



The Prevalence of TEM-1 gene causing resistance to beta-lactam antibiotics in *Klebsiella pneumoniae* isolates from clinical samples and plasmid curing

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ABSTRACT

Existence of extended spectrum B-lactamase (ESBL) genes plays an important role in causing B-lactam antibiotic resistance in the producing strains of these enzymes. The resistance of gram-negative bacteria, such as *Klebsiella pneumoniae*, to different antimicrobial agents, especially B-lactams and carbapenem, has increasingly been reported. This study was conducted to determine the prevalence of TEM-1 beta-lactamases in *Klebsiella pneumoniae* isolates through PCR method. In this descriptive-analytic study, 120 *Klebsiella pneumoniae* isolates collected from patients with Lung infection and UTI were subjected to bacteriological tests. The samples were cultured and identified according to standard methods. Then, frequency of the strains producing extended spectrum beta-lactamases was determined with Disk diffusion method. By using of kits, DNA was extracted and examined for the existence of TEM-1 gene by PCR. out of the 120 *Klebsiella pneumoniae* isolates, 13 (10.83 %) isolates were ESBL positive, 5.3 % of which were positive for TEM-1 B-lactamase resistance gene. Considering the increasing rate of the ESBLs producing strains, using the appropriate treatment protocol based on the antibiogram pattern of the strains is highly recommended.

Keywords: Extended-spectrum B-lactamases, ESBL, *Klebsiella pneumoniae*, TEM-1

INTRODUCTION

Klebsiella pneumoniae is one of the common causes of nosocomial infections and resistance to many antibiotics, including beta-lactams. Resistance to beta-lactam antibiotics in *Klebsiella pneumoniae* producing AmpC mainly due to chromosomal or non-enzymatic mechanisms such as changes in permeability or changes in the permeability of the outer membrane pump system is secretory. Beta-lactamase enzymes inhibitors are the most important medical agents (antibacterial) in bacteria that can hydrolyze beta-lactam band of beta lactam antibiotics connection to the purpose of preventing the emergence of resistance and will. To date, more than 340 beta lactamase enzymes have been identified. According to molecular classification based on nucleotide sequences and amino acid in the enzyme, respectively. Today, four classes (A-D) is known. Class A, C and D through mechanisms act on serine. While class B or MBL will need zinc for their activities. The majority of ESBL (Spectrum B-lactamases Extended) belong to the class of molecule A in the classification Ambler, ESBL enzymes derived from SHV and TEM related to Class A requirements. The first beta-lactamase TEM-1 plasmids in gram-negative bacteria described in the mid-sixties, TEM-1 from blood culture called (Temonera) in Greece was isolated from *E. coli*. Shortly after the separation of the beta-lactamase TEM, these enzymes were released quickly so that today around the world as the most common mechanism of resistance to beta-lactam drugs are considered in Gram-negative bacilli.

Due to the prevalence of antibiotic resistance in hospitals is abundant. However, the most common causes of nosocomial *Klebsiella pneumoniae* and the bacterium has a high resistance to a wide range of antibiotics is available. Check β -lactamase production as the main way of expressing resistance to beta-lactams (the most widely used antibiotics) can be considered decent view of the resistance situation in a drawing area. The aim of this study was to determine beta lactamase TEM-1 gene in *Klebsiella pneumoniae* by PCR is isolated in a hospital.

B-lactamases

B-lactamases have been recognized as the main defense of Gram Negative bacteria against antibiotics (1). B-lactamases are divided into four groups according to Bush-Jacoby, Amnler (2).

Beta-lactamases identified in Iran:

In the previous studies conducted in Iran, TEM, SHV and PER enzymes were diagnosed with phenotype methods and the gene which can produce them can be identified with molecular methods. Figure 2 shows distribution of Beta-lactamase genes in Iran.

