Original article

The prevalence and molecular characterization of extended-spectrum β-lactamases-producing *Klebsiella pneumoniae* isolates recovered from Kashan hospital university, Iran

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Abstract

Introduction and objective: *Klebsiella* is an opportunistic pathogen that is an important cause of nosocomial infections. The prevalence of extended spectrum beta-lactamases (ESBLs)-producing strains and their resistance to betalactam antibiotics has had a daily increase. Because of the importance of these enzymes in *Klebsiella pneumonia*, this study was carried out to investigate it's prevalence in Shahid Beheshti hospital of Kashan.

Materials and methods: This descriptive study was done on clinical samples collected from different wards of Shahid Beheshti hospital of Kashan. *K. pneumonia* was isolated on the basis of standard procedures and ESBLs producing strains were confirmed by double disk diffusion method. Extracted DNAs were investigated using specific primers for SHV-1 and TEM-1 genes by PCR method.

Results: Thirty two percent of all 100 isolated *K. pneumonia* had ESBL phenotype. Seven (21.8%) of isolates contained both SHV-1 and TEM-1 genes. Twelve (37.5%) had just TEM1 gene and 16(50%) had SHV1 gene.

Conclusion: Type and amount of antibiotic consumption and length of hospital stay has direct correlation with ESBL production. Because of more morbidity and mortality caused by ESBL isolates compared with infections caused by non-ESBL-producing organisms for treatment of a serious infection caused by an isolate confirmed for ESBL production, a carbapenem agent is indicated despite reports of treatment success with extended-spectrum cephalosporins.

Significance and impact of the study: The results of our study help to well define of ESBL producers prevalence in hospitalized and other patients.

Keywords: Extended spectrum β -lactamases (ESBLs); Nosocomial infection; *Klebsiella pneumonia*

Introduction

Since 1984, multi resistant *Klebsiella pneumonia* has been increasingly recognized as a cause of nosocomial infections [1]. Frequency of reservoirs of these bacteria increases dramatically in the hospital where colonization has direct relation with length of hospital stay. According to Selden *et al*. [2] investigations, the rate of reservoirs of *Klebsiella* among hospitalized patients is nearly 77% with colonization in feces, 19% in pharynx, 42% on hands which is directly associated with antibiotics administration. *Klebsiella* is the cause of 5 to 7.5% of all nosocomial infections and its infections in pediatrics and intensive care units lead to big problems.

Klebsiella is one of the four commonest pathogens in intensive care unit and also the rate of *Klebsiella* borne pneumonia and bacteremia is very high [3-6]. *K. pneumonia* strains resistant to the third generation of cephalosporins were first reported in 1983 in Germany by Knothe *et al*. [7]. In this way resistance to the third generation of cephalosporins especially ceftazidime was observed in most of the *K. pneumonia* and oxytoca isolates [8,9-11]. These organisms are resistant to some of antibiotics, including extended-spectrum cephalosporins and aminoglycosides, because of the acquisition of plasmids which code for the production of extended-spectrum beta-lactamases (ESBL) and aminoglycoside-modifying enzymes [12-15].

Extended spectrum beta -lactamases are mostly transmitted on plasmids. Because these plasmids transmit easily among different *Enterobacteriaceae*, accumulation of resistance genes leads to creation of multiresistant plasmids. Therefore, analysis of plasmid content is essential, because, in epidemiologic investigations, proper treatment, control and prevention of endemic infections and epidemiology of *Klebsiella* are very important. Nosocomial outbreaks caused by *K. pneumonia* are associated with transferable plasmids encoding for ESBLs.

The most frequent ESBLs reported from western and Asian countries include the various SHV and TEM enzymes transmitted on large plasmids that often carry other resistance determinants and are transferred to different strains of one species or other species of enterobacteriaceae family [16]. Studies show that SHV-1 is the commonest β -lactamase among clinical *Klebsiella* isolates and 11 to 73 percent of isolates have this enzyme [17]. TEM-1 b-lactamase also has been distributed in many other *Klebsiella* strains, accompanied by SHV-1 or alone [18].

In this study, we investigate the current condition of extended-spectrum beta-lactamase producer *Klebsiella* species isolated from Shahid Beheshti hospital of Kashan which more resistant to different kinds of antibiotics and caused nosocomial outbreaks.

Material and methods

Study protocol

From November 2007 to August 2008, 100 isolates of *K. pneumonia* from Shahid Beheshti hospital in Kashan were identified using standard biochemical tests and ESBL phenotype was detected using the five antibiotics discs containing aztreonam, cefepime, cefotaxime, ceftriaxone, amoxicillin/clavulanic acid (Becton Dickinson Microbiology System, England) according to CLSI criteria and with preparation of 0.5 McFarland suspension and culture on Mueller-Hinton agar (Merck, Germany) and antibiogram with double-disk diffusion method [19]. Then DNA of samples identified as ESBL were extracted with application of the boiling method and stored for PCR process in TE buffer at -20°C. PCR

reaction was maximized with adjusting the temperature of primers' attachment and their concentration.

Reaction mixture was prepared in 25µl volume containing Tris-HCl 10Mm (pH: 8.4), KCl 50Mm, MgCl2 2.5Mm, primers with 15pM concentration, optimal concentrated dNTPs (Sina gene, Iran), 0.5 unit single polymerase enzyme 1µl (Sina gene, Iran). 5µl of extracted DNA was added to it. Polymerase chain reaction (PCR) amplification for blaTEM was carried out on the isolates with a primary digestion for 4mins at 95°C and then in 35 PCR cycle, denaturation for 1min at 95°C, annealing for 1min at 50°C, extension for 1min at 72°C, and a final extension for 10mins at 72°C. Polymerase chain reaction (PCR) amplification for blaSHV was carried out on the isolates with a primary denaturation for 4mins at 95°C and then in 35 PCR cycle, denaturation for 1min at 95°C, annealing for 1min at 55°C, extension for 1min at 72°C, and a final extension for 10min at 72°C. The sequences of used primers for detection and identification of various genes are shown in table1. Subsequently 5µl of PCR product was recognized in 1.2% agarose gel and ethidium bromide in gel documentation machinery (InGenius model, Syngene Company, USA).

