Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jopr

Original Article

A randomized, open-label, prospective, multicenter phase-III clinical trial of Elores in lower respiratory tract and urinary tract infections

*Manu Chaudhary, Anurag Payasi**

Department of Cell Culture and Molecular Biology, Venus Medicine Research Centre, Baddi, Bhatoli Kalan, H.P 173205, India

ARTICLE INFO

Article history:
Received 20 March 2013
Accepted 10 April 2013
Available online 3 May 2013

Keywords:
Antibiotic adjuvant entity
Clinical trial
Elores
Phase-III

ABSTRACT

Background: Lower respiratory tract infections (LTRIs) and urinary tract infections (UTIs) are the leading causes of death world-wide. Treatment of these infections require the use of antibiotics with enhanced activity against a broad spectrum of respiratory and urinary pathogens. This study was designed to study clinical and bacteriological efficacy as well as tolerability of ceftiaxone + disodium edtate + sulbactam (the novel Antibiotic Adjuvant Entity; Elores) in adult patients in the treatment of lower respiratory tract infections (LRTIs) and urinary tract infections (UTIs).

Methods: A randomized, open-label, multicenter study was conducted on 297 patients which included 204 in UTIs and 93 in LRTIs. A total of 148 patients were there in Elores group with 102 cases of UTIs and 46 LRTIs; 149 in ceftriaxone group with 102 cases of UTIs and 47 LRTIs. The patients received 3–10 days of treatment with Elores 3.0 g twice daily and ceftriaxone 2.0 g twice daily in two divided doses.

Results and discussion: Clinical cure rates in ITT (Intend to treat) populations of Elores were 83.33% (85/102), 91.30% (42/46) in the UTIs and LRTIs, respectively, and 34.31% (35/102), 31.91% (15/47) in the ceftriaxone group for UTIs and LRTIs, respectively. The corresponding bacterial eradication rates were 95% (57/60) and 97.05% (33/34) for Elores in the UTIs and LRTIs, respectively and 80.64% (50/62) and 71.42% (10/14) for ceftriaxone in the UTIs and LRTIs, respectively. Adverse reaction were observed in 20.59% (21/102) and 15.22% (7/46) in Elores groups of UTIs and LRTIs, respectively and 36.27% (37/102) and 31.91% (14/47) in ceftriaxone groups of UTIs and LRTIs, respectively.

Conclusions: Results obtained in the present study, together with microbiological evaluation data suggest that Elores is more effective and safe antibacterial agent for the treatment of LRTIs and UTIs infections.

1. Introduction

Lower respiratory tract infections (LTRIs) are one of the leading causes of death world-wide.[1] Urinary tract infections (UTIs) are the second most commonly found in women and it has been estimated that about one-third of adult women have experienced UTIs at least twice.[2] A variety of bacterial pathogens are responsible for LRTIs and UTIs, but the most

* *Corresponding author*. Tel.: +91 1795 302068; fax: +91 1795 302133.
E-mail address: ccmb@venusremedies.com (A. Payasi).

0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.04.011

prominent are *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter* spp., and coagulase-negative *staphylococci*.[3,4] Resistance to antibiotics has increasingly been reported in recent years and most of the pathogens have become resistant to third-generation cephalosporins.[5] Antibiotic resistance being the first cause of failure of therapy particularly in *Acinetobacter baumannii*, *P. aeruginosa*, *K. pneumoniae*, *Klebsiella oxytoca*, *E. coli*. Extended-spectrum-beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs) are the main factors for antibiotic resistance. Till date, CTX-M, TEM, SHV, KPC are the most common ESBL genes. In MBL category VIM, IMP, and NDM-1 are the most spread ones in Asian region. Recently there have been reports of failure of β-lactam and β-lactamase inhibitors (BL + BLI) combinations and even penems to these MBL producing microbes.[4] This indicates the need to develop new antimicrobial agents.

