tested were cefxime (30 µg), ceftrixone (30µg), ceftazidime (30 µg), cefotaxime (30 µg), imipenem (10 µg), cefepime (30µg), cefoxitin (30µg), gentamycin (30µg), amikacine (30µg) meropenem (30 µg) tobramicine (30 µg) and piraktame(30ug) (Himedia, India). K.pneumoniae ATTC number 788 was used as quality control strain for antimicrobial susceptibility testing.

Detection of ESBLs Producing Isolates

ESBLs producing isolates were detected using the combination double disk test (CDDT) as a standard disk diffusion assay on Mueller Hinton agar (Himedia, India) (11). ESBLs presence was assayed using the following antibiotic disks: ceftazidime (CAZ) (30 µg), ceftazidime (30 µg) plus clavulanic acid (CAZ+CA) (10 µg), (MAST Chemical Co, England). The disk with CA and without CA was placed on the inoculated surface of the Mueller–Hinton agar (Himedia, India) plate by the standard disk diffusion method. The plates were then incubated overnight at 37°C in incubator. An increase of = 5 mm in zone diameter of CAZ with CA (CAZ-CA was considered positive for ESBLs K.pneumoniae ATTC number 788 was used as control strains for detection of ESBLs.

DNA Extraction and Amplification

The primers used for PCR amplification were blaCTX and bla TEM (Table 1). The PCR reactions were carried out in a Primus thermo cycler by using the PCR Master Kit (Takapozist Clone Inc., Iran) according to the manufacturer guideline. PCR condition was as follows: initial denaturation at 95°C for 4 minutes followed by 30 cycles of denaturation at 95°C for 1 minute, annealing for 1 minute at 60°C, extension at 72°C for 1 minute. The final extension step was continued for another 5 minutes at 72°C.

Statistical Analysis

Statistical analysis was carried out using the SPSS 16 statistical software. We used the Chi-squared analysis for comparison of data.

RESULTS

Total of 150 strains of K. pneumoniae (urine = 38%, sputum 27%, tracheal tube 22%, wound 8% and blood 5%) were collected from three hospitals in sari, Iran.

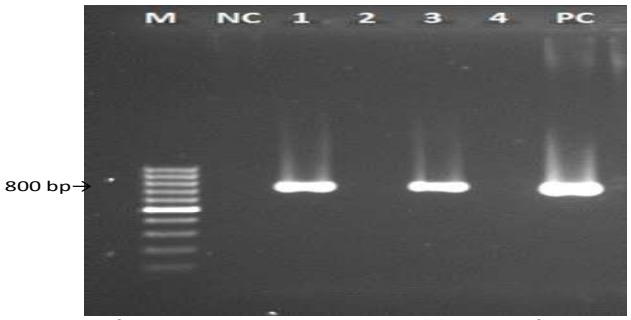
In this study, only two K. pneumoniae were resistant to imipenem, which were isolated from blood. Eighty-four percent of the isolates of K. pneumoniae were resistant to at least one antibiotic and 12 (16%) isolates were susceptible to all tested antibiotics.

More than 50 % of K. pneumoniae isolates were resistant, ceftazidime and gentamycin. Among 150 K. pneumoniae isolates 45 (30%) produced ESBL.

All ESBLs producer isolates were multi drug resistant and showed resistance to four different antibiotics .None of the AmpC ß-lactamases producing isolates were resistant to imipenem. The prevalence rate of blaCTX-M gene among ESBLs producing isolates of K. pneumoniae was 30 (66.66%) and 15 (33.333%) respectively.

Table 1: **The primers used for PCR amplification were blaCTX and bla TEM**

primer	primers5´-3´	Size product
TEM-F	GAGTATTCAACATTTCCGTGTC	800 bp
TEM-R	TAATCAGTGAGGCACCTATCTC	
CTX-MU1	ATGTGCAGTACCAGTAAGGT	594 bp
CTX-MU2	TGGGTAAAGTAGGTCACCAGA	



M= Marker
NC= Negative Control
PC= Positive Control
Positive sample= 1,3
Negative Sample=2,4
TEM

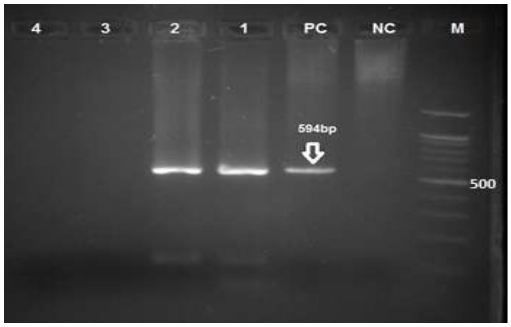


Figure 1 and 2: M= Marker, 1, 2= positive samples, 3,4= Negative samples , pc= positive control, NC= Negative control CTX

DISCUSSION

Appearance of ß-lactamase enzymes among Gram-negative bacteria especially those that have a key role in hospital infections, is an important concern. The prevalence of strains possessing several resistance enzymes concomitantly has caused serious problems for the treatment and the diagnosis of such strains [7]. Compared to the results of previous studies in 2002, the percentages of multi-resistance isolates increased in E. coli [15]. In general, ESBLs producing isolates are susceptible to imipenem [4]. In this study, similar to other reports, more than 99% of isolates were susceptible to imipenem. However, two isolates in our study were resistant to imipenem fortunately; carbapenem resistance is still very rare among *K. pneumoniae* in Iran [16-18]. These results can partially be explained by the concomitant presence of several resistance mechanisms in these isolates. It may also be inferred that the procedures available for the detection of ß-lactam resistance phenotypes are no longer practical. . In this study, the prevalence of the phenotypes in K. pneumoniae, this was 30%. The prevalence of ESBLs in K. pneumoniae as reported in this study is in agreement with that of an investigation from 2007 and 2009 in Tehran (16-18). This can partially show an invariant and uniform prevalence for ESBLs across Iran. There was a significant correlation between presence of blaCTX and resistance to cefotaxime and ceftizoxime in our isolates ($P < 0.05$). CTX producing isolates amongst *K. pneumoniae* have been reported from other parts of Iran such as Tehran and Kurdistan (16, 22, 23). Also, CTX-M producing isolates have also been identified in Korea, Italy, France, New Zealand, Egypt and other countries and carriers of CTX type of ß-lactamases were subsequently hospitalized (21, 24-27). This enzyme has now become the most prevalent ß-lactamases in hospitals and in the community (16).The emergence of the ESBLs, is increasing in Iran. The prevalence of isolates possessing several resistance enzymes concomitantly has caused serious problems for treatment and diagnosis. Consequently, regular epidemiological assessments on the drug resistance patterns of the isolates responsible for nosocomial infections and determination of the molecular resistance mechanisms can be useful for the empirical treatment of the infections and prevention of the drug resistance distribution.

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