Prevalence of blaTEM, blaSHV, and blaCTX-M Genes among ESBL-Producing Klebsiella pneumoniae and Escherichia coli Isolated from Thalassemia Patients in Erbil, Iraq

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Competing interests: The authors have declared that no competing interests exist.

Abstract. Background: Due to the recent appearance of organisms that are resistant to several drugs (multidrug-resistant) like Enterobacteriaceae that produce extended-spectrum β-lactamase (ESBL), concerns have remarkably increased regarding the suitable treatment of infections. The present study was an investigation into ESBL molecular characteristics among clinical isolates of Klebsiella pneumoniae and Escherichia coli resulting in urinary tract infections (UTIs) and their pattern of antimicrobial resistance in order to come up with helpful information on the epidemiology of these infections and risk factors accompanied with them. Methods: In order to conduct the study, 20 K. pneumoniae and 48 E. coli were isolated and retrieved from thalassemia center in Erbil, Iraq during July 2016 and September 2016. The collected strains were analyzed and the profile of their antimicrobial susceptibility was specified. In order to spot β-lactamase genes (i.e. blaTEM, blaSHV, and blaCTX-M), polymerase chain reaction was conducted. Results: The findings obtained from multiplex PCR assay showed that out of the collected strains of ESBL-producing E. coli, had 81% blaTEM, 16.2% blaSHV, and 32.4% blaCTX-M genes. Similarly, 64.7% blaTEM, 35.2% blaSHV, and 41.1% blaCTX-M genes existed in the isolates of K. pneumoniae. It was found that antibiotic resistance pattern of E. coli and K. pneumoniae isolates to 20 antibiotics varied widely. It was also concluded that the majority of the K. pneumoniae and E. coli isolates were multi-drug resistant (MDR). Moreover, 75% and 87.5% of respectively K. pneumoniae and E. coli isolates showed the MDR phenotypes. Conclusion: TEM prevalence was high among other types of ESBLs. Over all, the most active antimicrobial agents in vitro remained to be the carbapenems.

Keywords: Escherichia coli, Klebsiella pneumoniae, ESBL, blaTEM, blaSHV and blaCTX-M, Thalassemia.

Citation: Pishtiwan A.H., Khadija Kh.M. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing Klebsiella pneumoniae and Escherichia coli isolated from thalassemia patients in Erbil, Iraq. Mediterr J Hematol Infect Dis 2019, 11(1): e2019041, DOI: http://dx.doi.org/10.4084/MJHID.2019.041

Published: July 1, 2019 Received: March 8, 2019 Accepted: June 10, 2019

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Introduction. It has been reported that bacteria that belong to the Enterobacteriaceae family are etiologic factors of numerous nosocomial infections all over the world.1 It is difficult to control diseases induced by bacilli Enterobacteriaceae given the limitation of therapeutic possibilities caused by constantly rising resistance of such organisms to antibiotics. In fact, Ojdana et al. (2014) introduced ESBLs as one of the most well-known resistance mechanisms in Gram-negative bacilli.2 ESBLs are a group of enzymes that lead to resistance increase in Aztreonam, Ceftazidime, Cefotaxime, related Oxyimino-β-lactams,
enzymes which are most often found in of them are derivatives of SHV, TEM, and CTX, OXA, CTX, AmpC, and so forth exist; however, most of them are derivatives of SHV, TEM, and CTX-M enzymes which are most often found in *K. pneumoniae* and *E. coli*. In this regard, the current study was aimed at determining the prevalence of the ESBL phenotype and examines the existence of *bla*SHV, *bla*CTX-M, and *bla*TEM genes in isolates.

**Materials and Methods**

**Isolates of bacteria.** In total, 68 consecutive non-duplicate of *K. pneumoniae* and *E. coli* isolates (n = 20 and 48, respectively) were retrieved from specimens of urinary at a Thalassemia center in Erbil, Iraq. The samples were obtained from both outpatients and inpatients between July 2016 and September 2016. Standard microbiological techniques were used for isolation.10 Conventional microbiological procedures were employed to identify the isolates. Besides, the VITEK 2 compact system was utilized to re-identify them (BioMerieux, France).