Elores (ceftriaxone + disodium edtate + sulbactam) is a unique novel antibiotic adjuvant entity which has been engineered to take care of multiple mechanisms adopted by bacteria such as overexpression of efflux pump, membrane impermeability, biofilm etc. The in vitro, preclinical and microbiological studies on this product proved it to be more effective than pencillins, cephalosporins, BL + BLI combinations and provide a strong rationale for the study.[6–9] Current study is approved by Drug Controller General of India (DCGI) and has been performed in accordance with Good Clinical Practice (GCP) guidelines. Therefore, present study was planned to observe randomized, open-label, prospective, multicenter comparison of Elores versus ceftriaxone in the treatment of LRTIs and UTIs.

2. Material and methods

The study was conducted in accordance with International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (EC-6).[10]

2.1. Patients selection

Adult patients >18 and <65 years old with signs of LRTIs and UTIs were screened for enrollment. Approximately 306 patients were enrolled with clinical evidence of LRTIs and UTIs infection in the 9 centers across India of which 297 completed the study and 9 were dropped out.

2.2. Study design and antimicrobial therapy

This was a multicenter, prospective, randomized, open-label study. Patients were randomly assigned into two groups: those receiving Elores (3.0 g twice daily) and those administered ceftriaxone (2.0 g twice daily). Both of the drugs were administered intravenous infusion for 3–10 days.

2.3. Inclusion criteria

LRTI subjects included by the presence of signs and symptoms of an acute respiratory infection (cough, nasal discharge, oropharyngeal hyperemia, with or without fever), and lower respiratory signs (tachypnea, retractions, prolonged expiratory time, or crackles/wheezing on auscultation). Subjects with diagnosis of pneumonia (either mild to severe community-acquired pneumonia (CAP) or mild to severe hospital-acquired pneumonia (HAP)), bacterial pneumonia were included. All the subjects have undergone X-ray chest. Subjects in which culture report was negative were enrolled based on radiological examination results and clinical findings of related symptoms.

UTI subjects were included with pyuria determined by a midstream clean-catch (MSCC) or catheterized (indwelling or straight catheter) urine specimen with greater than or equal to 10 white blood cells (WBCs) per high-power field (hpf) on standard examination of urine sediment or greater than or equal to 10 WBCs/mm^3 in unspun urine, suspected pyelonephritis, dysuria, complicated lower UTIs.

2.4. Exclusion criteria

Subjects with clinically significant cardiovascular, renal, hepatic, gastrointestinal conditions, neurological, psychiatric, other severely immunocompromised, hematological or malignant disease and other condition which may interfere with the assessment, history of uncontrolled diabetes mellitus, HIV and hepatitis-B were excluded. Also, subjects with history of resistance to any of the investigational drugs, history of hypersensitivity, allergic response or any contraindications to penicillin, cephalosporin or carbapenem groups of drugs, history of hearing loss and participation in any clinical study within the previous 6 month, pregnant or lactating women were excluded from LRTI groups. Additionally in UTIs, subjects with perinephritic abscess or renal corticomedullary abscess, polycystic kidney disease, only one functional kidney, chronic vesicouretheral reflux, uncomplicated UTI, previous or planned renal transplantation or cystectomy, urinary tract surgery within 7 days prior to randomization or urinary tract surgery planned during the study period (except surgery to relieve obstruction, to place a stent or nephrostomy) were excluded.

2.5. Clinical laboratory tests

All the laboratory parameters (biochemical and hematological, urine analysis) were analyzed and reviewed by the Principal investigator. In addition, Ultrasound was also done as per investigator discretion.

