Tropical Biomedicine 36(2): 459–467 (2019)

Concurrent of *bla*CTX-M and *bla*NDM-1 genes in clinical isolates of *Escherichia coli* from northern Iran

Shahandeh, Z.[1], Kalantari, N.[2*], Sadighian, F.[1], Aram, H.[1] and Etminan, M.[1]
[1]Department of Laboratory Sciences, Faculty of Paramedical Sciences, Babol University of Medical Sciences, Babol, I.R. Iran
[2]Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran
[*]Corresponding author e-mail: n.kalantari@gmail.com
Received 12 August 2018; received in revised form 13 February 2019; accepted 19 February 2019

Abstract. The emergence of *E. coli* producing extended-spectrum β-lactamases and metallo β-lactamases has been reported as an important cause of treatment failure. The present study aimed to evaluate the existence of *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*NDM-1 and *bla*IMP-1 genes among *E. coli* isolated from patients in Babol, Northern Iran. The pattern of antibiotic resistance and the prevalence of multidrug-resistant (MDR) *E. coli* isolates were determined. *E. coli* isolates were separated from clinical specimens and antimicrobial susceptibility test (AST) was performed using the disk diffusion method. These isolates were further evaluated for the production of ESBLs and MBLs enzymes using cefotaxime (CTX), ceftazidime (CAZ) disks with and without clavulanic acid, and two CAZ with 2 mercaptopropionic acid disks, respectively. The ESBLs and MBLs positive isolates were analysed for the existence of *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*NDM-1 and *bla*IMP-1 genes with the conventional PCR method. Of 259 isolates, 117 β-lactamase producing *E.coli* were detected. MDR isolates were observed in 110/117 (94.9%) *E. coli*. Among 117 isolates, ESBLs, MBLs and coproduction of ESBL and MBL enzymes were observed in 45, 7 and 65 isolates, respectively. PCR analysis showed that the predominant genes were *bla*CTX-M-15(95.5%) and *bla*NDM-1 (31.9%) among ESBL and MBL producing *E. coli*, respectively. Also, the concurrent occurance of the *bla*CTX-M with *bla*TEM, *bla*SHV, *bla*NDM-1 and *bla*IMP-1 genes were demonstrated. In conclusion, high prevalence rate of MDR isolates, particularly ESBL and MBL producing *E. coli*, observed in the current study shows the necessity of control and management strategies for the aforementioned isolates. Also, the early detection of concurrent ESBLs and MBLs producing *E. coli* is necessary to avoid treatment failure and prevent the distribution of such bacteria.

INTRODUCTION

Escherichia coli is the most prevalent species in clinical laboratories and is the main cause of enteric infections in humans. This species also cause extraintestinal infections such as urinary tract infections, sepsis and neonatal meningitis (Allocati *et al.*, 2013). Therefore, it is considered as an important pathogen in health care settings. The main transmission route of enteric *E. coli* strains is fecal-oral, through the ingestion of contaminated food or water, and extraintestinal infections are mainly related to nosocomial and community-associated infections (Allocati *et al.*, 2013).

Over the past few decades, antibiotic resistance development has been frequently reported among various microorganisms. Several reports indicate that *E. coli* strains resistant to the first line of antibiotics are prevalent throughout the world (Nepal *et al.*, 2017). The resistance of *E. coli* against other antibiotics has become widespread which causes major challenges in the management of infectious diseases (Fair & Tor, 2014). Many investigations have been carried out to find the mechanisms of drug

resistance in bacteria, which show that the most common antimicrobial resistance mechanism is the production of hydrolytic enzymes, particularly β-lactamases (Singh-Moodley & Perovic, 2016; Moxon & Paulus, 2016; Shaikh *et al.*, 2015). Extended-spectrum β-lactamases (ESBLs) are frequently reported in *Enterobacteriaceae* such as *E. coli* (Nepal *et al.*, 2017) and other life-threatening bacteria like *Pseudomonas* spp. and *Acinetobacter baumannii* (Shaikh *et al.*, 2015). Several types of ESBLs genes have been identified and the most prevalent gene types around the world are *bla*CTX-M type, *bla*TEM-type, and *bla*SHV-type (Dallenne *et al.*, 2010; Shaikh *et al.*, 2015).

