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Full Length Research Paper

Occurrence of *Klebsiella oxytoca* producing extended-spectrum beta-lactamases in different seasons in Ilam Hospitals, Iran

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Our study focused on assessing frequency of *Klebsiella oxytoca*, to study incidence of *K. oxytoca* producing ESBLs, to investigate frequency of blaTEM,blaSHV and blaCTX-M and to determine resistance of *K. oxytoca* producing ESBLs toward non-beta-lactam antibiotics, in different seasons in Ilam hospital. Twelve clinical isolates of *K. oxytoca* were found during March 2007 to April 2008 in Ilam hospital in Ilam city in west of Iran. The strains were isolated from admitted patients in surgery wards, lesion and respiratory tract infection. ESBLs identified by phenotypic and genotypic methods. PCR had done for detection of blaSHV, TEM and CTX-M. *K. oxytoca* producing ESBLS were evaluated against non-beta-lactam antibiotics.of twelve *K. oxytoca* collected in Ilam hospitals, 16.67% (n=2), 16.67% (n=2) and 66.66% (n=8) were from the surgery wards, lesion, and respiratory tract infections (RTIs), respectively. 25% of *K. oxytoca* were ESBLs positive. blaSHV were found as responsible for ESBLs production. All the *K. oxytoca* producing ESBLs were susceptible to non-beta-lactam antibiotics. The highest frequency of *K. oxytoca* were found from patients with RTI (66.7%) and the lowest frequency of *K. oxytoca* had observed in admitted patients in surgery ward and patients with lesion infections, as an equal (16.6%). Our finding showed that resistant to ceftazidime was more than the others antibiotics (41.66%). We were found the most ESBLs production occurred in winter in *K. oxytoca* isolated in surgery ward (50%).

Key word: *K. oxytoca,* ESBLs, Ilam Hospitals, Iran.

INTRODUCTION

The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases. The first report of plasmid-encoded beta-lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 (Knothe et al., 1983). The gene encoding the beta-lactamase showed a mutation of a single nucelotide compared to the gene encoding SHV-1. Other closely related to TEM-1 and TEM-2 beta-lactamases which had the ability to confer resistance to the extended-spectrum cephalosporins were soon discovered (Sirot et al., 1987; Brun-Buisson et al., 1987). However, these new beta-lactamases were coined ESBLs. The total number of characterized ESBLs, for the

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Abbreviations: ESBL, Extended spectrum beta-lactamases; ***Klebsiella spp,*** *Klebsiella* species; **RTI,** respiratory tract infection; ***K. oxytoca,*** *Klebsiella oxytoca.*

Table 1. Primers for PCR of *K. oxytoca*.

Primers	Sequence of primers	Size of amplicon (bp)	References
blaTEM	F: 5-GAGTATCAACATTTCCGTGTC-3 R: 5-TAATCAGTGAGGCACCTTCTC-3	800	Shahcheraghi et al. (2007)
blaSHV	F: 5–AAGATCCACTATCGCCCAGCAG-3 R: 5-ATTCAGTTCCGTTTCCCAGCGG-3	200	Shahcheraghi et al. (2007)
blaCTX-m	F:5-ACGCTGTTGTTAGGAAGTG-3 R:5-TTGAGGCTGGGTGAAGT-3	750	Mansouri et al. (2009)

time being, exceeds two hundred. The introduction of the third-generation of cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against beta lactamase- mediated bacterial resistance to antibiotics. Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to penicillins, cephalosporins of the first, second and third generations and aztreonam via hydrolysis of the antibiotics. ESBL are inactivated by beta-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005). Objectives of our study were focused to: Determine frequency of *Klebsiella oxytoca*; to investigate incidence of *K. oxytca* producing ESBLs; to study frequency of blaTEM,blaSHV and blaCTX-M, and resistance of *K. oxytoca* producing ESBLs toward non-beta-lactam antibiotics, in different seasons in Ilam hospital.