2.6. Microbiological investigations

Sputum, blood and urine specimens for routine culture and pathogens resistant gene characterization were obtained within 24 h prior to start of treatment. Identification of causative organisms was done according to previously reported methods[11] and susceptibility studies were conducted according to Clinical Laboratory Standard Institute.[12]

2.7. Gene characterization

A PCR assay was performed to detect ESBL and MBL encoding genes using the specific primers, namely, TEM-1, TEM-2, TEM-

50, SHV-1, SHV-10, AMP-C, NDM-1, VIM-1 and IMP-1.[13–20] All of the respective primers were obtained from Sigma Aldrich Chemicals Pvt. Ltd., Banglore, India. For PCR amplifications, about 200 pg of DNA was added to 20 μl mixture containing 0.5 mM of dNTPs, 1.25 μM of each primer and 1.5 unit of Taq polymerase (Banglore Genei) in 1× PCR buffer. Amplification was performed in an Eppendorf thermal cycler (Germany). The amplified products were separated in 1.5% agarose gel containing 2.5 μl of 10 mg/ml ethidium bromide. The gel was run at 70 V for 1 h. The gel images were taken under ultraviolet light using gel documentation system (Bio-Rad, USA). A 100 bp ladder (Banglore Genie) was used to measure the molecular weights of amplified products. The images of ethidium bromide stained DNA bands were visualized using a gel documentation system (Bio-Rad, USA).

2.8. DNA isolation

DNA isolation from clinical isolates was carried out using the alkaline lysis method.[21]

2.9. Clinical and microbiological response of drugs

Clinical response was the primary efficacy variable in this study. Patients were evaluated for clinical symptoms and physical signs of infection prior to therapy, during treatment and 5–7 days post-treatment follow-up. Clinical assessment was made on the basis of changes in symptoms and signs from the initial (pretherapy) presentation, as well as by comparing the posttherapy. Patients were categorized as cured (resolution of sign and symptoms associated with active infection), improved (continued incomplete resolution of signs and symptoms with no deterioration or relapse during the follow-up period) and failure (no response of drug).

Bacterial response to treatment was a secondary efficacy variable in this study, and its evaluation included assessment of pathogens isolated from clinical specimens of urine and sputum cultures. A culture was considered microbiologically evaluable if it was adequate and obtained at the appropriate time and if the patient was clinically evaluable. A count of 10^3 was considered as sterile. Microbiological responses after

Table 1 – Pathogens associated with clinically evaluable patients along with their antibiotic resistants.