Carbapenems are the last option for the treatment of infections caused by multidrug-resistant gram negative bacteria. These antibiotics are suggested for treatment of infectious diseases caused by ESBL producing bacteria (Ibadin *et al.*, 2017; Wan Nor Amilah *et al.*, 2012). Carbapenemases are another group of β-lactamases which are less frequent than ESBLs, however they have been reported in different regions throughout the world. Carbapenemase producing bacteria cause serious problems for the treatment of infectious diseases since they are usually highly resistant to the most β-lactams and other antibiotics considered for the treatment of multidrug-resistant (MDR) isolates. Metallo β-lactamases (MBLs) including the New Delhi metallo β-lactamase-1 (NDM-1) and IMP-1 are class B carbapenemases which are very important as they cause often resistance to all β-lactam antibiotics (Kost *et al.*, 2017). The rate of MBL producing *Enterobacteriaceae*, particularly in *E. coli*, has rapidly increased around the world, which is considered a serious global public health problem (Nepal *et al.*, 2017).

The concurrent presence of ESBLs and MBLs in bacteria limits the choices of antibiotic therapy and often leads to failure in treatment (Nepal *et al.*, 2017).

Furthermore, these genes are located on plasmids and other mobile genetic elements which can transfer quickly and become widespread around the world (Kost *et al.*, 2017). Moreover, the lack of a standard phenotypic test for the detection of MBLs in clinical laboratories increases the difficulty of diagnosis (Ibadin *et al.*, 2017).

The prevalence of ESBLs and MBLs encoding genes in *E. coli* strains are different based on geographical location, therefore the current study was carried out to investigate distribution of *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*NDM-1 and *bla*IMP-1 genes among *E. coli*, isolated from patients at Babol University of Medical Sciences affiliated Hospitals, in the North of Iran. Furthermore, antibiotic susceptibility of ESBL and MBL strains were determined.

MATERIALS AND METHODS

Phenotypic methods

In total, 259 *E. coli* isolates were obtained from clinical samples. Antimicrobial susceptibility test (AST) for cefotaxime (CTX), ceftazidime (CAZ), cefpodoxime (CPD), cefepime (CPM), ertapenem (ETP) and aztreonam (ATM), (Mast disks,UK) were performed using the disk diffusion method according to CLSI standard protocol (Shahandeh *et al.*, 2015; Wanyne, 2014). Then, all isolates were examined for ESBLs and MBLs production using screening and confirmatory phenotypic methods as described below. For ESBL, all isolates which were resistant to CAZ and CTX (CAZ $\leq$ 22 mm, CTX $\leq$ 27 mm), were examined by CAZ and CTX disks with and without Clavulanic acid (CA). These isolates were also tested using CAZ disks alongside 2-mercaptopropionic acid (2-MPA) disk to evaluate MBLs production (Shahandeh *et al.*, 2015). The interpretation of these confirmatory methods was performed based on Wins *et al.*, 2006 and Arakawa *et al.*, 2000, respectively. In brief, for ESBLs producing *E. coli*, a considerable increase of growth inhibitory zone presents around the disks containing Clavulanic acid and CAZ or CTX, and for MBLs producer, a distinct growth inhibitory zone appears between the disk containing CAZ and the filter disk containing 2-MPA. No change is observed for non-ESBLs and non-MBLs

isolates (Figure 1). *E. coli*, ATCC25922, was used as a control strain.

An AST using cefoxitin (FOX), ciprofloxacin (CIP), cotrimoxazole (TS), and gentamicin (GM) was carried out for the bacteria which were confirmed positive for ESBLs and MBLs production by confirmatory phenotypic methods. Bacterial isolates which were resistant to at least three antibiotics of different families, were considered multi-drug resistance (MDR).