METHODS

Bacterial isolates

Twelve clinical isolates of *k. oxytoca* were found during March 2007 to April 2008 in Ilam hospital in Ilam city in west of Iran. The strains were isolated from admitted patients in surgery wards, patients with lesion and respiratory tract infection.

Detection of ESBL by phenotypic method

ESBL screening methods

In-vitro sensitivity testing was performed using established NCCLS procedure with ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg) and cefpodoxime (30 µg). The zone diameters were read using the revised NCCLS (National Committee for Clinical Laboratory Standards, 1998). Any zone diameter within the “grey zone” was considered a probable ESBL producing strain requiring phenotypic confirmatory testing.

Phenotypic confirmatory method

The combined disk method for phenotypic detection was utilized using cefpodoxime (30 µg), ceftazidime (30 µg) and cefotaxime (30 µg) disks, alone and in combination with clavulanic acid (10 µg) (Hi Media, India). The tests were carried out in Mueller-Hinton agar (Merck, Germany) and interpreted according to the standards established by the CLSI (Clinical and Laboratory Standards Institute) (NCCLS, 2003, 2005). An increase of more than 5 mm in the diameter of the inhibition halos around disks containing clavulanic acid as compared to the diameters of around disks free of this inhibitor indicated ESBL activity. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

Effect of non beta-lactame antibiotics against K. oxytoca. Producing ESBLs

Amikacin (30 µg), cotrimoxazol (30 µg) ciprofloxacin (30 µg), imipenem (30 µg) were used to find out resistance to non beta-lactam antibiotics in *Klebsiella spp.* Producing ESBLs (Paterson et al., 2000).

Detection of ESBL by genotypic method

Polymerase Chain Reaction (PCR) was used for detection of blaSHV, blaTEM and blaCTX-M (Table 1).

RESULTS

Of twelve *K. oxytoca*, 16.67% (n=2), 16.67% (n=2) and 66.66% (n=8) were from the surgery wards, lesion, and respiratory tract infections, respectively. Generally, resistance to ceftazidime, cefotaxime, cefteriaxone, cefpodoxime and aztreonam were 41.66, 16.66, 33.3, 25 and 25%, respectively (Table 2). Generally, 25% of *K. oxytoca* were ESBLs production.

Screening stage

Of two *K. oxytoca* isolated from the patients in the surgery wards of Ilam hospitals, 50% (n=1), 50% (n=1), 100% (n=2), 50% (n=1) and 100% (n=2) were resistant to aztreonam, cefpodoxime, cefteriaxone, cefotaxime and ceftazidime, respectively. All the isolates collected in winter. Therefore, in the screening stage in winter, 50% (n=1) of *K. oxytoca* were prone to produce ESBLs (Table 3 and Figure 1).

Table 2. 3th generations of cephalosporins and aztreonam resistance of *K.oxytoca* isolated in Ilam hospitals.

	K. oxytoca	Ceftazidime resistance	Cefotaxime resistance	Cefteriaxone resistance	Cefpodoxime resistance	Aztreonam resistance
Total	12	5 (41.66%)	2 (16.66%)	4 (33.3%)	3 (25%)	3 (25%)

Table 3. Screening stage for detection of *K.oxytoca* producing ESBLs from patients in surgery wrad in Ilam hospitals.

	K. oxytoca frompateints in surgery ward	Ceftazidime resistance	Cefotaxime resistance	Cefteriaxone resistance	Cefpodoxime resistance	Aztreonam resistance
Winter	2 (100%)	2 (100%)	1 (50%)	2 (100%)	1 (50%)	1 (50%)

