

# Phenotypic Detection of ESBL, MBL (IMP-1), and AmpC Enzymes, and Their Coexistence in *Enterobacter* and *Klebsiella* Species Isolated From Clinical Specimens

Zahra Shahandeh,<sup>1,2</sup> Farahnaz Sadighian,<sup>2,\*</sup> and Khadije Beigom Rekabpor<sup>2</sup>

<sup>1</sup>Infectious Disease and Tropical Medicine Research Center, Babol University of Medical Sciences, Babol, IR Iran

<sup>2</sup>Department of Laboratory Medicine, Paramedical Faculty, Babol University of Medical Sciences, Babol, IR Iran

\*Corresponding author: Farahnaz Sadighian, Department of Laboratory Medicine, Paramedical Faculty, Babol University of Medical Sciences, Babol, IR Iran. Tel: +98-9111183149, Fax: +98-132234367, E-mail: F.sadigh@gmail.com

Received 2015 August 30; Revised 2015 November 02; Accepted 2015 December 23.

## Abstract

**Background:** Bacterial resistance to beta-lactam is a major problem in all developed and developing countries. The genera of *Klebsiella* and *Enterobacter* are associated with opportunistic and nosocomial infections. All beta-lactamase genes can cause dissemination of resistance to beta-lactams.

**Objectives:** The present study aimed to investigate the phenotypic detection of beta-lactamases in *Enterobacter* and *Klebsiella* species isolated from clinical specimens.

**Materials and Methods:** This cross-sectional study was performed on 59 *Klebsiella* spp. and 49 *Enterobacter* spp. isolated from clinical samples. They were confirmed using API 20E. These bacteria were evaluated for the production of extended-spectrum beta lactamase (ESBL), metallo-beta-lactamase (MBL) (IMP-1), and the pAmpC and iAmpC enzymes. This was done using the Clinical and Laboratory Standards Institute (CLSI) method, 2-mercaptopyronic acid, the cefoxitin method, and the use of imipenem as an enzyme inducer, respectively. Mask-ESBL production was also identified, using different concentrations of 3-amino-phenyl boronic acid compound. Data were analyzed with SPSS version 22.

**Results:** In total, 38 (64.4%) *Klebsiella* spp. and 41 (83.7%) *Enterobacter* spp. produced at least one type of beta-lactamase. AmpC-producing *Enterobacter* spp. (71.4%), and ESBL-producing *Klebsiella* spp. (42.4%) had the highest prevalence of beta-lactamase types in each genus. There were two bacteria in both types that were resistant to all antibiotics without producing any type of beta-lactamase.

**Conclusions:** According to our findings, it is necessary to pay special attention to ESBL production in *Klebsiella* spp., while in *Enterobacter* spp., it is essential to search for AmpC production (chromosomal and plasmid). In addition, the genotypic evaluation of beta-lactamase variety in these bacteria may be necessary in different geographical areas.

**Keywords:** *Klebsiella*, *Enterobacter*, AmpC beta-Lactamase

## 1. Background

The genera of *Klebsiella* and *Enterobacter*, of the *Enterobacteriaceae* family, are associated with opportunistic and nosocomial infections, such as wound and urinary tract infections. *K. pneumoniae* is a common cause of lower respiratory tract infections among hospitalized patients, and the prevalence of infection is increased with long hospital stays. In humans, *K. oxytoca* can produce infections similar to *K. pneumoniae*. *E. cloacae* and *E. aerogenes* have been isolated from wounds, urine, blood, and CSF more often than the other *Enterobacter* spp. *E. sakazakii* has been identified in brain abscesses and in respiratory and wound infections (1).

Bacterial resistance to beta-lactam antibiotics is a major problem in all developed and developing countries (2). Antimicrobial resistance in the *Enterobacteriaceae* family

has increased in recent years; the most severe type is probably carbapenemase-producing *K. pneumoniae* (1). The main mechanism of resistance to beta-lactams (penems, cefems, carbapenems, and monobactam) in gram-negative bacteria is the production of beta-lactamase enzymes (3).