DNA extraction, PCR and Sequencing

Total DNA was extracted from isolates which were identified as ESBLs and MBLs producing bacteria using a commercial DNA extraction kit according to manufacturer's instructions (Signage, Iran). Then, PCR analysis of *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*IMP-1 and *bla*NDM-1 genes was performed. Details of the genes encoding β-lactamases and the primers pairs are listed in Table 1. The PCR conditions for *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*IMP-1 and *bla*NDM-1 were adjusted according to previous publication (Dallenne *et al.*, 2010; Shahcheraghi *et al.*, 2013). *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* (Azizi *et al.*, 2016) and *E. coli* K12 (Solgi *et al.*, 2017b) were used as positive control strains for *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*IMP-1 and *bla*NDM-1 genes, respectively.

PCR products were electrophoresed on a 2% agarose gel and the gels were evaluated under UV light and photographed by gel documentation system (Vilber, Lourmat, France).

Furthermore, the PCR product of selected amplicons for each gene was subjected to sequencing with forward primers (Bioneer Company, South Korea). The sequencing results were evaluated with Chromas (v.2.6.4) software and BLAST analysis (www.ncbi.nlm.nih.gov/blast).

Ethical consent

This work was approved by the ethical committee of Babol University of Medical Sciences, Babol, Iran (Ethical number: MUBABOL.REC.1394.223).

RESULTS

One-hundred and seventeen out of 259 (45.2%) isolates were recognised as ESBL and MBL producing bacteria by confirmatory phenotypic methods (Figure 1). Of 117 isolates, 45(38.4%) produced only ESBLs and 7(6%) produced only MBLs. The concurrence of ESBL and MBL production

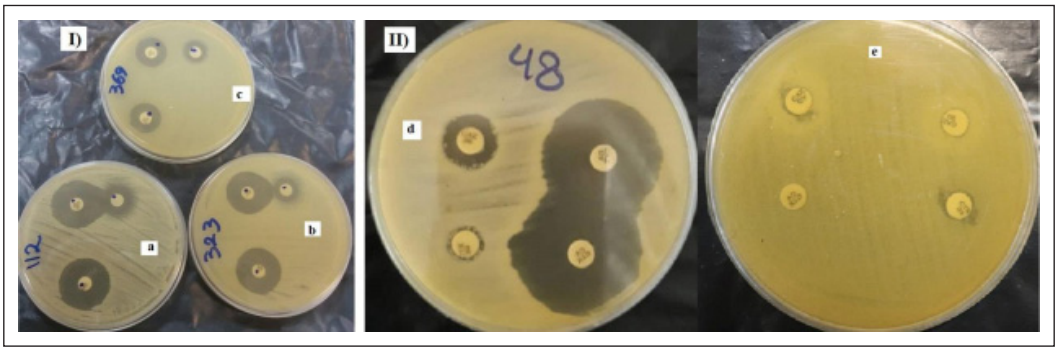

Figure 1. I) Inhibitory effects of 2-mercaptopropionic acid (2-MPA) on MBLs and non-MBLs producing *E. coli*, and II) Inhibitory effects of Clavulanic acid on ESBLs producing *E. coli* isolated from clinical samples. For MBLs producing *E. coli*, a distinct growth-inhibitory zone appeared between the disk containing CAZ and the filter disk containing 2-MPA (a and b). No change was observed around the two disks containing CAZ the filter disk with 2-MPA for non-MBLs producers (c). For ESBLs producing *E. coli*, a considerable increase of growth inhibitory zone appeared around the disks containig Clavulanic acid and CAZ or CTX(d). No change was seen around the two disks containing CAZ or CTX with and without Clavulanic acid for non-ESBLs producing isolates (e).

Table 1. β-lactamase genes and group-specific primers used for the PCR amplification

β-lactamase genes	Primer name	Sequence (5'–3')	Amplicon size (bp)	References
TEM-1&2	MultiTSO-T, F MultiTSO-T, R	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	800	8
SHV-1	MultiTSO-S, F MultiTSO-S, R	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	713	8
CTX-M	CTX-M-F CTX-M-R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	552	16
IMP-1	MultiIMP-1, F MultiIMP-1, R	TTGACACTCCATTTACAG GATTGAGAATTAAGCCACTCT	139	8
NDM-1	NDM-1, F NDM-1, R	ACCGCCTGGACCGATGACCA GCCAAAGTTGGGCGCGGTTG	263	17

Table 2. Antimicrobial susceptibility test of 117 β-lactamase producing *E. coli* isolates