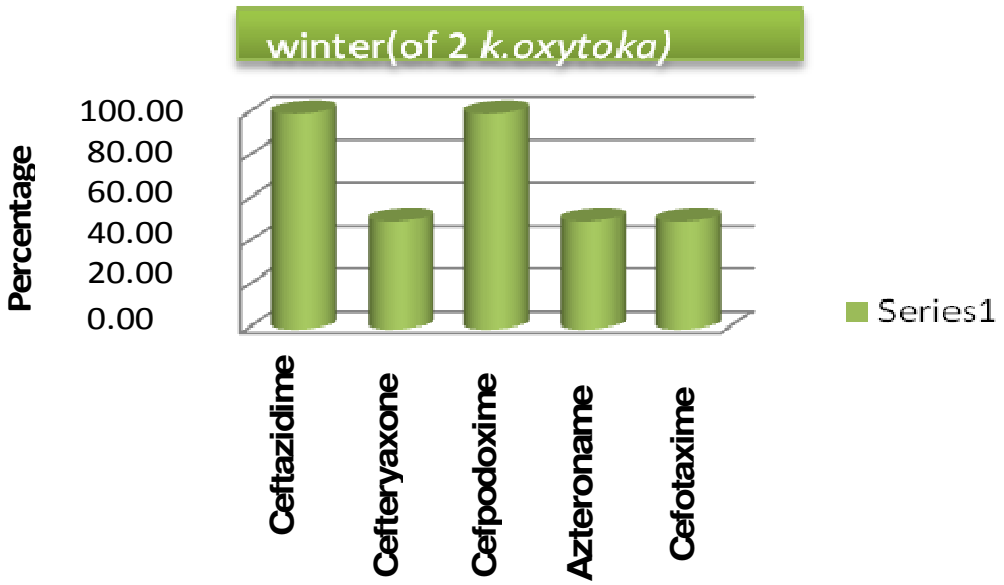

Figure 1. screening stage for *K.oxytoca* isolated from patients in surgery ward in winter.

Table 4. Screening stage for detection of *K.oxytoca* producing ESBLs from patients with lesion infection in Ilam hospitals.

	K. oxytoca	Ceftazidime resistance	cefotaxime resistance	cefteriaxone resistance	cefpodoxime resistance	aztreonam resistance
Spring	2 (100%)	0	0	0	0	0

of two *K. oxytoca* isolated from the patients with lesion infections, both obtained in spring with no sign of resistance to any sort of the antibiotics (Table 4, Figure 2).

Of eight *K. oxytoca* isolated from the patients with RTI, 32.5% (n=3), and 62.5% (n=5) were obtained in fall and winter, respectively. Of three *K. oxytoca* isolated in fall, 33.4% (n=1), 33.4% (n=1), 33.4% (n=1), and 33.4% (n=1) proved resistant to aztreonam, cefpodoxime, cefteriaxone and ceftazidime, respectively. All the isolates were susceptible to cefotaxime. Therefore, in the screening stage in fall, 33.4% (n=1) of *K. oxytoca* were prone to produce ESBLs. Of five *K. oxytoca* isolated in winter, 20% (n=1), 20% (n=1), 20% (n=1), 20% (n=1), and 40% (n=2) were resistant to aztreonam, cefpodoxime, cefteriaxone, cefotaxime and ceftazidime, respectively.

Therefore, in the screening stage in winter, 20% (n=1)

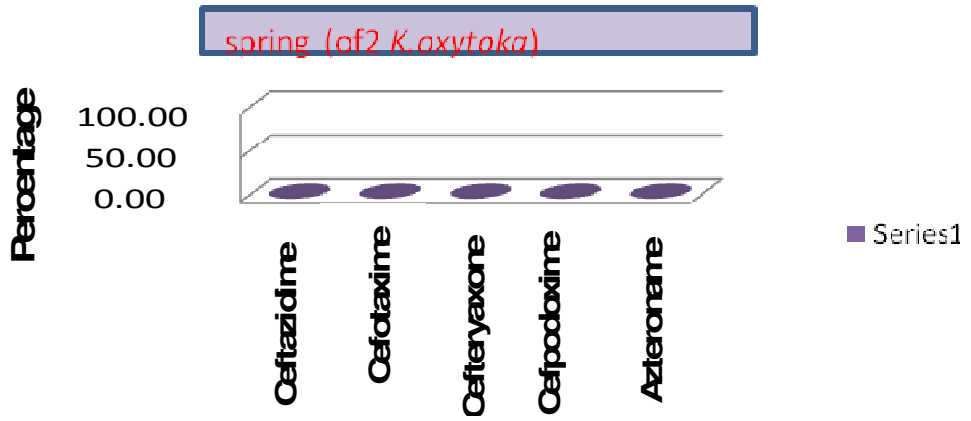

Figure 2. Screening stage for *K. oxytoca* isolated from patients with lesion infection in spring.