Beta-lactamases are divided into A, B, C, and D molecular classes in the Ambler classification system (1, 4). The extended spectrum beta-lactamase (ESBL) enzyme is a type of beta-lactamase that hydrolyzes penicillins, cephalosporins, and aztreonam, and that is coded by movable genes (5). ESBLs play a major role in the creation of multidrug resistance (MDR) among gram-negative bacilli. Rapid diagnosis of ESBL-producing bacteria can be important in limiting the spread of MDR pathogens and infections (6). The CLSI (Clinical and Laboratory Standards Institute) has provided a protocol to identify ESBLs (A and D Ambler classes) in the *Enterobacteriaceae* family

(7). Plasmid-mediated metallo-beta-lactamases (MBLs, belonging to Ambler class B) are classified into three main groups: IMP, VIM, and SPM (8). The CLSI recommends a method for the detection of small quantities of MBL, which has not yet been approved (9). There are different techniques for the isolation of MBL-producing bacteria, including the double-disk synergy test (DDST) using EDTA, or 2-mercaptopropionic acid with imipenem or ceftazidime (10). Among these compounds, 2-mercaptopropionic acid (2MPA) is able to detect even small amounts of the enzyme (11).

The production of AmpC enzymes (Ambler class C) in gram-negative bacteria is controlled by genes on the chromosome (inductive type) or by a plasmid (permanent type) (12). They confer resistance to cephalosporins, oxyimino groups, and 7- $\alpha$ -methoxy cephalosporins, and are not inhibited by ESBL inhibitors, such as clavulanic acid (13).

Generally, production of AmpC enzymes in *Enterobacter* species is controlled by chromosomal AmpC genes (3) and are considered in serious infections dependent on *E. aerogenes* and *E. cloacae* (14). Plasmid-mediated AmpC strains of *K. pneumoniae* and *K. oxytoca* are widely associated with MDR phenotypes (13). Plasmid genes (transferable) have been acquired by bacterial species, such as *E. coli* and *K. pneumoniae*, which lack chromosomal genes (14).

Various methods have been introduced for the detection of chromosomal AmpC using different inducers, such as imipenem, tazobactam, and ceftaxime (15, 16). Plasmid-mediated AmpC beta-lactamases are detected in enzyme extracts of bacteria or with techniques based on using enzyme-inhibitor compounds, such as boronic acid (17).

## 2. Objectives

Since all beta-lactamase genes can be distributed by transmissible genetic elements in bacteria and can cause dissemination of resistance to beta-lactam antibiotics, the present study aimed to investigate the phenotypic detection of different kinds of beta-lactamase enzymes in *Enterobacter* and *Klebsiella* species isolated from clinical specimens.

## 3. Materials and Methods

This cross-sectional study was performed from November 2011 to December 2014 on 108 *Klebsiella* and *Enterobacter* spp. isolated from urine (77, 71.3%), blood (2, 1.9%), wounds (2, 1.9%), secretions (7, 6.5%), feces (5, 4.6%), and other samples (sputum, CSF, discharge, Sheldon catheter) (15, 13.9%). The samples were taken from patients who were referred to the microbiology labs of the affiliated hospitals at Babol University of Medical Sciences in northern Iran.

### 3.1. Diagnosis of Bacteria and Antimicrobial Susceptibility Testing

The isolates were identified with API 20E (bioMérieux SA, France) and differential tests, including KIA, SIM, and MR (Himedea). Antimicrobial susceptibility testing (AST) was done based on the CLSI 2014 protocol. Antibiotics were used for the disk diffusion method on MHA medium (7), including cefotaxime (CTX), ceftazidime (CAZ), cefpodoxime (CPD) (cephalosporin III), cefepime (CPM) (cephalosporin IV), ertapenem (ETP) (carbapenem), and aztreonam (ATM) (monobactam) (MASTDiscs, UK).

### 3.2. Phenotypic Methods

#### 3.2.1. ESBL

The bacteria that were resistant to at least two antibiotics (CAZ  $\leq$  22 mm, CTX  $\leq$  27 mm) were suspected to produce ESBL enzymes. They were examined by confirmatory testing (7).

#### 3.2.2. Mask-ESBL

The Mask-ESBL test was performed on the isolates for which the confirmatory test of ESBL was negative, despite being resistant to two antibiotics, or if the growth-inhibitory zone was not increased around only one type of combination disk (18).

### 3.3. Evaluation of pAmpC using Cefoxitin Agar Medium (CAM AmpC)

#### 3.3.1. Enzyme Extraction

For enzyme extraction, 25  $\mu$ L of bacterial suspension (equal to 0.5 McFarland) was inoculated into 6 mL of Trypticase Soy Broth and incubated for 4 hours at 35°C. Then, it was centrifuged at 3,000 rpm for 15 minutes, and seven freeze/thaw cycles were performed on the precipitate (19).