**Antimicrobial susceptibility testing.** According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), the isolates were screened by the disc diffusion method (Kirby-Bauer disc diffusion method) on Mueller-Hinton agar (MHA) plates in order to test their antimicrobial susceptibility.11 The utilized antimicrobials included Amoxicillin+Clavulanic acid (20+10 µg), Amikacin (10 µg), Azithromycin (15 µg), Cefixime (5 µg), Cefotaxime (30 µg), Chloramphenicol (30 µg), Cefazidime (30 µg), Ciprofloxacin (10 µg), Doxycycline (30 µg), Imipenem (10 µg), Gentamicin (10 µg), Kanamycin (30 µg), Nalidixic acid (30 µg), Meropenem (10 µg), Nitrofurantoin (100 µg), Norfloxacin(10 µg), Ofloxacin (5 µg), Streptomycin (25 µg), Pipercillin (100 µg), and Tobramycin (10 µg).

**Testing for production of ESBL (MDDST).** Using a disc of Amoxicillin-Clavulanate (20/10 µg) with four cephalosporins of Ceftriaxone, 3GC-Cefotaxime, 4GC-Cefepime, and Cefpodoxime, the Modified Double Disc Synergy Test (MDDST) was employed to test all strains in terms of their production of Extended Spectrum Beta-Lactamase (ESBL). A lawn culture belonging to the organisms was created on a Mueller-Hinton agar plate following the recommendations by CLSI.11 A disc that contained Amoxicillin-Clavulanate (20/10 µg) was put in the middle of the plate. The 3GC and 4GC discs were placed respectively 15 mm and 20 mm center-to-center apart from the center of the amoxicillin-clavulanate disc.12 Any increase or distortion in the zone toward the Amoxicillin-Clavulanate disc was regarded positive for the production of ESBL. According to CLSI guidelines, the combined disc test was used to confirm ESBL production.

**Detection of ESBL genotypes by multiplex PCR amplification.** Using the method utilized by Monstein *et al.* (2007) with slight modifications, multiplex PCR
was employed to examine the positive isolates in the initial screening test for ESBL production for the existence of \textit{bla}SHV, \textit{bla}CTX-M, and \textit{bla}TEM genes.\textsuperscript{13} Freshly cultured isolates bacteria were used to prepare template deoxyribonucleic acid (DNA) was prepared using PrestoTM Mini gDNA bacterial kit. All reactions of PCR were conducted by utilizing 2 µl DNA template (density of 10 ng/µl), the Master Mix consisting of 3 mM MgCl\textsubscript{2}, 0.2% Tween\textsuperscript{®} 20, 20 mM Tris-HCl pH 8.5, (NH\textsubscript{4})2SO\textsubscript{4}, 0.4 mM of each dNTP, 0.4 µM of each primer, and 0.2 units/µl Amplion Taq DNA polymerase. The conditions of polymerase chain reaction amplification were set up as follow: primary denaturation step for 10 minutes at 95°C; 30 denaturation cycles for 30 seconds at 94°C, annealing 30 seconds at 60°C for, extension for 2 minutes at 72°C, and a final extension step for 10 minutes at 72°C. Using agarose gel electrophoresis, size separation PCR amplicons were utilized to detect respective genes (Table 1).

Results.

\textbf{Antimicrobial susceptibility profile.} In total, 68 consecutive non-duplicate of \textit{K. pneumoniae} and \textit{E. coli} isolates (n = 20 and 48, respectively) were retrieved, and their antimicrobial resistance profile against 20 different antimicrobial agents was tested. The current results revealed that \textit{K. pneumoniae} and \textit{E. coli} isolates vary widely to different antimicrobials.