Table 5. Screening stage for detection of *K.oxytoca* producing ESBLs from patients with RTI in Ilam hospitals

	***K. oxytoca* frompateints with RTI**	**Ceftazidime resistance** (%)	**Cefotaxime resistance**	**Cefteriaxone resistance** (%)	**Cefpodoxime resistance** (%)	**Aztreonam resistance** (%)
Fall	3	1 (33.4)	0	1 (33.4)	1 (33.4)	1 (33.4)
Winter (%)	5 (62.5)	2 (40%)	1 (20%)	1 (20)	1 (20)	1 (20)
Total (%)	8 (100)	3 (37.5%)	1 (12.5%)	2 (25)	2 (25)	2 (25)

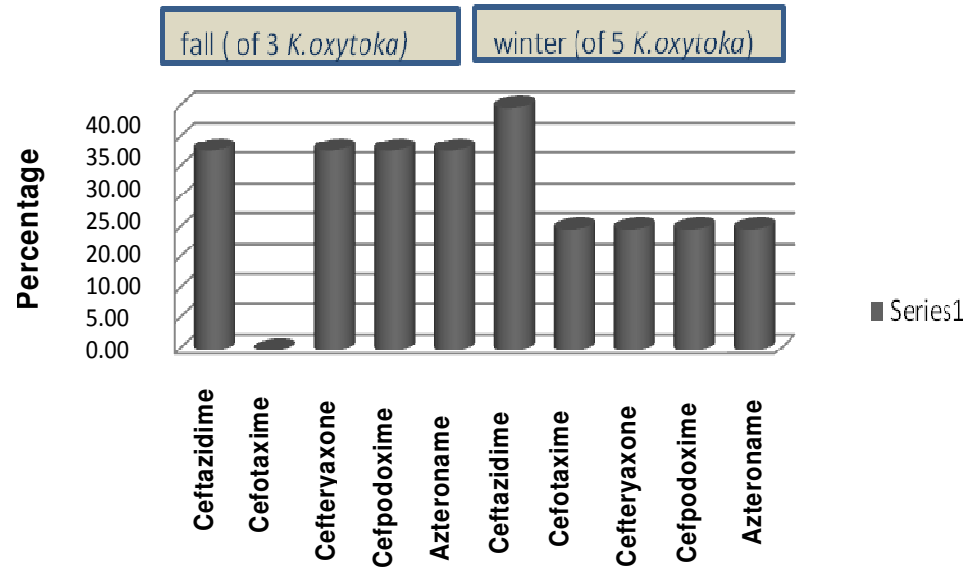

Figure 3. screening stage for *K. oxytoca* isolated from patients with RTI in fall and winter.

Of *K. oxytoca* were suspected to produce ESBLs (Table 5 and Figure 3).

Confirming stage

Of two *K. oxytoca* collected from the patients in surgery wards in winter, 50% (n=1) were suspected to produce ESBLs in winter. It confirmed by ceftazidim/clavulanic acid and cefpodoxime/clavulanic acid in the confirming stage (Table 6 and Figure 4). Of three *K. oxytoca* collected from the patients with RTI in fall, one isolate confirmed by ceftazidime/clavulanic acid and cefpodoxime/clavulanic acid in the confirming stage was

Table 6. Confirming stage and effect of non-beta-lactam antibiotics toward *K.oxytoca* producing ESBLs isolated from patients in surgery wards of Ilam hospitals.