Antimicrobial agent	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
Cefpodoxime	3 (2.5)	5 (4.3)	109 (93.2)
Cefotaxime	7 (5.9)	1 (0.9)	109 (93.2)
Aztreonam	5 (4.3)	4 (3.4)	108 (92.3)
Ceftazidime	5 (4.3)	18 (15.4)	94 (80.3)
Cotrimoxazole	23 (19.6)	3 (2.5)	91 (77.9)
Ciprofloxacin	21 (17.8)	5 (4.3)	91 (77.9)
Cefepime	8 (6.8)	9 (7.7)	100 (85.5)
Gentamicin	44 (37.6)	0 (0)	73 (62.4)
Cefoxitin	71 (60.6)	23 (19.7)	23 (19.7)
Ertapenem	93 (79.5)	11 (9.4)	13 (11.1)

was observed in 65 (55.6%) isolates. AST results showed that 109 out of 117 (93.2%) *E. coli* isolates were resistant to CPD and CTX. Ninety-three out of 117 (79.5%) were susceptible to ETP. In total, 111 out of 117 (94.9%) isolates were detected as MDR bacteria (Table 2).

PCR analysis revealed that the predominant ESBL gene was *bla*CTX-M-15 [105/110(95.5%)] followed by *bla*TEM-1[48/110(43.6%)] and *bla*SHV-33 [8/110(7.3%)] in ESBL producing *E. coli* isolates. Forty-one and eight isolates carried two and three genes simultaneously, in that order. Figure 2 shows the PCR analysis of CTX-M, SHV, TEM, and NDM-1 genes from *E. coli* isolates.

The *bla*CTX-M-15 gene was exclusively detected in 51.8% (57/110) isolates and a combination of this gene with the other studied genes was observed in 58/110 (52.7%) isolates (Table 3). All isolates which were positive for *bla*CTX-M-15 gene were resistant to at least one of the following antibiotics in addition to the β-lactam group: GM, CIP and TS. Fifty-three (50.5%, 53/105) isolates were considered MDR as a result of resistance to the aforementioned antibiotics.

PCR analysis of 72 MBLs producing *E. coli* showed the existence of *bla*NDM-1 and *bla*IMP-1 genes among 21 (29.2%) and 6 (8.3%) isolates, respectively. Also, 2/72 (2.8%) isolates were positive for *bla*NDM-1

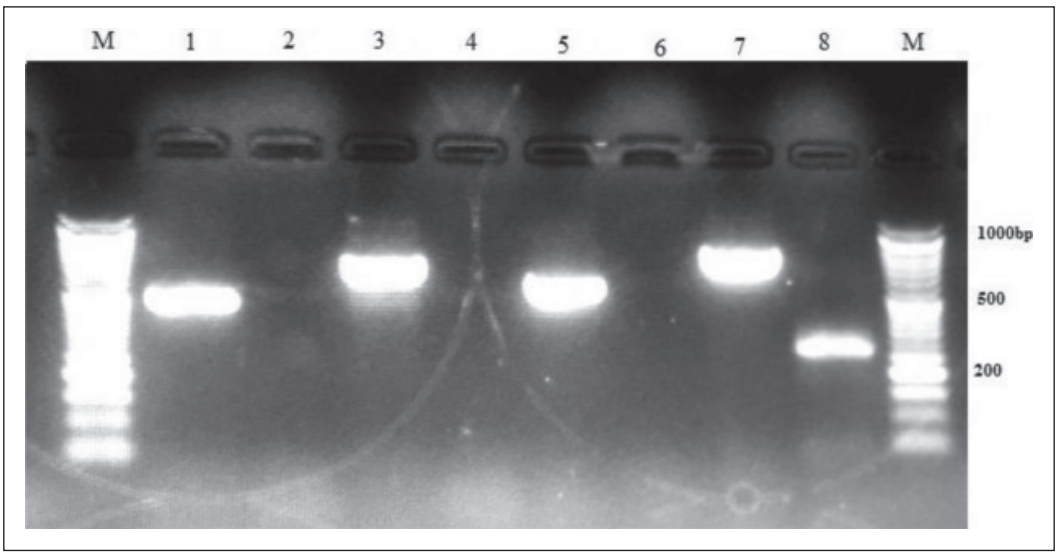

Figure 2. Gel electrophoresis of PCR products of *bla*CTX-M, *bla*SHV, *bla*TEM, and *bla*NDM-1 genes from *E. coli* isolates. Lanes: M, 50 bp DNA size marker; 1&5, CTX-M(500 & 550 bp); 2, *bla*CTX-M negative isolate; 3&4, *bla*SHV positive (713 bp)and negative isolates; 6&7, *bla*TEM negative and positive isolates (800 bp); 8, *bla*NDM-1 positive isolate.