#### 3.3.2. Evaluation

In this method, an MHA medium containing 6  $\mu$ g/mL of ceftaxime (TAB/0.4 mg, Mast Diagnostics, UK, 325304) was used. A suspension of *E. coli* ATCC 25922 (equivalent to 0.5 McFarland) was cultured using the bacterial lawn technique. A well (5 mm) was created in the agar, to which 30  $\mu$ L of bacterial extract was added. After one night incubation at 35°C, the bacteria that grew around the well were considered plasmid AmpC enzyme producers (Figure 1) (20).

### 3.4. Evaluation of Chromosomal AmpC (iAmpC) by the Disk Method

The isolated bacteria were cultivated on MHA medium by the bacterial lawn technique. Imipenem (as the inducer), CTX, and CTX containing 3APBA (at a concentration of 60 g/L in DMSO) disks were placed on the surface of the

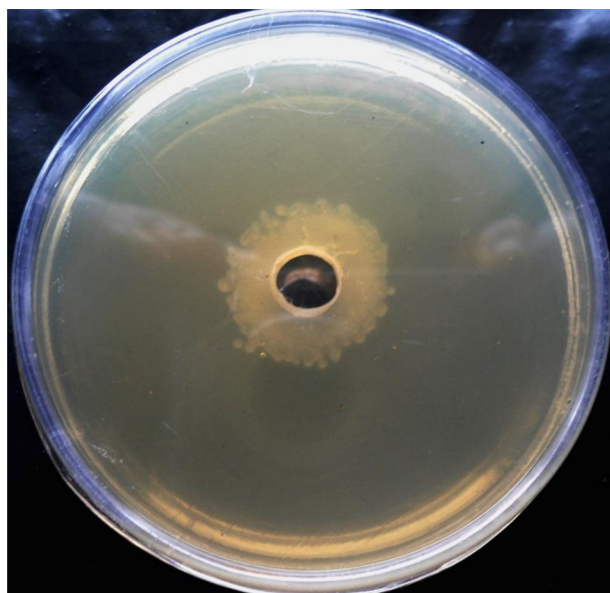


Figure 1. Positive Plasmid AmpC Producing Isolate

agar. After one night incubation at 35°C, the deformity of the growth inhibition zone around the CTX, along with the growth inhibition zone around the CTX + BA disk without any changes, indicated iAmpC-producing bacteria (Figure 2) (21).

#### 3.4.1. Metallo-Beta-Lactamase (IMP-1)

The isolates that were resistant to at least two antibiotics in the first stage (screening) (CAZ  $\leq$  22 mm, CTX  $\leq$  27 mm), were suspected to produce IMP-1 enzymes, and were examined for confirmatory testing (18).

The results were analyzed with SPSS version 22. Descriptive statistics were used.

## 4. Results

Fifty-nine isolated *Klebsiella* spp. bacteria and 49 *Enterobacter* spp. bacteria from the clinical samples (Table 1) were confirmed with API 20E and with differential tests, including KIA, SIM, and MR.

Among the 59 *Klebsiella* spp., 52 (88.1%) and seven (11.9%) were *K. pneumoniae* and *K. oxytoca*, respectively. There were also 37 (75.5%) *E. cloacae*, 10 (20.4%) *E. aerogenes*, and two (4.1%) *E. sakazakii* among the 49 *Enterobacter* spp.

On the first AST, *Klebsiella* spp. were resistant to cefpodoxime (50.8%), ertapenem (13.6%), aztreonam (54.2%), ceftazidime (52.5%), cefotaxime (55.9%), and cefepime (45.8%). The *Enterobacter* spp. were resistant to cefpodoxime (65.3%), ertapenem (28.6%), aztreonam (46.9%), ceftazidime

(57.1%), cefotaxime (63.3%), and cefepime (32.7%). In total, seven (11.9%) of the *Klebsiella* spp. and four (8.2%) of the *Enterobacter* spp. showed resistance to all of the beta-lactam antibiotics. Overall, 38 (64.4%) of the *Klebsiella* spp. and 41 (83.7%) of the *Enterobacter* spp. produced at least one type of beta-lactamase enzyme.