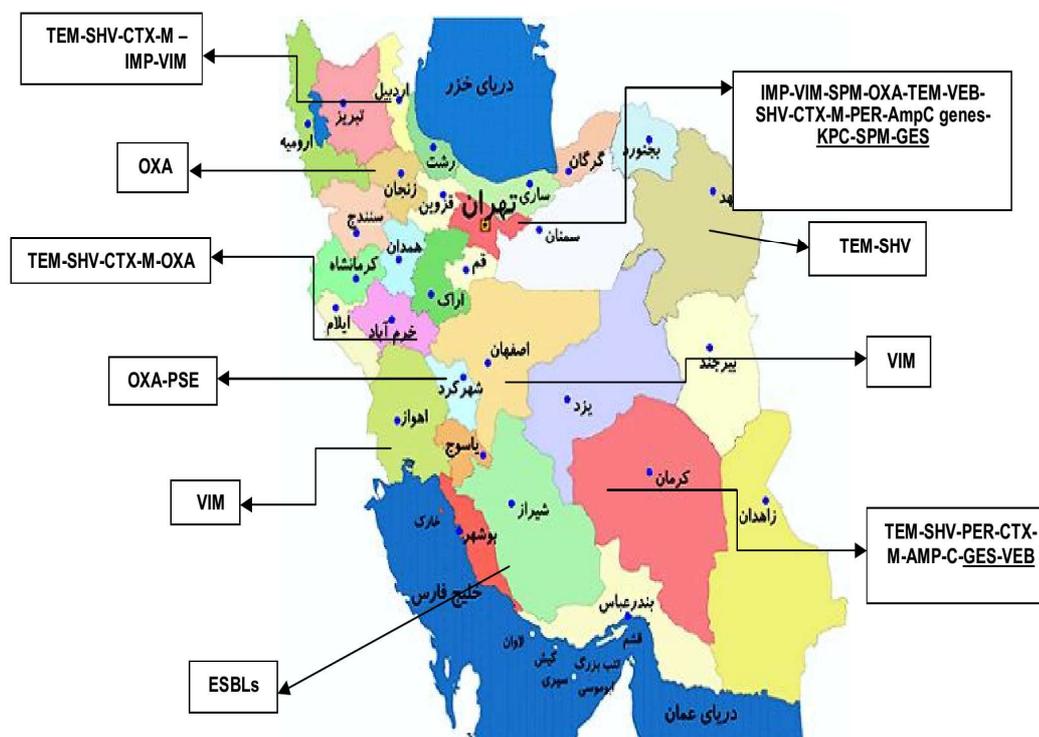


Figure 1- Distribution of Beta-lactamase genes in Iran

MATERIALS AND METHODS

In this study, there were 120 isolates of klebsiella pneumonia from the clinical samples including urine, mucus and blood based on creation of Eosin methylen-blueagar medium (EMB) and also differential tests and non-fermentative condition in TSI(Triple Sugar Iron Agar) medium . Isolates of klebsiella pneumonia were studied based on Disk Diffusion method for the presence of extended-spectrum Beta-lactamase. In this method, 11 antibiotic disks including cefoxitin(30 µg) ,Ceftriaxone(30 µg), Colistin(10 µg), Meropenem(10 µg), Imipenem(10 µg), gentamicin(10 µg),Ampicillin(10 µg),Ciprofloxacin(5 µg), Fosfomycin(200 µg),Piperacillin(100 µg) and Amoxicillin (25 µg), prepared from Padtan Teb Company were placed in Mueller hintonagar medium in distance of 15 mm . In case the inhibition zone diameter around the antibiotic disk particularly imipenem and meropenem disks exceeds 5 mm, its resistant bacteria is regarded as antibiotic and is a part of bacteria producing extended-spectrum Beta-lactamase. Percentage of each of the antibiotics is given in Table 2. We studied the isolates which were identified as the strain producing extended-spectrum Beta-lactamase in this stage with PCR method for the presence of TEM-1 gene.

For this purpose, one colony of the resistant bacteria was placed in 5 ml of the sterile TSB culture medium containing 50 to 100 µg/ml of ampicilin antibiotic (for protection of plasmid)and was cultured for 24 hours and then the resistant bacteria were isolated with Plasmid extraction kit of Sina Clon Company and then plasmid extraction is done for 13 isolates with electrophoresis loading buffer for more assurance.

Now, PCR (polymeras chain reaction) test was used to determine TEM-1 genotype. In this method, a specific primer synthesized by Sina Clon Company relating toTEM-1 gene has been used in Klebsiella pneumonia bacteria and its sequence and temperatures used inPCR are given in Table3(3).