Table 3. The frequency of *bla*CTX-M-15, *bla*TEM-1 and *bla*SHV-33 genes in ESBL- producing *E. coli* (110 isolates)

Genes	*bla*CTX-M-15	*bla*TEM-1	*bla*SHV-33
*bla*CTX-M	57 (51.8%)	40 (36.4%)	1 (0.9%)
*bla*TEM	40 (36.4%)	1 (0.9%)	7 (6.3%)
*bla*SHV	1 (0.9%)	7 (6.3%)	–
Total	98 (89.1%)	48 (43.6%)	8 (7.3%)

and *bla*IMP-1 genes concurrently. Twenty-one out of 29 (72.4%) isolates which carried *bla*NDM-1 or *bla*IMP-1 genes, were susceptible to ETP.

The nucleotide sequences of *bla*CTX-M-15, *bla*TEM-1, *bla*SHV-33 and *bla*NDM-1 genes were deposited in the Gene Bank database under accession numbers. MG770114, MG745167, MG745166, MG770113 and MG995853.

DISCUSSION

Recent studies on drug resistance mechanisms against β-lactam antibiotics in bacteria have demonstrated that the production of β-lactamase is the most important strategy involved in this phenomenon (Ibadin *et al.*, 2017; Sadeghi *et al.*, 2016). In the current study, high resistance rates (88.1%) to third and fourth generation cephalosporins were observed while resistance to ertapenem was low (11.1%) (Table 2). These results are supported by other studies, which found that the resistance rate to cephalosporins was 76.8% and 94.8%, whereas resistance to ertapenem was 13.3% and 1.9% in Azarbaijan, Iran (Sadeghi *et al.*, 2016) and Lebanon (Dandachi *et al.*, 2016), respectively.

Our findings showed that 94.9% of the studied isolates were MDR. Different prevalence rates of MDR are reported

among various bacteria throughout world (Ibadin *et al.*, 2017; Nepal *et al.*, 2017).

*bla*CTX-M-15 and *bla*CTX-M-14, are among the most important *bla*CTX-M genes in bacterial species affecting humans and animals, which have worldwide distribution (Allocati *et al.*, 2013). In the present study, PCR analysis showed that the most prevalent gene in this region was *bla*CTX-M-15 (95.5%). Several studies indicate that *bla*CTX-M-15 is the most prevalent gene among ESBL producing bacteria (Araque & Labrador, 2018; Khaleque *et al.*, 2017; Sadeghi *et al.*, 2016) but this is in contrast with other studies indicating that the *bla*CTX-M gene has low prevalence in Europe (Dallenne *et al.*, 2010; Karanika *et al.*, 2016; Valenza G, 2014). This dissimilarity may result from the differences in geographical regions and health care systems. According to Allocati *et al.*(2013), possible explanations for the high prevalence of CTX-M are as follow: (1) the insertion of *bla*CTX-M gene in plasmids and transposons results in rapid transmission between several kinds of bacteria; (2) the concurrency of this gene alongside other genes which are responsible for resistance to aminoglycosides and fluoroquinolones (Allocati *et al.*, 2013). In line with previous studies, our study showed that more than 50% of the *E. coli* isolates which were resistant to aminoglycosides and fluoroquinolones, carry the CTX-M-15 gene. In addition, the frequency of concurrent *bla*CTX-M and *bla*TEM genes obtained in the present study was higher than the surveys conducted by Haghighatpanah *et al.*, in Iran and Singh *et al.*, in India. However the frequency of coexistence between *bla*CTX-M, *bla*TEM and *bla*SHVgenes was lower than those studies (Singh *et al.*, 2012).