Results

Thirty two percent of isolates had ESBL phenotype belonging to 21(52.5%) male and 19(47.5%) female with mean age of 39.27±19.2. Nineteen (28.1%) were 40 years, and 31(45.6%) were above 40 years old. From all 32 isolated strains, 12(37.5%) had TEM1 gene (Fig. 1) and 16(50%) had SHV1 gene (Fig. 2). Seven samples (21.87%) contained both genes simultaneously.

Table 1: Used primers and proliferated parts

Target of primers	Sequence of primers	The size of the proliferated part	Ref
blaTEM	5' ATA AAATTCTTGAAGACGAAA 3' 5' GCAAGTTACCAATGCTTAATCA3'	1080 bp	20
blaSHV	5'TGGTTATGCGTTATATTCGCC 3' 5'GGTTAGCGTTGCCAGTGCT3'	865 bp	21

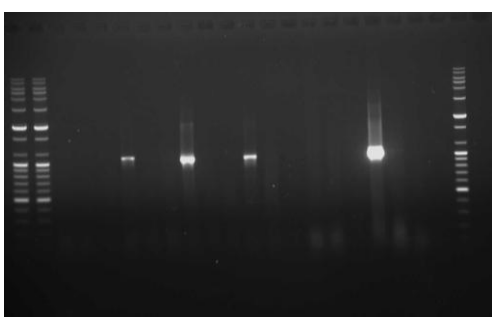

Fig. 1: Proliferation of 1080 base pair part for TEM-1 gene. lane 1&2 DNA ladder (100bp). Lane 3&4&5 negative samples. Lane 6 positive control. Lane 7&8 negative control. Lane 9 positive control

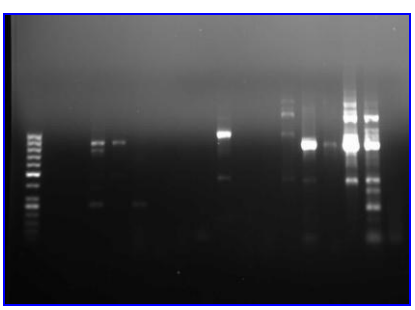

Fig. 2: Proliferation of 865 pair base part for SHV-1 gene. lane 1 DNA ladder (100bp). Lane 2&3 negative samples. Lane 4&5 positive samples. Lane 6&7 negative control

Discussion

Infectious diseases and their treatment are important problems in mankind's life and daily increase in bacterial resistance has raised patients' expenses in recent years. ESBL production rate by *Enterobacteriaceae* has increased noticeably in two recent decades. Most of the hospitalized patients have immune deficiency and underlying disease and *K. pneumonia* as an opportunistic pathogen is one of the most important causes of nosocomial infections especially in intensive care units. In this study from all 100 isolated *K. pneumonia* samples 32(32%) were ESBL producing.

There are different reports from all over the world in the case of prevalence of ESBL bacteria. In a study done in Japan prevalence of 40% [22] and in another similar one in Berkley state of America prevalence of 44% has been reported [23]. In a study in France, the incidence of this phenotype has been reported to be 30-40% in hospitalized patients and 6% in ambulatory patients [24]. Although the prevalence of ESBL has been reported to be 20% in some studies in southern east of Asia, in some regions it has been more than 60% [25]. A study in the year 2004 has shown the prevalence of ESBL in Europe to be 18.4% which is 40% in Netherland and 3% in Sweden [26].

In a study in 2005, the most important isolated genes recovered from hospitals in Turkey were SHV-1 and SHV-5 [27]. The prevalence of this phenotype has been various in Iranian studies too. In a study in Alzahra hospital in Esfahan 218 strains of bacteria were studied with 70(32%) *Klebsiella* from which 49(70%) were ESBL-producing *K. pneumonia* [28]. In a study in Tehran university of Iran 76% of *K. pneumonia* samples had ESBL which is representative of high infectious potential of these strains in different hospital wards [29]. Irajian *et al*. [30] in Semnan indicate that *K. pneumoniae* ESBL producers in urinary tract infection is 28.9%. In another study in Tehran Feizabadi *et al*. [31] showed that 72.1% of isolated *K. pneumonia* was ESBL producer in nosocomial infections.

In the present study it was revealed that 12(37.5%) of samples had TEM-1 and 16(50%) of samples had SHV-1. In other studies, the prevalence of these genes in *K. pneumonia* has been assessed. Although TEM-1 gene has been the most frequent among ESBL producers in 1980s and early 1990, today SHV-1 has been the most prevalent gene in most of the regions and also New York [32]. Similarly, in the present study the prevalence of SHV-1 has been more than TEM-1in ESBL-producing *K. pneumonia* strains. Fecal carriers of ESBL are increasing in Asia and it seems to be due to the lack of proper repelling of sewage. Moreover urinary catheters usage and misuse of antibiotics can lead to higher prevalence and colonization of ESBL-producing organisms.

Conclusion

The prevalence of ESBL is very low in some countries in which accurate nosocomial infection controls have been applied and there is proper control on antibiotic consumption. Therefore, to control these strains, it is necessary to supervise the hospitals and health centres strictly.

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