Pathogens	Drugs (eradication/total pathogens)		Genotyping		
	UTI	LRTI	ESBL	ESBL + MBL	MBL
A. baumannii	C (3/3)	C (0/2)	SHV-1, TEM-1, TEM-1, SHV-1, TEM-1, SHV-1, KPC-2	TEM-1, SHV-1, NDM-1	–
	E (6/6)	E (8/9)	TEM-1, SHV-1, TEM-50, SHV-10, OXA-11	IMP-1, SHV-1, SHV-1, TEM-1, NDM-1, VIM-1, TEM-1, KPC-2, VIM-1	–
E. coli	C (29/36)	C (7/7)	KPC-2, SHV-1, KPC-2, TEM-50, SHV-1, TEM-50, CTXN-9, OXA-11, SHV-10	CTXM-9, NDM-1, IMP-1, SHV-1, IMP-1, SHV-1, TEM-2, IMP-1, TEM-2, NDM-1, TEM-2, IMP-1, SHV-1, TEM-1, NDM-1, VIM-1, SHV-1, KPC-1,	VIM-1, IMP-1
	E (33/36)	E (0/0)	CTXM-9, OXA-11, SHV-10, TEM-1, VIM-1, VIM-1, IMP-1, NDM-1, IMP-1,	IMP-1, SHV-1, NDM-1, SHV-1, NDM-1, SHV-1, TEM-2, TEM-1, SHV-1, VIM-1, TEM-2	
E. cloacae	E (1/1)	E (0/0)	–	TEM-2, IMP-1, NDM-1	–
P. mirabilis	C (1/1)	C (0/0)	SHV-1	–	–
	E (2/2)	E (0/0)	TEM-1, SHV-1	–	–
K. pneumoniae	C (4/4)	C (1/2)	TEM-2, SHV-1, TEM-1, KPC-2, TEM-4, CTXM-10	–	–
	E (6/6)	E (5/5)	TEM-2, SHV-1	TEM-1, SHV-1, NDM-1, VIM-1, SHV-1, KPC-1, IMP-1, SHV-1, TEM-2	–
K. oxytoca	C (0/0)	C (0/1)	SHV-1	–	VIM-1, NDM-1
	E (0/0)	E (3/3)	–	IMP-1, SHV-1, SHV-1, VIM-1, KPC-1, TEM-2, SHV-1, IMP-1	–
P. aeruginosa	C (4/4)	C (2/2)	SHV-1, TEM-1, TEM-1, SHV-1, TEM-50	–	–
	E (1/1)	E (6/6)	KPC-2, TEM-50, SHV-1, TEM-1	VIM-1, SHV-1, KPC-1	
S. aureus	C (0/0)	C (2/2)	TEM-1, TEM-1, SHV-1	–	–
	E (0/0)	E (4/4)	Bla-Z, Van-B, TEM-1, SHV-1, mec-A, TEM-2, SHV-1	–	–
Coagulase-negative	C (1/1)	C (0/0)	TEM-1	–	–
staphylococci	E (2/2)	E (0/0)	–	TEM-1, VIM-1, SHV-1, NDM-1	–
E. faecalis	C (3/5)	C (0/0)	TEM-1, TEM-1, SHV-1	–	VIM-1, IMP-1, VIM-1, NDM-1
	E (2/2)	E (0/0)	–	TEM-1, SHV-1, NDM-1, TEM-1, VIM-1	–
S. pneumoniae	E (0/0)	E (11/11)	TEM-1, SHV-1	CTXM-9, NDM-1, SHV-1, IMP-1, SHV-1, TEM-2, IMP-1, TEM-1, VIM-1	

Where C and E stand for ceftriaxone and Elores, respectively.

Table 2 – Clinical response rates at the end of therapy.

Indications	Clinical response %					
	Cure		Improved		Failed	
	Elores	Ceftriaxone	Elores	Ceftriaxone	Elores	Ceftriaxone
LRTI	91.30 (42/46)	31.91 (15/47)	8.69 (4/46)	46.80 (22/47)	(0/46)	14.89 (7/47)
UTI	83.33 (85/102)	34.31 (35/102)	15.68 (16/102)	54.90 (56/102)	(0/102)	8.8 (9/102)

completion of therapy were defined as eradication (admission pathogens were absent), negative (inability to produce a colony) and failure (admission pathogens were failed to produce response against drug). Superinfection was defined as a new infection causing organisms, found at any site during therapy which required a change in antimicrobial therapy.

2.10. *Safety evaluations*

All patients who received at least one dose of the study drug were evaluated for drug safety. Adverse events were categorized by the investigators according to their intensity (mild, moderate or marked) and their relationship to the study drug.

2.11. *Statistical analysis*

The continuous variables were summarized by using *N*, mean, standard deviation, median and range. Categorical variables were summarized by using frequency distributions and percentages. The intention to treat population was included all subjects who were enrolled, dosed with the investigational product (minimum duration of treatment should be three days).

3. Results

3.1. *Patient selection*

The study included 297 patients enrolled at 9 centers: 148 were treated with Elores (102 cases of UTIs and 46 LRTIs) and 149 were treated with ceftriaxone (102 cases of UTIs and 47 LRTIs). The demographic characteristics of both groups were comparable (data not shown).

3.2. *Study design and antimicrobial therapy*

Patients were randomly assigned into two groups: Elores (3.0 g BID) and ceftriaxone (2.0 g BID) IV in patients with LRTIs and UTIs. The mean total duration of treatment for both treatment groups was 5–10 days.