### Table 1. List of primers used for Multiplex PCR amplification.

| Target gene | Primer  | Sequence (5’-3’) | Amplicon size | References |
|-------------|---------|------------------|---------------|------------|
| \textit{bla}TEM | Forward | TCG CCG CAT ACA CTA TTT TCA GAA TGA | 445-bp | [30] |
| | Reverse | ACG CTC ACC GCC TCC AGA TTT AT | 747-bp | [12] |
| \textit{bla}SHV | Forward | ATG CGT TATATT CGC CTG TG | 593-bp | [30] |
| | Reverse | TGC TTT GTT ATT CGG GCC AA | | |
| \textit{bla}CTX-M | Forward | ATG TGC AGY ACC AGT AAR GTK ATG GC | | |
| | Reverse | TGG GTR AAR TAR GTS ACC AGA AYC AGC GG | | |

### Table 2. Antibiotic resistance pattern of \textit{K. pneumoniae} and \textit{E. coli} isolates.

| Name of Antibiotic | Symbol | \textit{E. coli} | \textit{K. pneumoniae} |
|--------------------|--------|------------------|-----------------------|
| Amikacin,          | AK     | 95.8             | 4.2                   |
| Amoxicillin-Clavulanic acid | AMC | 18.75            | 81.25                 |
| Azithromycin       | AZM    | 89.5             | 10.5                  |
| Cefixime           | CFM    | 16.6             | 83.4                  |
| Cefotaxime         | CTX    | 16.6             | 83.4                  |
| Cefazidime         | CAZ    | 37.5             | 62.5                  |
| Chloramphenicol    | C      | 85.4             | 14.6                  |
| Ciprofloxacin      | CIP    | 95.8             | 4.2                   |
| Doxycycline        | DOX    | 39.5             | 60.5                  |
| Gentamicin         | CN     | 95.8             | 4.2                   |
| Imipenem           | IPM    | 100              | 0                     |
| Kanamycin          | KAN    | 62.5             | 37.5                  |
| Meropenem          | MEM    | 100              | 0                     |
| Nalidixic acid     | NA     | 50               | 50                    |
| Nitrofurantoin     | F      | 75               | 25                    |
| Norfloxacin        | NOR    | 89.5             | 10.5                  |
| Ofloxacin          | OFL    | 93.75            | 6.25                  |
| Piperacillin       | PIPER  | 33.3             | 66.7                  |
| Streptomycin       | S      | 89.5             | 10.5                  |
| Tobramycin         | TOB    | 95.2             | 4.8                   |

The resistance rates of isolates of \textit{K. pneumoniae} and \textit{E. coli} against the selected 20 antimicrobial agents obtained from urine samples. It was found that a majority of the \textit{K. pneumoniae} and \textit{E. coli} isolates were resistant to several drugs (multi-drug resistant: MDR) where a total of 87.5% and 75% of respectively \textit{E. coli} and \textit{K. pneumoniae} isolates indicated MDR phenotypes.

Furthermore, the results of the antimicrobial susceptibility test against \textit{E. coli} revealed that \textit{E. coli} showed 81.25% resistance to Amoxicillin+Clavulanic acid (Table 2), whereas susceptibility to doxycycline decreased to 39.5%. Similar patterns were observed for Piperacillin. Substantial decrease of 16.6–37.5% was observed in the susceptibility for all Cephalosporins. Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tobramycin and Ofloxacin with susceptibility rates of respectively 100%, 100%, 95.8%, 95.8%, 95.8%, 95.2%, and 93.75% were the most active agents against \textit{E. coli}. Resistance to Azithromycin Norfloxacin and Streptomycin was comparatively less (10.5%, for them). On the other hand, \textit{E. coli} showed a different sensitive rate to Chloramphenicol, Kanamycin, Nalidixic acid and Nitrofurantoin with 85.4%, 62.5%, 50% and 75%, respectively. Meanwhile, similar results were observed for \textit{K. pneumoniae} which revealed that \textit{K. pneumoniae} showed 65% resistant to Amoxicillin+Clavulanic acid, whereas susceptibility to Piperacillin dropped to 50% (Table 2).
Similar patterns were noticed for Nalidixic acid. In addition, a substantial drop of 30–40% was observed in the susceptibility for all Cephalosporins. Nevertheless, *K. pneumoniae* showed a different sensitive rate to Chloramphenicol, Doxycycline, Streptomycin, Azithromycin, Kanamycin and Nitrofurantoin with 95%, 95%, 95%, 90%, 90% and 70% respectively.

**ESBL screening of E. coli and K. pneumoniae.** Out of the 48 *E. coli* isolates, a total of 37 isolates (77%) showed positive results in initial screening test of ESBL production by MDDST and phenotypic confirmatory test of ESBL production. Meanwhile, out of the 20 *K. pneumoniae* isolates, a total of 17 isolates (85%) showed positive results in initial screening test of ESBL production and phenotypic confirmatory test of ESBL production.