Table 2: resistance percent of antibiotics

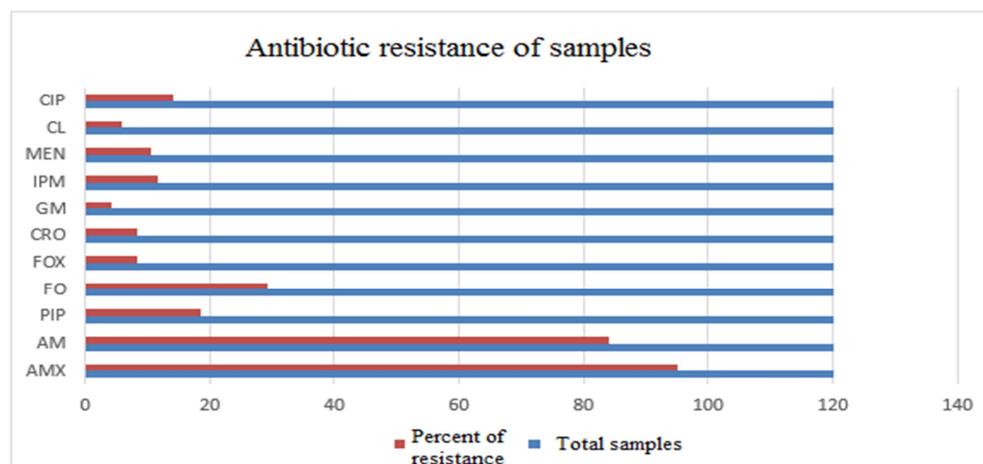


Table 3- sequence of primer and temperatures used in PCR

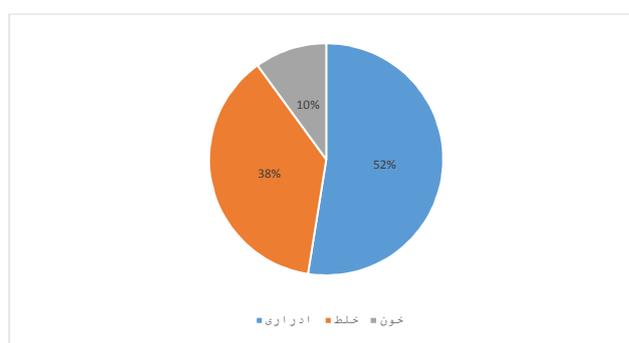
Gene (base pair)	Number of cycle	Stages of reaction PCR			Sequence of primer	Primer
		extension	Annealing	Denaturation		
1079bp	30	72C	52C	94C	ATA AAA TTC TTG AAG ACG AAA	TEM-1-(F)
		30 sec	30 sec	30 sec	GACAGTTAC CAA TGC TTA ATC A	TEM-1-(R)

After performing PCR, plasmid curing test has been done for studying the presence of Beta-lactamase genes. Plasmid curing is the process which loses its plasmid with different compounds such as ethidium-bromide or acridine orange or with physical conditions such as high temperatures and by performing centrifuge. For this purpose, antibiogram was prepared from the resistant bacteria to ensure that our bacteria contain plasmid and antibiotic resistant genes. Then, 400 µg/ml was added to TSB culture medium which contains resistant bacteria with ethidium bromide poisonous paint which is a very poisonous matter and cultured for 24 hours at 35 to 37 °C. After 24 hours, antibiogram is done with sterile cotton swap on Mueller hinton agar medium and heated for 18 to 24 hours at 35 to 37°C and we can see the results. In case the bacteria lose its plasmid, bacteria will be found sensitive to antibiotics and inhibition zone will be created around antibiotic disks.

Findings:

In this study, 120 *klebsiella pneumonia* isolates collected from different clinical samples including 63 urinary samples, 45 mucus samples, and 12 blood samples are shown in Figure 3.

Figure 2- percent of different clinical samples



After 13 isolates (10.83%) of extended-spectrum Beta-lactamase were positive after determining 120 isolates from the clinical samples with disk diffusion method and 107 isolates (89.17%) were reported as negative phenotype. confirmatory phenotypical test with disk diffusion method Assessment of PCR results among 13 strains of extended-spectrum Beta-lactamase shows that 6 isolates (5.3%) have TEM-1 gene.



Figure 3: PCR of 5 samples ; Visible bands with 1080 bp size were seen in positive samples in comparison with DNA Ladder

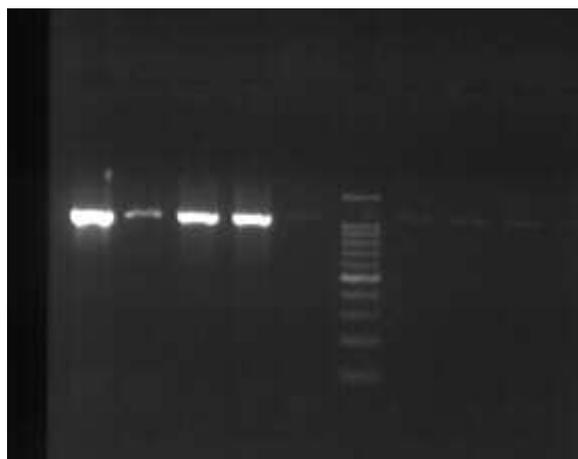


Figure 4: PCR of 8 samples; Visible bands with 1080 bp size were seen in positive samples in comparison with DNA Ladder

After obtaining PCR results , plasmid curing test was performed and the related bacteria which had plasmid containing extended-spectrum Beta-lactamase genes lost its plasmid and had become sensitive to all Beta-Lactam antibiotics.