3.3. *Clinical laboratory tests*

There were no significant changes in the hematological as well as biochemical parameters before and at the end of therapy (data not shown).

3.4. *Microbiological investigations*

The details of pathogens obtained from patients along with their characterization is shown in Table 1. A total of one hundred and seventy bacterial pathogens were isolated among which gram-negative bacteria were predominant (80.58%, 137/170) followed by gram-positive 19.41% (33/170). Out of which *E. coli* were 46.47% (79/170), followed by *A. baumanni* 11.76% (20/170), *K. pneumoniae* 10% (17/170), *P. aeruginosa* 7.64% (13/170), *K. oxytoca* 2.3% (4/170) prevailed in both LRTIs and UTIs, *P. mirabilis* 1.76% (3/170) and *E. cloacae* 0.6% (1/170) from UTI only. Gram-positive pathogens were mainly *S. pneumoniae* 10% (17/170) from both LRTIs and UTIs samples followed by *E. faecalis* 4.11% (7/170), *S. aureus* 3.52% (6/170) and coagulase-negative *staphylococci* 1.76% (3/170) from UTIs only.

3.5. *Clinical evaluation*

Elores eradicated all gram-positive and gram-negative organisms except 4 pathogens, one *A. baumannii* recovered from

Table 3 – Adverse events observed during study in patients with LRTIs.

Based on system organ class			Based on severity			Based on casual relationship		
System organ class	Elores ($N = 46$)	Ceftriaxone ($N = 47$)	Severity	Elores ($N = 46$)	Ceftriaxone ($N = 47$)	Casual relationship	Elores ($N = 46$)	Ceftriaxone ($N = 47$)
Ear and labyrinth disorders	0 (0%)	1 (2.13%)	Mild	7 (15.22%)	1 (2.13%)	Definitely	1 (2.17%)	3 (6.38%)
Gastrointestinal disorders	3 (6.52%)	4 (6.38%)	Moderate	0 (0%)	13 (27.66%)	Possibly	2 (4.34%)	2 (4.25%)
General disorders and administration site conditions	1 (2.17%)	8 (17.02%)	Total	7 (15.22%)	14 (29.78%)	Probably	1 (2.17%)	2 (4.25%)
Nervous system disorders	3 (6.52%)	2 (4.26%)				Unlikely	1 (2.17%)	2 (4.25%)
Total	7 (15.22%)	14 (29.79%)				Unrelated	2 (4.34%)	6 (12.76%)
						Total	7 (15.22%)	14 (29.79%)

Table 4 – Adverse events observed during study in patients with UTI.

Based on system organ class			Based on severity			Based on casual relationship		
System organ class	Elores (N = 102)	Ceftriaxone (N = 102)	Severity	Elores (N = 102)	Ceftriaxone (N = 102)	Casual relationship	Elores (N = 102)	Ceftriaxone (N = 102)
Gastrointestinal disorders	3 (2.94%)	7 (6.86%)	Mild	16 (15.69%)	26 (25.49%)	Definitely	8 (7.84%)	14 (13.72%)
			Moderate	5 (4.90%)	11 (10.78%)	Possibly	5 (4.90%)	6 (5.88%)
General disorders and administration site conditions	16 (15.69%)	27 (26.47%)	Total	21 (20.59%)	37 (36.27%)	Probably	3 (2.94%)	5 (4.90%)
Nervous system disorders	2 (1.96%)	3 (2.94%)				Unlikely	1 (0.98%)	5 (4.90%)
Total	21 (20.59%)	37 (36.27%)				Unrelated	4 (3.92%)	7 (6.86%)
						Total	21 (20.59%)	37 (36.27%)

LRTIs and 3 *E. coli* recovered from UTIs. Contrary to this, ceftriaxone failed to eradicate 16 pathogens, 2 of *A. baumannii* (recovered from LRTIs), 7 of *E. coli* (recovered from UTI), 2 each of *E. faecalis* and *S. pneumoniae* obtained from UTIs and one each of *K. pneumoniae*, *K. oxytoca* (recovered from LRTI) and *P. mirabilis* (recovered from UTIs).