	***K. oxytoca* suspected toproduce ESBLs**	**Ceftazidime /clavulanic acid**	**Cefotaxime /clavulanic acid**	**Cefpodoxime /clavulanic acid**	**Amikacin**	**Ciprofloxacin**	**Cotrimoxazol**	**Imipenem**
winter	1(100%)	1(100%)	0	1(100%)	0	0	0	0

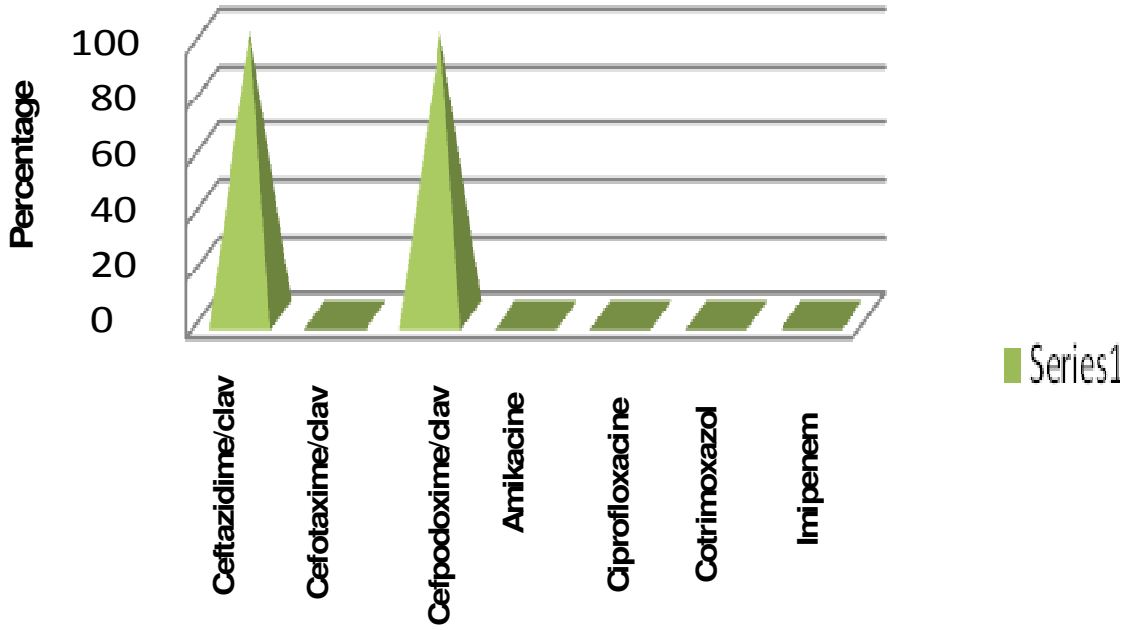

Figure 4. Confirming stage and effect of non-beta-lactam antibiotics from patients in surgery ward in winter.

respiratory tract infections in winter, one isolate confirmed by ceftazidime/clavulanic acid and cefpodoxime/clavulanic acid in the confirming stage which was suspected to produce ESBLs. Of five *K. oxytoca* collected from the patients with respiratory tract infections in winter, one isolate confirmed by ceftazidime/clavulanic acid and cefpodoxime/clavulanic acid in the confirming stage was suspected to produce ESBLs (Table 7 and Figure 5). There was no resistance demonstrated against non-beta-lactam antibiotics in *K. oxytoca* producing ESBLs.

PCR results

Of three *K. oxytoca* producing ESBLs in phenotypic stage, all of them were positive for blaSHV and negative for blaTEM and blaCTX-M (Figure 6).

DISCUSSION

Nowadays, ESBLs is considered a problem among the hospitalized patients throughout the world. The prevalence of ESBLs among the clinical isolates, which is rapidly changing over time, varies greatly and geographically worldwide. Patients suffering from infections caused by ESBL- producing organisms are at increasing risks of treatment failures with broad-spectrum beta-lactam antibiotics. Therefore, it is recommended that any organisms confirmed for ESBL production experimentally be reported as resistant to the entire broad-spectrum beta-lactam antibiotic, regardless of any susceptibility test results.