The last option of β-lactam antibiotics for the treatment of infections caused by resistant bacteria are carbapenems (Ibadin *et al.*, 2017). Recently, the emergence of MBLs producing bacteria isolated from clinical specimens and their distribution throughout the world has raised a major public health concern (Nepal *et al.*, 2017). One important MBLs is the New Delhi metallo-β-lactamase (NDM-1) and its variants (Khan *et al.*, 2017). The present study showed that the prevalence of *E. coli* encoding the *bla*NDM-1 gene was high in our region (23 out of 72, 31.9%). In Iran, the first report of NDM-1 producing *K. pneumoniae* isolate was reported in 2013 (Shahcheraghi *et al.*, 2013). Subsequently, the *bla*NDM-1 gene was detected in 6 of 40 (15%) (Shahcheraghi *et al.*, 2017), 17 of 54 (31.5%) of *Enterobacteriaceae* isolates (Solgi *et al.*, 2017a), and 4 of 170 (2.4%) of *K. pneumoniae* isolates (Shoja *et al.*, 2017) in Iran.

Our findings demonstrated that the studied isolates were resistant to a broad range of antimicrobial agents. The coexistence of *bla*CTX-M-15 with *bla*NDM-1 and *bla*IMP-1 genes was observed in 22 out of 65 (33.9%) and 6 out of 65 (9.2%) of the *E. coli* isolates, respectively. The coexpression of ESBLs and MBLs in *Enterobacteriaceae*, particularly in *E. coli*, and *K. pneumoniae* isolates, has been reported by several studies (Shoja *et al.*, 2017; Solgi *et al.*, 2017a). However, ESBLs producing isolates like *E. coli* are resistant to other families of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones in addition to β-lactam antibiotics (Tewari *et al.*, 2018). Furthermore, MBLs producing bacteria are usually resistant to aminoglycosides and fluoroquinolones (Ibadin *et al.*, 2017). On the other hand, *E. coli* is the most common gram negative bacilli isolated from clinical specimens (Dumaru *et al.*, 2019). Therefore, the co-harboring of different β-lactamase encoding genes causes therapeutic problems to clinicians as there are limited treatment choices (Ibadin *et al.*, 2017). In addition, the production of these enzymes are largely undetected by routine tests (Ibadin *et al.*, 2017). Our results showed that 72.4% of isolates which carried MBLs genes, were susceptible to ertapenem using the AST method which is an important result obtained from the present study. This is supported by results obtained by Singh-Moodley and Perovic, 2016 which found that the *bla*NDM gene in some *Entero-*

bacteriaceae isolates were susceptible to ertapenem by the AST method (Singh-Moodley & Perovic, 2016).

CONCLUSION

The current study demonstrated that the prevalence of ESBLs and MBLs producing MDR *E. coli* isolates are high in the studied region. Also, the high co-occurrence of *bla*CTX-M-15 with *bla*TEM-1 and *bla*NDM-1 genes are alarming and suggest that these genes may rapidly distribute throughout our country. Furthermore, isolates carrying the NDM-1 and IMP-1 genes were susceptible to ertapenem, which demonstrates the necessity of a new phenotypic method for the accurate detection of these bacteria. These findings indicate the need for the establishment of adequate strategies to control the increase of ESBLs and MBLs producing bacteria.

Acknowledgement. We are grateful to Ms. Masoumeh Ghasemi at the Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran for her kind help. We also thank Ms. Taraneh Ghaffari for language editing. This study was approved by the Ethics Committee of Babol University of Medical Sciences through grant number 9441049.

Conflict of interest
There are no conflict of interest.

REFERENCES

Allocati, N., Masulli, M., Alexeyev, M.F. & Di Ilio, C. (2013). *Escherichia coli* in Europe: an overview. *International Journal of Environmental Research and Public Health* **10**: 6235-6254.

Araque, M. & Labrador, I. (2018). Prevalence of Fecal Carriage of CTX-M-15 β-Lactamase-Producing *Escherichia coli* in Healthy Children from a Rural Andean Village in Venezuela. *Osong Public Health and Research Perspectives* **9**: 9-15.