In UTIs, the bacterial eradications rates were 95% (57/60) and 80.64% (50/62) for Elores and ceftriaxone, respectively and bacteriological failure rates were 5% (3/60) and 19.37% (12/62), for Elores and ceftriaxone, respectively. Similarly for LRTIs, the bacterial eradication rates were 97.05% (33/34) and 71.42% for Elores and ceftriaxone, respectively, and bacteriological failure rates were 2.94% (1/34) and 28.57% (4/14) for Elores and ceftriaxone, respectively.

In UTIS, the clinical cure rates were 83.33% (85/102) and 34.31% (35/102) for Elores and ceftriaxone, respectively. Similarly for LRTI, the clinical cure rates were 91.30% (42/46) and 31.91% (15/47) for Elores and ceftriaxone, respectively, suggesting that Elores is superior than ceftriaxone. In UTIs, 6.86% (7/102) and 8.8% (9/102) patients were failed to respond to Elores and ceftriaxone, respectively. In LRTI, 100% (91.3% cured and 8.69% improved) and 4.89% (7/47) patients of failed to respond to Elores (Table 2). Approximately, 20.59% (21/102) and 15.22% (7/46) for Elores in the UTIs and LRTIs, respectively compared to 36.27% (37/102) and 31.91% (15/47) of the patients for ceftriaxone in the UTIs and LRTIs, respectively were experienced at least one adverse reactions (Tables 3 and 4).

4. Discussion

Treatment of patients with LRTIs and UTIs represents a significant therapeutic challenge since these patients often have multiple underlying risk factors. The prime objective of this study was to compare clinical and bacteriological efficacy of Elores compared with ceftriaxone. Most of infections are caused by gram-negative bacteria. 58.8% (100/170) in UTI and 22.35% (38/170) in LRTI.

Overall, clinical cure rate was high in the group of patients treated with Elores in comparison to ceftriaxone. The enhanced susceptibility of Elores (ceftriaxone plus EDTA plus sulbactam) against gram-positive and gram-negative organisms are likely to be associated with synergistic activity of ceftriaxone plus sulbactam plus disodium edetate. Disodium edetate chelates the divalent ions required for the activity of MBLs and alters the outer membrane permeability which in turn increased penetration of drug inside the bacterial cells, thus enhancing the susceptibility of Elores.[22,23]

It is important to mention here Elores was resistant only to those strains which were positive with TEM-50, OXA-11 and CTXM-9, whereas ceftriaxone was resistant to all isolates which were positive with MBL genes including NDM-1, VIM-1, KPC-2, IMP-1 and ESBL genes such as TEM-50, SHV-10, OXA-11 and CTXM-9. However, Elores appeared to be highly susceptible to all isolates positive with MBL genes NDM-1, VIM-1, KPC-2, IMP-1.

5. Conclusion

Results obtained in the present study, together with microbiological evaluation study suggest that Elores should be considered as antibacterial agents for the treatment of LRTI and UTI caused by these organisms.

Conflicts of interest

All authors have none to declare.

Acknowledgments

Authors are thankful to sponsor, Venus Medicine Research Center India and Germany, for providing assistance to carry out this study. Also thanks to all investigators, centers and patients who participated in the study.

REFERENCES

1. World Health Organization. *The 10 Leading Causes of Death by Broad Income Group*; 2008.
2. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. *Int J Gen Med*. 2011;4:333–343.
3. Numazaki K, Chiba S, Umetsu M, et al. Etiological agents of lower respiratory tract infections in Japanese children. *In Vivo*. 2004;18:67–71.