Discussion. As a global challenge, antimicrobial resistance in pathogenic bacteria is accompanied with high rates of mortality and morbidity. In addition, because of multidrug resistant patterns, infections have been reported to be difficult or even impossible to treat with conventional antimicrobials. Because many healthcare centers fail to diagnose causative microorganisms and their patterns of antimicrobial susceptibility timely in patients with bacteremia and other serious infections, antibiotics are broadly, liberally and mostly unnecessarily used.

In the current study, high prevalence of MDR isolates of *K. pneumoniae* and *E. coli* was noticed in the clinical samples. The overall prevalence of MDR phenotypes in *K. pneumoniae* and *E. coli* isolates was respectively 75% and 87.5%. Among the MDR isolates of *E. coli* and *K. pneumoniae*, a majority of them were producers of ESBL. Similar to the results of the present study, also in a research by Bora et al. (2014) reported the same ratios.

In the current study, the antimicrobial susceptibility patterns were determined in all isolates, and the results obtained from the test of antimicrobial susceptibility against *E. coli* and *K. pneumonia* revealed that isolated bacteria were different in their susceptibility to the tested antimicrobials. Liao et al. (2017) and Tabar et al. (2016) reported similar results.

Carbapenems are often the final influential therapy that exists for infections resulting from MDR Enterobacteriaceae. According to other studies, 100% sensitivity was seen with Imipenem and Meropenem, which has been reported to be the most effective antibiotic including the isolates that produce ESBLs. This is an important result of the present study because many infections can be treated with Carbapenemes. This result can be relevant to the fact that these antibiotics are more expensive and thus used less in this region.

Paterson et al. (2001) stated that even if ESBL producers show an *in vitro* susceptibility, they are intrinsically resistant to all cephalosporins. In the
present study, 9% and 13% of the producers of ESBL were found to have false susceptibilities respectively to cefotaxime and Ceftazidime. This can be attributed to the fact that different ESBL enzymes possess various optimal substrate profiles.19

In fact, ESBLs are reported to be a challenge among hospitalized patients all over the world. It has also been reported that ESBLs have different prevalence rates among clinical isolates in different parts of the world, and there is a rapid continuous change in their prevalence rate over time.20 Given the increased prevalence of ESBLs-producing Enterobacteriaceae, it is highly crucial to develop laboratory testing methods in order to accurately diagnose the existence of such enzymes in clinical isolates.21 Among all ESBL detection methods, modified double disc synergy tests were the most sensitive ones.22 A study carried out by23 presented similar findings and indicated positive MDDST in 40/40 isolates, while it was positive in 25/40 and 39/40 isolates respectively in double disk synergy test (DDST) and phenotypic confirmatory disc diffusion test (PCDDT).

By following the MDDST screening criteria for ESBL production, respectively 85% and 77% of K. pneumoniae and E. coli isolates were screened for detecting production of ESBL. Existence of one or more ESBL genes in all screened positive isolates revealed that K. pneumoniae and E. coli isolates that produce ESBL are highly frequent in the geographical region under investigation. In India, Kaur et al. (2013) observed that 63.4% E. coli and 60.3% K. pneumoniae isolates produced ESBL.24 Phenotypic tests for detection of ESBL can only confirm ESBL production but fail to recognize the subtypes of ESBL. As reported by Nüesch-Inderbinen et al. (1996), molecular methods have been proved to be sensitive, but they costly and conducting them requires a long time, expertise, and specialized equipment.25 Ultimate identification is only probable through methods of molecular detection. The results of a study conducted by (Navon-Venezia et al., 2003) revealed that it is necessary to periodically evaluate these phenotypic tests because introduction of new enzyme can change their performance.26 In their study of phenotypic and genotypic methods of ESBL detection, (Grover et al., 2006) stated that PCR is a reliable method for detecting ESBL.27 In the present study, multiplex PCR amplification assay was utilized to detect blaCTX-M, blaSHV, and blaTEM genes in the retrieved clinical isolates of K. pneumoniae and E. coli because one of the advantages of this assay rapid screening of large numbers of clinical isolates, moreover, if it is required, further molecular epidemiological studies can take advantage of the DNA that is isolated via this assay.13

Furthermore, it is essential to identify beta-lactamase in order to conduct a reliable epidemiological investigation into antimicrobial resistance. The current study was conducted to survey antimicrobial drug resistance, ESBL phenotypes, and blaSHV, blaTEM and blaCTX-M genes detection in K. pneumoniae and E. coli isolates retrieved from urinary tract infections in Erbil, Iraq.