As seen in Table 2, ESBL production was identified in 17 (34.7%) *Enterobacter* spp. and 25 (42.4%) *Klebsiella* spp. (MESBL + ESBL).

Of the *Enterobacter* spp., seven (14.3%) bacteria produced iAmpC, pAmpC, and IMP-1 enzymes simultaneously. In addition, AmpC/MESBL co-production was seen in four (8.2%) of the *Enterobacter* spp. and in one (1.7%) of the *Klebsiella* spp. (Table 3).

It is noteworthy that among the 21 non-lactose-fermenter *Enterobacter* species, 12 (57.1%) produced at least one kind of beta-lactamase enzyme. There were also two bacteria in each genus that were resistant to all antibiotics, without producing any type of beta-lactamase.

## 5. Discussion

MDR microbial strains may occur via mutations of beta-lactamase due to the use of antimicrobial agents (22). The present study showed that 17 (34.7%), 21 (42.8%), 19 (38.8%), and 14 (28.6%) of the *Enterobacter* spp. produced ESBL, pAmpC, IMP-1, and iAmpC, respectively. However, the frequency of beta-lactamase-producing *Klebsiella* spp. were 25 (42.4%), 9 (15.2%), and 12 (23.1%), respectively. Other studies carried out in the different parts of the world, such as India, Nigeria, and Iran, confirm these results, with slight differences (12, 22-25).

There were 22 (42.2%) and 8 (15.4 8%) ESBL and pAmpC enzymes, respectively, produced by *K. pneumoniae* in the current study, which was similar to the results of research into on ESBL-producing *K. pneumoniae* in Mashhad, north-eastern Iran, which found 55 (43%) such species (24), and in Ahvaz, southern Iran, which identified 26 (47.3%) such species (26). However, in Zabol, southeastern Iran, Saeide reported 20 (66.6%) ESBL-producing *K. pneumoniae* in urine samples (27). Yusuf reported the production of ESBL in 10 (20%) and of pAmpC in two (4%) *K. pneumoniae* spp. in Nigeria (25), while Varsha reported production rates of these enzymes at 56 (56%) and 32 (32%), respectively, in India (13).

Paterson reported a high percentage of ESBL-producing *K. pneumoniae* and stated that infections caused by these bacteria must be considered as risk factors for bacterial infections due to ESBL producers in the community (28). Therefore, it seems that performing routine screening tests to determine their prevalence is necessary.

Among the *Enterobacter* spp. isolated in the present study, the AmpC-producing bacteria had the highest preva-

**Figure 2.** Positive Inducible AmpC Producing Isolate

1, imipenem; 2, CTX + boronic acid; 3, CTX.

**Table 1.** Frequency of *Klebsiella* and *Enterobacter* spp. in Clinical Samples

Bacteria	Clinical Specimen						Total
	Urine	Blood	Wound	Secretion	Feces	Other <sup>a</sup>	
<i>Klebsiella</i> spp.	47	1	0	1	2	8	59
<i>Enterobacter</i> spp.	30	1	2	6	3	7	49

<sup>a</sup>Sputum, CSF, discharge, Sheldon catheter.

**Table 2.** Frequency of ESBL, MESBL, pAmpC, iAmpC, and IMP-1 Production in Isolated *Klebsiella* and *Enterobacter* spp.<sup>a</sup>

Bacteria	Enzyme					Total
	ESBL	MESBL <sup>b</sup>	pAmpC	iAmpC	IMP-1	
<i>K. pneumonia</i>	20 (38.5)	2 (3.4)	8 (15.4)	0	12 (23.1)	52 (100)
<i>K. oxytoca</i>	3 (42.9)	0	1 (14.3)	0	0	7 (100)
<i>E. aerogenes</i>	4 (40)	0	3 (30)	1 (10)	5 (50)	10 (100)
<i>E. cloacae</i>	6 (16.2)	7 (18.9)	18 (48.6)	12 (32.4)	14 (37.8)	37 (100)
<i>E. sakazakii</i>	0	0	0	1 (50)	0	2 (100)

<sup>a</sup>Values are expressed as No. (%).