Azizi, O., Shakibaie, M.R., Badmast, I.F., Modarresi, F., Ramazanzadeh, R., Mansouri, S. & Shahcheraghi, F. (2016). Class 1 integrons in non-clonal multidrug-resistant *Acinetobacter baumannii* from Iran, description of the new *bla*IMP-55 allele in In1243. *Journal of Medical Microbiology* **65**: 928-936.

Dallenne, C., Da Costa, A., Decré, D., Favier, C. & Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important â-lactamases in *Enterobacteriaceae*. *Journal of Antimicrobial Chemotherapy* **65**: 490-495.

Dandachi, I., Salem, Sokhn, E., Najem, E., Azar, E. & Daoud, Z. (2016). Carriage of β-lactamase-producing *Enterobacteriaceae* among nursing home residents in north Lebanon. *International Journal of Infectious Diseases* **45**: 24-31.

Dumaru, R., Baral, R. & Shrestha, L.B. (2019). Study of biofilm formation and antibiotic resistance pattern of gram-negative bacilli among the clinical isolates at BPKIHS, Dharan. *BMC Research Notes* **12**: 38. doi: 10.1186/s13104-019-4084-8.

Fair, R.J. & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry* **6**: 25-64.

Haghighatpanah, M., Mozaffari, Nejad, A.S., Mojtahedi, A., Amirmozafari, N. & Zeighami, H. (2016). Detection of extended-spectrum β-lactamase (ESBL) and plasmid-borne *bla*CTX-M and *bla*TEM genes among clinical strains of *Escherichia coli* isolated from patients in the north of Iran. *Journal of Global Antimicrobial Resistance* **7**: 110-113.

Ibadin, E.E., Omoregie, R., Anogie, N.A., Igbarumah, I.O. & Ogefere, H.O. (2017). Prevalence of extended spectrum β-lactamase, AmpC β-lactamase and metallo-β-lactamase among gram negative bacilli recovered from clinical specimens in Benin City, Nigeria. *International Journal of Enteric Pathogens* **5**: 85-91.

Karanika, S., Karantanos, T., Arvanitis, M., Grigoras, C. & Mylonakis, E. (2016). Fecal colonization with extended-spectrum β-lactamase – producing *Enterobacteriaceae* and risk factors among healthy individuals: a systematic review and metaanalysis. *Clinical Infectious Diseases* **63**: 310-318.

Khaleque, M., Akter, S., Mondal, D., Akhter, H., Khan, S. & Begum, A. (2017). Molecular characterization of extended spectrum β-lactamase producing bacteria isolated from urinary tract infected patients, Bangladesh. *Tropical Biomedicine* **34**: 512-523.

Khan, A.U., Maryam, L. & Zarrilli, R. (2017). Structure, genetics and worldwide spread of new delhi metallo-β-lactamase (ndm): a threat to public health. *BMC Microbiology* **17**: 101. doi: 10.1186/s12866-017-1012-8.

Kost, K., Yi, J., Rogers, B. & Jerris, R. (2017). Comparison of clinical methods for detecting carbapenem-resistant *Enterobacteriaceae*. *Practical Laboratory Medicine* **8**: 18-25.

Moxon, C.A. & Paulus, S. (2016). β-lactamases in *Enterobacteriaceae* infections in children. *Journal of Infection* **72**: Suppl, S41-S49.

Nepal, K., Pant, N.D., Neupane, B., Belbase, A., Baidhya, R., Shrestha, R.K., Lekhak, B., Bhatta, D.R. & Jha, B. (2017). Extended spectrum β-lactamase and metallo β-lactamase production among *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. *Annals of Clinical Microbiology and Antimicrobials* **16**: 62. doi: 10.1186/s12941-017-0236-7.

Sadeghi, M.R., Ghotaslou, R., Akhi, M.T., Asgharzadeh, M. & Hasani, A. (2016). Molecular characterization of extended-spectrum β-lactamase, plasmid-mediated AmpC cephalosporinase and carbapenemase genes among *Enterobacteriacea*e isolates in five medical centres of East and West Azerbaijan, Iran. *Journal of Medical Microbiology* **65**: 1322-1331.