The most globally common type of ESBL appeared to be CTX-M-type ESBLs with their higher incidence in most locations compared to SHV and TEM ESBLs.28 Among the three ESBL genotypes included in this study, the most prevalent one was found to be blaTEM (81%) and blaTEM (64.7%) respectively in ESBL-producing isolates of E. coli and K. pneumoniae. The less prevalent ESBL genotype was blaSHV, and the prevalence rate of blaSHV in ESBL-producing K. pneumoniae isolates (35.2%) was higher than E. coli isolates (16.2%). Also, the prevalence rate of blaCTX-M in ESBL-producing K. pneumoniae isolates (41.1%) was higher than E. coli isolates (32.4%). It was found that all of the ESBL-producing isolates of both organisms were positive for one or more ESBL genotypes. It was observed that blaTEM alone was more prevalent in E. coli (62.16%, 23/37), and in K. pneumoniae (41.17%, 7/17), while blaCTX-M and blaTEM together predominated in E. coli (8.1%), while blaSHV, blaTEM, and blaCTX-M together predominated in isolates of K. pneumoniae (11.76%). A study conducted by Manoharan et al. (2011) reported similar findings.29 In the present study; however, TEM ESBL was the prevalent genotype and CTX-M-type ESBL was not prevalent. The discrepancy is assumed to be because of regional variations, since the strains collected and evaluated in the current study were only from Erbil, Iraq.

Furthermore, in another study, Moghnieh et al. (2018) have reported that E. coli and Klebsiella spp resistance to third-generation cephalosporins is usual in whole countries, with outbreak reaching over 50% in Egypt and Syria30 and in our study, 30–40% was observed in the susceptibility for all Cephalosporins, which this prevalence is close to other Arabia countries, as well as in Moghnieh study, they reported that carbapenem resistance is emerging, albeit with a prevalence of less than 10%.30 In parallel, we have found that the most active antimicrobial agents in vitro remained to be the carbapenems. Khalaf and Al-Ouqaili et al. (2018) in Baghdad, during a period one year demonstrated that SHV gene was detected only in 12.5% E. coli, and 56.25% in K. pneumoniae.31 Approximately, we found close to findings above that 16.2% SHV genes in E. coli and 35.2% SHV genes existed in the isolates of K. pneumoniae. Of course, according the above findings several studies by Teawtrakul et al. (2015) Girmenia et al. (2016), Ricciardi et al. (2016) and Devrim et al. (2018) have shown that the rates and types of Klebsiella and Escherichia strains isolated are differed in other countries.32-35 These outcomes highlight require for
References:

1. Gaynes R, Edwards JR. National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis. 2005;41(6):848-54. https://doi.org/10.1086/432803.

2. Ojdana D, Sacha P, Wieczorek P, Czaban S, Michalska A, Jaworskowsa J. The Occurrence of blaCTX-M, blaSHV, and blaTEM Genes in Extended-Spectrum β-Lactamase-Positive Strains of Klebsiella pneumoniae, Escherichia coli, and Proteus mirabilis in Poland. Intern J Antibi Volume 2014, Article ID 935842, 7 pages. https://doi.org/10.1155/2014/935842.

3. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamasas: a clinical update. Clin Microbiol Rev. 2005;18(4):657-86. https://doi.org/10.1128/CMR.18.4.657-686.2005.

4. Liao K, Chen Y, Wang M, Guo P, Yang Q, Ni Y, Yu Y, Hu B, Sun Z, Huang W. Molecular characteristics of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae causing intra-abdominal infections from 9 tertiary hospitals in China. Diagn Microbiol Infect Dis. 2017;87(1):45-48. https://doi.org/10.1016/j.diagmicrobio.2016.10.007.

