Infection Epidemiology & Medicine
Vol 1, No 1, Summer 2013
Original Article

Identification of Extended Spectrum Beta-lactamase producing *Klebsiella pneumoniae* isolated from Intensive Care Unit (ICU) patients in three hospitals in Tehran

S. Derakhshan[1], S. Najar Peerayeh*[1], F. Fallah[2], B. Bakhshi[1], M. Rahbar[3], M. Mohammad-Zadeh[4]

1. Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Pediatric Infections Research Center, Tehran, Iran
3. Department of Microbiology, Iranian Reference Health Laboratory, Ministry of Health and Medical Education, Tehran, Iran
4. Department of Microbiology, Milad Hospital, Tehran, Iran

Original Submission: 03 Jan. 2013 Revised Submission: 27 Mar. 2013 Accepted: 17 Apr. 2013

Abstract

Aim of Study: Multiple drug resistance has significantly increased in recent years. The aim of our study was to determine the antibiotic susceptibility of *Klebsiella pneumoniae* isolated from Intensive Care Unit (ICU) patients in three hospitals in Tehran, Iran.

Methods: A total of 116 *K. pneumoniae* isolates were collected from different clinical samples of patients admitted to the ICUs of three hospitals in Tehran, Iran between March and December 2011. Most strains were isolated from tracheal secretions (75%), and urine (16%). Identification was carried out based on biochemical tests and the PCR method to detect the 16S–23S internal transcribed spacer unit of *K. pneumoniae subsp. pneumoniae*. Susceptibility of isolates to 14 different antibiotic disks was determined using agar disk diffusion method. The combination disk method was used for phenotypic detection of the ESBL- producing isolates.

Results: All of the 116 isolates (except one isolate) were susceptible to imipenem. Most of the isolates showed high level of antibiotic resistance: amoxicillin-clavulanic acid and tobramycin (64%), cefotaxime and ceftriaxone (63%), aztreonam (61%), ceftazidime and gentamicin (60%), and trimethoprim-sulfamethoxazole (58%). Sixty percent of the isolates were detected as ESBL producers by the phenotypic confirmatory test.

Conclusion: This study showed that the frequency of ESBL producing strains of *K. pneumoniae* was high in ICU. Carbapenems were the most effective drugs against ESBL-producing strains. Because ICU patients are at high risk, continued monitoring of drug resistance and restriction of antibiotics usage are necessary in clinical settings.

Keyword: *Klebsiella pneumoniae*, Antibiotic resistance, Intensive Care Unit

Introduction

Beta-Lactam antimicrobial agents represent the most common treatment for bacterial infections. Widespread use of β-lactams has induced continuous production and mutation of β- lactamases, expanding their activity against the newly developed β-lactam antibiotics among Gram-negative bacteria worldwide. These enzymes are known as extended-spectrum β-lactamases (ESBLs) (*Sarojamma and Ramakrishna* 2011), commonly found in the family of Enterobacteriaceae mainly *Escherichia*

***Corresponding author**. S. Najar peerayeh, Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Tel: +98 21 82883870
E-mail: najarp_s@modares.ac.ir

coli and *Klebsiella pneumoniae (K. pneumoniae)* (*Alipourfard and Yeasmin Nili* 2010). *K. pneumoniae* is an important Gram-negative pathogen, frequently associated with nosocomial infections. It is involved in urinary tract infections, pneumonia, bacteremia, septicemia, and diarrhoea. *K. pneumoniae* is a part of commensal gastrointestinal flora in humans and disruption of this ecosystem by antibiotics, probably contributes to colonization by the strains that are highly resistant to antibiotics (*Muzaheed et al.* 2009).

Infections caused by ESBL-producing *K. pneumoniae* often involve immune-compromised patients that make infections by these bacteria very difficult to eradicate in high-risk wards, such as intensive care units (*Bonnet et al.* 2000). It is necessary to know the frequency of ESBL positive strains in hospitals so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms are much higher (*Roopa and Sudha* 2010). Compared with infections caused by susceptible organisms, infection with these ESBL positive and resistant strains results in increased mortality, hospital stay and hospital charges (*Dumpis et al.* 2010).

Hence, the aim of our study was to determine the antibiotic susceptibility and prevalence of ESBL production in *K. pneumoniae* isolated from different clinical samples of ICU patients in three hospitals in Tehran, Iran.

Materials and Methods

Bacterial isolates and identification

A total of 116 *K. pneumoniae* isolates were collected from different clinical specimens of patients admitted to the ICUs of three hospitals in Tehran, Iran between March and December 2011. They were mostly isolated from tracheal secretions (90), urine (19), wound (5), and blood (2). Identification was based on the routin biochemical tests. The PCR method was used to detect the 16S–23S internal transcribed spacer unit of *K. pneumoniae subsp. pneumoniae*, facilitating identification of this subspecies, as described previously (*Turton et al.* 2010): Pf: 5′-ATTTGAAGAGGTTGCAAACGAT-3′ and Pr1:5′-TTCACTCTGAAGTTTTCTTGTGTTC-3′ (amplicon size: 130 bp). Cycling conditions were as follows: Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min followed by a final extension at 72°C for 7 min. *K. pneumoniae* ATCC13883 was used as positive control.