The highest frequency of *K. oxytoca* were found from patients with RTI (66.7%) and the lowest frequency of *K. oxytoca* had observed in admitted patients in surgery ward and patients with lesion infections, as an equal (16.6%).

Our finding showed resistant to ceftazidime was more than the others antibiotics (41.66%). We were found the most ESBLs production had occurred in winter in *K. oxytoca* isolated in surgery ward (50%).

The highest resistance to 3th generation of cephalosporins in admitted patients in surgery ward had occurred in ceftazidime and cefteriaxone (100%) and the lowest resistance to 3th generation of cephalosporins had

Table 7. Confirming stage and effect of non-beta-lactam antibiotics toward *K.oxytoca* producing ESBLs isolated from patients with RTI in Ilam hospitals.

	k. oxytoca suspected to produce ESBLs (%)	Ceftazidime /clavulanic acid (%)	Cefotaxime /clavulanic acid	Cefpodoxime /clavulanic acid (%)	Amikacin	Ciprofloxacin	Cotrimoxazol	Imipenem
Fall	1(50)	1 (100%)	0	1 (100%)	0	0	0	0
Winter	1(50%)	1(100%)	0	1(100%)	0	0	0	0
Total	2 (100%)	2 (100%)	0	2 (100%)	0	0	0	0