DISCUSSION

In this study, 120 *klebsiella pneumonia* isolates were studied. Among them, there were 63 isolates from urinary culture, 14 isolates from mucus culture and 12 isolates from blood culture.

In this study, 10.83% of the isolated strains containing(TEM-1) gene, produced extended-spectrum Beta-lactamase. The detection rate of broad-spectrum beta lactamase phenotype of *P. aeruginosa* strains isolated in 2001 in Thailand Chanavng and colleagues in the study, 20.6% were reported(4). In the study by Li and colleagues in Korea in 2005, 25.4 percent were positive for ESBL(5).In 2006 in Bolivia and colleagues in a study conducted by Slnza 23.6% were positive for *Pseudomonas aeruginosa* strains ESBL phenotype (6).In a survey conducted by Jiang and colleagues in 2006, 45.33 percent in China and also in Iran Mir Salehian study in 2008, 40 percent were positive for *Pseudomonas aeruginosa* strains broad-spectrum (7,8).Also, another research by the Dr. Shabani and his colleagues in Damghan town of Iran in 2012, It was found that of 70 samples of *E.coli*, beta-lactamase enzyme producing by 27 samples and 10 samples (37/04) have TEM-1 gene (3).

CONCLUSION

Considering importance of *klebsiella pneumonia* in nasocomial infections and also high prevalence of strains containing producing extended-spectrum Beta-lactamasegenes particularly TEM-1should be used rapid diagnostic methods for determination of these strains in laboratories routinely, because these results can be regarded as a strategy for physicians to use extended-spectrum cephalosporins in treatment of this infections.

REFERENCES

- [1]Galas M, Decousser J, Breton N, Godard T, Allouch P, Pina P, et al. Nationwide study of the prevalence, characteristics, and molecular epidemiology of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in France. *Antimicrob Agents Chemother.* 2008 Feb; 52(2):786-9.
- [2]Yan J, Tsai S, Chuang C, Wu J. OXA-type beta-lactamases among extended-spectrum cephalosporin-resistant *Pseudomonas aeruginosa* isolates in a university hospital in southern Taiwan. *J Microbiol Immunol Infect.* 2006 Apr; 39(2):130-4.
- [3]Shabani A. A, Ebrahimi M, Aghaei, S. S., Nasr, R. Prevalence of TEM-1 gene in *Escherichia coli* isolated from clinical specimens in Damghan, 2012 ; *Zist Fanavary J.* 3(11); 15-22.
- [4]Chanawong A, M'Zali F, Heritage J, Lulitanond A, Hawkey P. SHV-12, SHV-5, SHV-2a and VEB-1 extended-spectrum beta-lactamases in Gram-negative bacteria isolated in a university hospital in Thailand. *J Antimicrob Chemother.* 2001 Dec; 48(6): 839-52
- [5]Lee S, Park Y, Kim M, Lee H, Han K, Kang C, et al. Prevalence of Ambler class A and D beta-lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. *J Antimicrob Chemother.* 2005 Jul;56(1):122-7.
- [6]Celenza G, Pellegrini C, Caccamo M, Segatore B, Amicosante G, Perilli M. Spread of blaCTX-M-type and blaPER-2 beta-lactamase genes in clinical isolates from Bolivian hospitals. *Journal of Antimicrobial Chemotherapy.* 2006 May;57(5):975-8.
- [7]Mirsalehian A, Feyzabadi M, Akbari Nakhjavani F, Jabal Ameli F. Prevalence of extended spectrum beta lactamases among strains of *pseudomonas aeruginosa* isolated from burn patients. *Tehran University Medical Journal (TUMJ).* 2008;66(5):333-7.
- [8]Jiang X, Zhang Z, Li M, Zhou D, Ruan F, Lu Y. Detection of extended-spectrum beta-lactamases in clinical isolates of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2006 Sep; 50(9):2990-5.