4. Horvath DJ, Dabdoub SM, Li B, VanderBrink BA, Justice SS. New paradigms of urinary tract infections: implications for patient management. *Indian J Urol*. 2012;28:154–158.
5. Carlet J, Jarlier V, Harbarth S, Voss A, Goossens Herman, Pittet D, the Participants of the 3rd World Healthcare-Associated Infections Forum. Ready for a world without antibiotics? The Pensières antibiotic resistance call to action. *Antimicrob Resist Infect Control*. 2012;1:11.
6. Chaudhary M, Payasi A. Disodium edtate as a conjugation inhibitor: a novel approach to curb growing conjugal transfer of vanA in gram positive bacteria. *J Pharm Res*. 2013;7:70–74.
7. Chaudhary M, Payasi A. Rising antimicrobial resistance of *Pseudomonas aeruginosa* isolated from clinical specimens in India. *J Proteomics Bioinform*. 2013;6:005–009.
8. Chaudhary M, Payasi A. Prospective study for antimicrobial susceptibility of *Escherichia coli* isolated from various clinical specimes in India. *J Microb Biochem Technol*. 2012;4(7):157–160.
9. Chaudhary M, Payasi A. Molecular characterization and antimicrobial susceptibility study of *Acinetobacter baumannii* clinical isolates from Middle East, African and Indian patients. *J Proteomics Bioinform*. 2012;5:265–269.
10. *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guideline for Good Clinical Practice, E6(R1)*; 1996.
11. Bergeys DH, Buchanan RE, Gibbons NE. *Bergey's Manual of Determinative Bacteriology*. Baltimore: Williams & Wilkins Company; 1975.
12. Clinical Laboratory Standard Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing, Approved Standards CLSI M100–s20*. PA: USA: Wayne; 2010.
13. Feria C, Ferreira E, Correia JD, Goncalves J, Canica M. Patterns and mechanisms of resistance to β-lactams and β-lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. *J Antimicrob Chemother*. 2002;49:77–85.
14. Gheldre YD, Avesani V, Berhin C, Delm? M, Glupczynski Y. Evaluation of oxoid combination discs for detection of extended-spectrum β-lactamases. *J Antimicrob Chemother*. 2003;52:591–597.
15. Colom K, Perez J, Alonso R, Fernandez-Aranguiz A, Larino E, Cisterna R. Simple and reliable multiplex PCR assay for detection of blaTEM, blaSHV and blaOXA1 genes in *Enterobacteriaceae*. *FEMS Microbiol Lett*. 2003;223:147–151.
16. Henquell C, Chanal C, Sirot D, Labia R, Sirot J. Molecular characterization of nine different types of mutants among 107 inhibitor-resistant TEM β-lactamases from clinical isolates of *Escherichia coli*. *Antimicrobial Agents Chemother*. 1995;39:427–430.
17. Chaves J, Ladona Margarita G, Segura C, Coira A, Reig R, Ampurdanes C. SHV-1 -lactamase is mainly a chromosomally encoded species-specific enzyme in *Klebsiella pneumoniae*. *Antimicrobial Agents Chemother*. 2001;45:2856–2861.
18. Chanawong A, Mzali FH, Heritage J, Lulitanond A, Hawkey PM. Discrimination of SHV β-lactamase genes by restriction site insertion-PCR. *Antimicrobial Agents Chemother*. 2001;45:2110–2114.
19. Poirel L, Walshb TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70:119–123.
20. Ellington Matthew J, Kistler J, Livermore David M, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. *J Antimicrob Chemother*. 2007;59:321–322.
21. Sambrook J, Russell DW. *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press; 2001.
22. Chaudhary M, Payasi A. Role of EDTA and CSE1034 in curli formation and biofilm eradication of *Klebsiella pneumoniae*: a comparison with other drugs. *J Antibiot*. 2012;65:631–633.
23. Chaudhary M, Payasi A. Role of CSE1034 in bacterial lipids and polysaccharides involved in biofilm formation: a comparison with other drugs. *Afr J Microbiol Res*. 2012;6: 6525–6531.