Antibiotic susceptibility testing

The antibiotic susceptibility was determined by disk diffusion method on Mueller-Hinton agar plates (Merck, Darmstadt, Germany) as recommended by the Clinical Laboratory Standards Institute (CLSI) (CLSI 2010). The disks containing the following antibiotics were used (Mast, UK): cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), amoxicillin-clavulanic acid (30 µg), aztreonam (30 µg), ciprofloxacin (5 µg), tobramycin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), gentamicin (10 µg), cefepime (30 µg), cefoxitin (30 µg), and amikacin (30 µg). *E. coli* ATCC 25922 was used as quality control for antimicrobial susceptibility test.

ESBL screening and confirmation by phenotypic method

The isolates showing reduced susceptibility to ceftazidime or cefotaxime or both were tested for ESBL production by the combination disk method according to CLSI guidelines (CLSI 2010). Combination disk method was performed using four disks: cefotaxime (CTX) (30 µg), cefotaxime + clavulanic acid (10 µg), ceftazidime (CAZ) (30 µg), and ceftazidime +clavulanic acid (10 µg). The inoculum and incubation conditions were the same as for standard disk diffusion recommendations. A ≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive.

Quality control for the production of ESBL was performed using *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 as negative and positive controls, respectively.

Results

The *K. pneumoniae* Pf/*K. pneumoniae* Pr1 primer pair was used for identification and confirmation of *K. pneumoniae* on the basis of 16S–23S internal transcribed spacer region. All *K. pneumoniae* isolates positive for biochemical tests also showed positive PCR results for the 16S–23S internal transcribed spacer region.

Analysis of the antimicrobial susceptibility profile of the isolates showed that all (except one isolate) were susceptible to imipenem. This isolate showed resistance to other tested antibiotics. Of 116 isolates, 64% of the isolates were resistant to amoxicillin-clavulanic acid and tobramycin (n= 74), 63% were resistant to cefotaxime and ceftriaxone (n=73), 61% were resistant to aztreonam (n= 71), and 60% were resistant to ceftazidime and gentamicin (n= 70) (Table 1).

Table 1. Antimicrobial resistance of clinical isolates of *Klebsiella pneumoniae* [a]

Antimicrobial resistance trait	No. (%) of isolates
Amoxicillin-clavulanic acid	74(64)
Tobramycin	74(64)
Cefotaxime	73 (63)
Ceftriaxone	73 (63)
Aztreonam	71(61)
Gentamicin	70 (60)
Ceftazidime	70 (60)
Trimethoprim-sulfamethoxazole	67(58)
Cefepime	61 (52.5)
Ciprofloxacin	61 (52.5)
Amikacin	43(37)
Tetracycline	24 (20)
Cefoxitin	8 (7)
Imipenem	1 (0.8)

a Shown are the numbers and percentages of isolates exhibiting resistance to the tested antimicrobials.

Among the 116 *K. pneumoniae* isolated from ICU patients, 66 isolates showed simultaneous resistance to 7 antibiotics [(amoxicillin-clavulanic acid, tobramycin, cefotaxime, ceftriaxone, aztreonam, ceftazidime, gentamicin (57%)]. The phenotypic confirmatory test detected 70 from 116 isolates (60%) as ESBL producer. ESBL isolates demonstrated higher degree of multidrug resistance as compared with non-ESBLs.

Discussion

Multidrug resistant and ESBL-producing *Klebsiella pneumoniae* have been increasingly responsible for infections among the patients admitted to the ICU in many countries (*Jain et al.* 2003; *Patterson* 2010; *Girish et al.* 2012). ICU patients are ideal reservoirs for multidrug resistant *K. pneumoniae*, presumably because in these areas, patients are exposed to a considerable number of potential risk factors for colonization or infection (*Asensio et al.* 2000). Problems associated with ESBL producing isolates include multidrug resistance, difficulty in detection and treatment, and increased mortality of patients (*Alipourfard and Yeasmin* 2010).

In the present study, among the 116 *K. pneumoniae* isolates collected from ICU patients, 66 isolates were simultaneously resistant to 7 antibiotics (57%). Of these 116 isolates, the 70 isolates showed ESBL production (60%). All of the 116 isolates (except one isolate) showed susceptibility to imipenem .

Mirsalehian and colleagues (2007) reported the prevalence of ESBL producing *K. pneumoniae* isolated from ICU of three hospitals in Tehran, Iran was 76% (*Mirsalehian et al.* 2007). In another study high level of resistance was observed for third generation cephalosporins in *Klebsiella* (92%) isolated from ICU patients; however, microorganisms were less resistant to imipenem and cefepime (*Talebi Taher et al.* 2009).