Shahandeh, Z., Sadighian, F. & Rekabpou, K.B. (2015). Phenotypic study of extended-spectrum β-lactamase, AmpC and carbapenemase among *E. coli* clinical isolates in affiliated hospitals of Babol University of Medical Sciences. *International Journal of Health System and Disaster Management* **3**: 74-78.

Shahcheraghi, F., Aslani, M.M., Mahmoudi, H., Karimitabar, Z., Solgi, H., Bahador, A. & Alikhani, M.Y. (2017). Molecular study of carbapenemase genes in clinical isolates of *Enterobacteriacea*e resistant to carbapenems and determining their clonal relationship using pulsed-field gel electrophoresis. *Journal of Medical Microbiology* **66**: 570-576.

Shahcheraghi, F., Nobari, S., Rahmati, Ghezelgeh, F., Nasir, I.S., Owlia, P., Nikbin, V.S. & Imani Fooladi, A.A. (2013). First report of New Delhi metallo-β-lactamase-1-producing *Klebsiella pneumoniae* in Iran. *Microbial Drug Resistance* **19**: 30-36.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M.D. & Kamal, M.A. (2015). Antibiotic resistance and extended spectrum β-lactamases: types, epidemiology and treatment. *Saudi Journal of Biological Sciences* **22**: 90-101.

Shoja, S., Ansari, M., Faridi, F., Azad, M., Davoodian, P., Javadpour, S., Farahani, A., Douzandeh Mobarrez, B. & Karmostaji, A. (2017). Identification of carbapenem-resistant *Klebsiella pneumoniae* with emphasis on new delhi metallo-β-lactamase-1 (*bla* NDM-1) in Bandar Abbas, South of Iran. *Microbial Drug Resistance* **24**: 447-54.

Singh-Moodley, A. & Perovic, O. (2016). Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in *Enterobacteriaceae* in South Africa. *BMC Infectious Diseases* **16**: 536. DOI: 10.1186/s12879-016-1858-7.

Singh, A., Shahid, M., Sobia, F. & Umesh, Khan, H.M. (2012). Occurrence and molecular epidemiology of *bla*CTX-M, including co-occurrence of *bla*TEM and *bla*SHV genes, and sul1 association in Indian *Enterobacteriaceae*. *Inter-*

national Journal of Antimicrobial Agents **39**: 184-185.

Solgi, H., Badmasti, F., Aminzadeh, Z., Giske, C., Pourahmad, M., Vaziri, F., Havaei, S. & Shahcheraghi, F. (2017a). Molecular characterization of intestinal carriage of carbapenem-resistant *Enterobacteriaceae* among inpatients at two Iranian university hospitals: first report of co-production of *bla*NDM-7 and *bla*OXA-48. *European Journal of Clinical Microbiology and Infectious Diseases* **36**: 2127-2135.

Solgi, H., Badmasti, F., Aminzadeh, Z. & Shahcheraghi, F. (2017b). Gastrointestinal colonisation with three different NDM-1-producing enterobacterial species isolated from an inpatient in Tehran, Iran. *Journal of Global Antimicrobial Resistance* **12**: 53-54.

Tewari, R., Mitra, S.D., Ganaie, F., Venugopal, N., Das, S., Shome, R., Rahman, H. & Shome, B.R. (2018). Prevalence of extended spectrum β-lactamase, AmpC β-lactamase and metallo β-lactamase mediated resistance in *Escherichia coli* from diagnostic and tertiary healthcare centers in south Bangalore, India. *International Journal of Research in Medical Sciences* **6**: 1308-1313.

Valenza, G.N.S., Pfeifer, Y., Eller, C., Krupa, E., Lehner-Reindl, V. & Höller, C. (2014). Extended-spectrum-β-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. *Antimicrobial Agents and Chemotherapy* **58**: 1228-1230.

Wan Nor Amilah, W.A., Noor Izani, N.J., Ng, W.K. & Ashraful Haq, J. (2012). A simple screening test for the detection of metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Acinetobacter* in a tertiary care hospital. *Tropical Biomedicine* **29**: 588-97.

Wanyne, P.I.C.a.l.s.i. (2014). CLSI. Performance standards for antimicrobial susceptibility testing. 24 informational supplement, CLSI document M100-S24.