5. Malloy AM, Campos JM. Extended-spectrum beta-lactamases: a brief clinical update. Pediatr Infect Dis J. 2011;30(12):1092-3. https://doi.org/10.1097/INF.0b013e318232c0e9d.

6. Akpaka PE, Legall B, Padman J. Molecular detection and epidemiology of extended-spectrum beta-lactamase genes prevalent in clinical isolates of Klebsiella pneumoniae and E.coli from Trinidad and Tobago. West Indian Med J. 2010;59(6):591-6.

7. Fraser A, Paul M, Almanareh N, Taconelli E, Frank U, Cauda R, Borok S, Cohen M, Andressen N, Nielsen AD, Leibovici L. TREAT Study Group. Benefit of appropriate empirical antibiotic treatment: thirty-day mortality and duration of hospital stay. Am J Med. 2006;119(11):970-6. https://doi.org/10.1016/j.amjmed.2006.03.034.

8. Livermore DM, Paterson DL. Pocket Guide to Extended-spectrum [beta]-lactamases in Resistance. Current Medicine Group, London, UK. 2006.

9. Sharrar S, Sharrar M, Ray P. Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates in a tertiary care hospital from India. Indian J Med Res. 2010;132:332-6.

10. Collee JG, Miles RS, WB. Tests for the identification of bacteria In: Collee JG, Fraser AG, Marimon BP, and S. A. (eds.), Mackie & McCartyen practical medical microbiology. Churchill Livingstone, Edinburgh, 1996.

11. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2011.

12. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA; International Klebsiella Study Group. Extended-spectrum β-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: Dominance and widespread prevalence of SHV-and CTX-M-type β-lactamases. Antimicrob Agents Chemother. 2003;47(11):3554-60. https://doi.org/10.1128/AAC.47.11.3554-3560.2003.

13. Monstein IJ, Ostholm-Balkhed A, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX - M genes in Enterobacteriaceae. APMIS. 2007;115(12):1400-8. https://doi.org/10.1111/j.1600-0463.2007.00722.x.

14. Akova M. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence, 2016;7:252-266. https://doi.org/10.1080/21505594.2016.1159366.

15. Bora, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of blaTEM, blaSHV and blaCTX-M genes in clinical isolates of Escherichia coli and Klebsiella pneumoniae from Northeast India. Indian J Pathol Microbiol. 2014;57(2):249-54. https://doi.org/10.4103/0377-4929.134698.

16. Tabar MM, Mirkalantari S, Amoli RJ. Detection of ctx-M gene in ESBL-producing E. coli strains isolated from urinary tract infection in Semnan, Iran. Electron Physician. 2016;8(7):2686-90. https://doi.org/10.19082/2686.

17. Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, Bonomo RA, Rice LB, McCormack JG, Yu VL. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol. 2001;39(6):2206-12. https://doi.org/10.1128/JCM.39.6.2206-2212.2001.

18. Paterson DL, Bora, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates from India. Indian J Med Res. 2010;132:332-6.

19. Wong-Beringer A. Therapeutic challenges associated with extended-spectrum, beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Pharmacotherapy. 2001;21(5):583-92. https://doi.org/10.1592/phco.21.6.635.34357.

20. Bhabayadmini S, Appalaraju B. Extended-spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol. 2004;22(3):172-4.

21. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14(4):933-51. https://doi.org/10.1128/CMR.14.4.933-951.2001.

22. Modi D, Patel D, Patel S, Jain M, Bhatt S, Vagad M. Comparison of various methods for the detection of extended spectrum beta lactamase in Klebsiella pneumoniae isolated from neonatal Intensive Care Unit, Ahmedabad. Nat J Med Res. 2012; 2(3): 348-53.

23. Khan MK, Thukral SS, Gaind R. Evaluation of a modified double-disc synergy test for detection of extended spectrum β-lactamases in AMPC β-lactamase-producing Proteus mirabilis. Indian J Med Microbiol. 2008;26(1):58-61. https://doi.org/10.4103/0255-0857.38860.

24. Kaur J, Chopra S, Sheevani, Mahajan G. Modified double disc synergy test to detect ESBL production in urinary isolates of Escherichia coli and Klebsiella pneumoniae. J Clin Diagn Res. 2013;7(2):229-33. https://doi.org/10.7860/JCDR/2013/4619.2734.