Mobin and colleagues (2007) reported that 90% of *K. pneumoniae* isolated from ICU of Children's Hospital in Tabriz, Iran were ESBL producers (*Mobin et al.* 2007). The antibiotic susceptibility tests showed that among 16 tested antibiotics, the highest resistance rate

was seen for carbenicillin and ciprofloxacin was the most effective antibiotic.

In recent years, a significant increase in ESBL-producing *Klebsiella* spp. was also reported from USA 4.2–44%, Canada 4.9%, Spain 20.8%, Taiwan 28.4%, Turkey 78.6%, Algeria 20%, and China 51% (*Sarojamma and Ramakrishna* 2011). The use of third-generation cephalosporins is an important risk factor for the development of ESBL-producing organisms. Of all available anti-microbial agents, carbapenemes are the most effective and reliable treatment options for infections caused by ESBL producing isolates. Hence, decreasing the use of third-generation cephalosporins and increasing the use of imipenem, should significantly decrease the prevalence of ESBL-producing bacteria. However, overuse of carbapenemes may lead to resistance of other Gram-negative organisms (*Alipourfard and Yeasmin Nili* 2010). Therefore, restricting the use of third-generation cephalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL producing isolates.

In conclusion, our study highlights the prevalence of ESBL-producing *K. pneumoniae* in patients admitted to ICU in the hospitals in Tehran, Iran. There is a need to emphasize on the rational use of antimicrobials to decrease the spread of ESBL producing bacteria.

Acknowledgement

This study was supported by a grant from Tarbiat Modares University, Faculty of Medical Sciences, Tehran, Iran.

References

Alipourfard I, Yeasmin Nili N. Antibiogram of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospital samples. *Bangladesh J Med Microbiol* 2010; 4:32-6.

Asensio A, Oliver A, González-Diego P, Baquero F, Pérez-Díaz JC, Ros P, et al. Outbreak of a multiresistant *Klebsiella pneumoniae* strain in an intensive care unit: antibiotic use as risk factor for colonization and infection. *Clin Infect Dis* 2000; 30:55-60.

Bonnet R, Sampaio JLM, Chana LC. A novel class A extended spectrum β-lactamase (BES-1) in *Serratia marcescens* isolated in Brazil. *Antimicrob Agents Chemother* 2000; 44:3061–8.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 20th informational supplement (M100-S20). Volume 30, No. 1. Wayne Pa: Clinical and Laboratory Standards Institute; 2010.

Dumpis U, Iversen A, Balode A, Saule M, Miklasevics E, Giske CG. Outbreak of CTX-M-15-producing *Klebsiella pneumoniae* of sequence type 199 in a Latvian teaching hospital. *APMIS* 2010; 118:713–6.

Girish N, Saileela K, Mohanty SK. Extended-Spectrum β-Lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* in Neonatal Intensive Care Unit. *J Bacteriol Parasitol* 2012; 3:141.

Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended spectrum β lactamase producing Gram-negative bacteria in septicemic neonates in a tertiary care hospital. *J Med Microbiol* 2003; 52:421-5.

Mirsalehian A, Nakhjavani F, Peymani A, Jabal Ameli F, Mirafshar SM, Hamidian M. Frequency of extended spectrum β-Lactamase producing Enterobacteriaceae in intensive care units. *Tehran University Medical Journal* 2007; 65:33-8.

Mobin H, Nahaie MR, Amir mozafari N, Sadeghi J, Rasouli M. *Enterobacteriaceae* producing extended-spectrum β-Lactamases (ESBL) and plasmid patterns in the intensive care unit of Children's Hospital in Tabriz. *Med J Tabriz Univ Med Sci* 2007; 28:95-101. Muzaheed, Doi Y, Adams-Haduch JM, Shivannavar CT, Paterson DL, Gaddad SM. Faecal carriage of CTX-M-15-producing *Klebsiella pneumoniae* in patients with acute gastroenteritis. *Indian J Med Res* 2009; 129:599-602.

Patterson JE. Extended spectrum β-lactamases: A therapeutic dilemma. *Pediatr Infect Dis J* 2002; 21: 957-9.

Roopa TJ, Sudha SS. Antimicrobial susceptibility of Extended Spectrum β-Lactamase (ESBL) producing Uropathogens isolated from ICU patients. *Int J Biological Technology* 2010; 1:23-31.

Sarojamma V, Ramakrishna V. Prevalence of ESBL-producing *Klebsiella pneumoniae* isolates in tertiary care hospital. *ISRN Microbiology* 2011; 5:91-5.

Talebi Taher M, Golestanpour A. Symptomatic nosocomial urinary tract infection in ICU patients: identification of antimicrobial resistance pattern. *Iran J Clin Infect Dis* 2009; 4:25-9.

Turton JF, Perry C, Elgohari S, Hampton CV. PCR characterization and typing of *Klebsiella pnumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. *J Med Microbiol* 2010; 59:541-7.