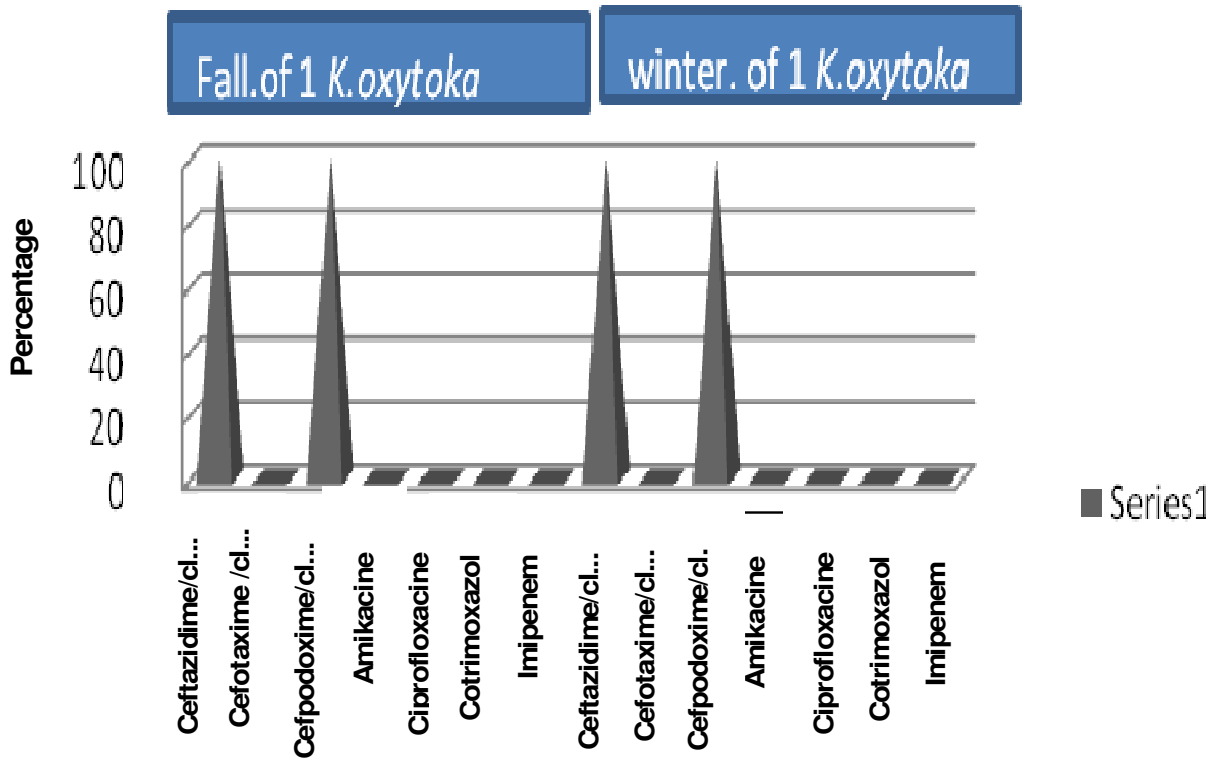

Figure 5. Confirming stage and effect of non-beta-lactam antibiotics from patients with RTI in fall and winter.

observed in cefotaxime and cefpodoxime (50%). However, all the *K. oxytoca* isolated from patients with lesion infections were susceptible to 3th generation of cephalosporins.

Interestingly, all *K. oxytoca* isolated in cold seasons (fall and winter) were ESBLs positive but *K. oxytoca* isolated in spring from patients with lesion infections were susceptible toward 3th generation of cephalosporins, thus they were not ESBLs productions. Fortunately, all *K. oxytoca* producing ESBLs were susceptible to non-beta-lactames antibiotics in our study; therefore, they were found as effective antibiotics. Only blaSHV was responsible for ESBLs productions.

Of two tertiary-care teaching hospitals in Brazil from August 2003 to August 2004, 24.1% *k. oxytoca* producing ESBLs were found by phenotypic assays (Nogueira et al., 2006).

In Makati City in Philippine, 38.5% *K. oxytoca* producing ESBLs were obtained (Villanueva et al., 2003). In 2008 in India, 33.3% of *K. oxytoca* were ESBLs positive (Bhattacharjee et al., 2008). In zil between April 2005 to September 2006, 25% *K. oxytoca* were confirmed for ESBLs production that 25% of them were blaSHV positive, too (Oliveira et al., 2010).

In Iran, no study had been done about *K. oxytoca* ESBLs production and our study was the first survey for determination of *K. oxytoca* producing ESBLs in Iran.

Our study had determined incidence of *K. oxytoca* in Iran and showed to need more study about prevalence of *K. oxytoca* producing ESBLs in all part of Iran.

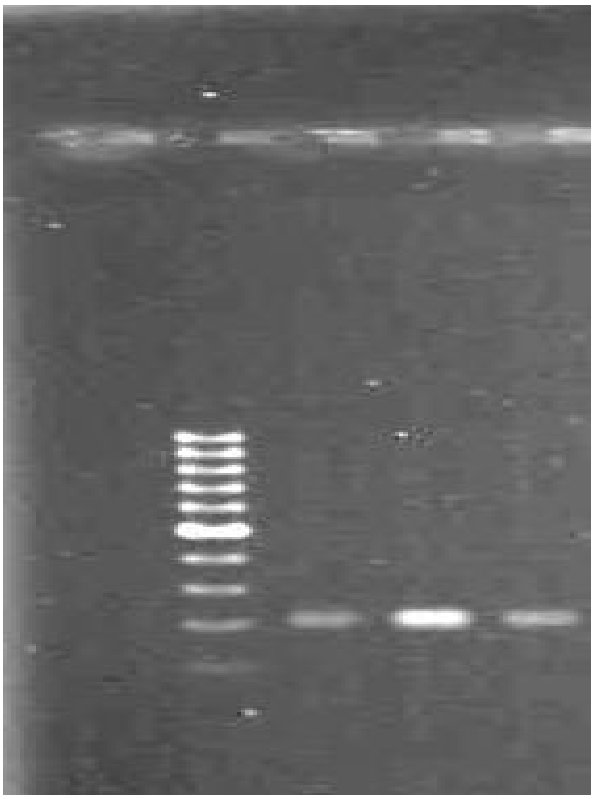

Figure 6. Left to right negative control, marker=100 bp, blaSHV=200 bp.

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