25. Nüesch-Inderbitzin MT, Hächer H, Kayser FH. Detection of genes coding for extended-spectrum SHV beta-lactamases in clinical isolates by a molecular genetic method, and comparison with the E test. Eur J Clin Microbiol Infect Dis. 1996;15(5):398-402. https://doi.org/10.1007/BF01690097.

26. Navon-Venezia S, Hamner-Munz O, Schwartz D, Turner D, Kuzmenko
B, Carmeli Y. Occurrence and phenotypic characteristics of extended-spectrum β-lactamases among members of the family Enterobacteriaceae at the Tel-Aviv Medical Center (Israel) and evaluation of diagnostic tests. J Clin Microbiol. 2003;41(1):155-8. https://doi.org/10.1128/JCM.41.1.155-158.2003 PMid:12517841 PMCid:PMC149636

27. Grover SS, Sharma M, Chattopadiya D, Kapoor H, Pasha ST, Singh G. Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in Klebsiella pneumoniae: emergence of high resistance against cefepime, the fourth generation cephalosporin. J Infect. 2006;53(4):279-88. https://doi.org/10.1016/j.jinf.2005.12.001 PMid:16488476

28. Jorgensen JH, McElmeel ML, Fulcher LC, Zimmer BL. Detection of CTX-M-type extended-spectrum beta-lactamase (ESBLs) by testing with MicroScan overnight and ESBL confirmation panels. J Clin Microbiol. 2010;48(1):120-3. https://doi.org/10.1128/JCM.01507-09 PMid:19889896 PMCid:PMC2812268

29. Manoharan A, Premalatha K, Chatterjee S, Mathai D; SARI Study Group. Correlation of TEM, SHV and CTX-M extended-spectrum beta-lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility. Indian J Med Microbiol. 2011;29(2):161-4. https://doi.org/10.4103/0255-0857.81799 PMid:21654112

30. Moghnieh RA, Kanafani ZA, Tabaja HZ, Sharara SL, Awad LS, Kanj SS. Epidemiology of common resistant bacterial pathogens in the countries of the Arab League. Lancet Infect Dis. 2018;18(12):e379-e394 https://doi.org/10.1016/S1473-3099(18)30814-6

31. Khalaf EA, Al-Ouqali MTS. Molecular detection and sequencing of SHV gene encoding for extended-spectrum β-lactamases produced by multidrug resistance some of the Gram-negative bacteria. Intern J Green Pharm 2018;12(4): S918 https://doi.org/10.22377/ijgp.v12i04.2274

32. Teawtrakul N, Jetsirisuparb A, Sirijerachai C, Chansung K, Wanitpongpun C. Severe bacterial infections in patients with non-transfusion-dependent thalassemia: prevalence and clinical risk factors. Int J Infect Dis. 2015;39:53-6 https://doi.org/10.1016/j.ijid.2015.09.001 PMid:26358855

33. Ricciardi W, Giubbini G, Laurenti P. Surveillance and Control of Antibiotic Resistance in the Mediterranean Region. Mediterr J Hematol Infect Dis. 2016;8(1):e2016036 https://doi.org/10.4084/mjhid.2016.036 PMid:27413528 PMCid:PMC4928537

34. Devrim F, Serdaroglu E, Calgr Y, Oruç Y, Demiray N, Bayram N, Ağın H, Çalkavur S, Sorguç Y, Dinçel N, Ayhan Y, Yılmaz E, Devrim I. The Emerging Resistance in Nosocomial Urinary Tract Infections: From the Pediatrics Perspective. Mediterr J Hematol Infect Dis. 2018 Sep 1;10(1):e2018055. https://doi.org/10.4084/mjhid.2018.055 PMid:30216748 PMCid:PMC6131100

35. Girmenia C, Serra A, Canichella M. Epidemiology of Carbapenem Resistant Klebsiella pneumoniae Infections in Mediterranean Countries. Mediterr J Hematol Infect Dis. 2016;8(1):e2016032. https://doi.org/10.4084/mjhid.2016.032 PMid:27441063 PMCid:PMC4943068