<sup>b</sup>Mask ESBL.

lence (71.4%). In addition, 39.3% of the *Enterobacter* spp. were susceptible to cephalosporins. Some cephalosporins lead to the production of mutant strains that contain the constitutive production of high levels of AmpC enzyme (5, 28, 29). The most common cause of AmpC overexpression

in bacteria isolated from clinical specimens is the mutation of AmpD enzyme inside the cytoplasm of the bacterial cell (20). The transfer of AmpC genes to plasmids can increase pAmpC-producing bacteria.

In this study, the prevalence of *E. cloacae* isolates pro-



**Table 3.** Frequency of ESBL, MESBL, pAmpC, iAmpC, and IMP-1 Co-Producing *Klebsiella* and *Enterobacter* spp.

Bacteria	Enzyme									
	E + IM	E + pA	ME + pA	iA + ME	pA + IM	iA + IM	iA + pA	E + pA + IM	iA + pA + ME	iA + pA + IM
<i>Klebsiella</i> spp.	1 (1.7)	1 (1.7)	1 (1.7)	0 (0)	3 (5.1)	0	0	1 (1.7)	0	0
<i>Enterobacter</i> spp.	1 (2)	3 (6.1)	3 (6.1)	1 (2)	0	2 (4.1)	2 (4.1)	0	0	7 (14.3)

Abbreviations: E, ESBL; iA, inducible AmpC; IM, IMP-1; ME, mask-ESBL; pA, pAmpC.

**Table 4.** Frequency of Pure ESBL, MESBL, pAmpC, iAmpC, and IMP-1 Production in Isolated *Klebsiella* and *Enterobacter* spp.

Bacteria	Enzyme				
	ESBL	IMP-1	pAmpC	Mask-ESBL	iAmpC
<i>Klebsiella</i> spp.	21 (35.5)	7 (11.9)	3 (5.1)	0	0
<i>Enterobacter</i> spp.	6 (12.2)	8 (16.3)	4 (8.2)	2 (4)	2 (4.1)

ducing iAmpC was 32.4%. In Lindsay's study in 2011, the incidence of iAmpC production in this genus, after treatment with broad-spectrum antibiotics, was reported as 10% - 19% (30). It appears that over the years from 2011 to 2014, the prevalence of these bacteria significantly increased.

Another important finding of this study was the presence of bacteria that simultaneously produced various beta-lactamases (*Enterobacter* 38.7%, *Klebsiella* 11.9%). These bacteria can limit clinical use of beta-lactam antibiotics and lead to laboratory diagnostic problems (31). This means that the identification of bacteria producing single enzymes or combinations of them is necessary for the control and treatment of infections (20).

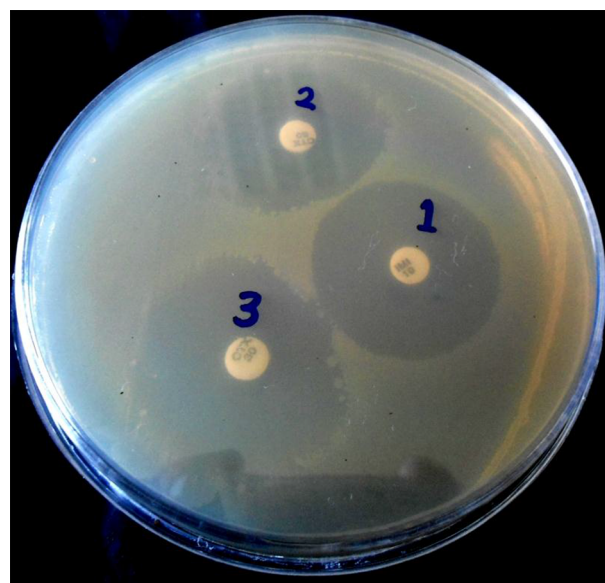
According to the present study, *Enterobacter* spp. co-production of iAmpC along with other beta-lactamases was found in 12 (24.5%) strains. These bacteria can lead to an increased rate of treatment failure in infected patients (5).

Another notable finding of this study was the fairly remarkable percentage of IMP-1-producing bacteria in combination with other beta-lactamase enzymes (*Klebsiella* 8.5%, *Enterobacter* 20.4%), and the proportion of *Enterobacter* spp. that produced plasmid and chromosomal AmpC along with IMP-1 enzymes was 14.3% (Table 3). The simultaneous production of carbapenemase and pAmpC can occur in strains that may lack outer membrane porin (3). Identifying the production of each mentioned enzyme in a bacterium can help physicians in choosing the appropriate antibiotic for treating infections caused by these organisms.

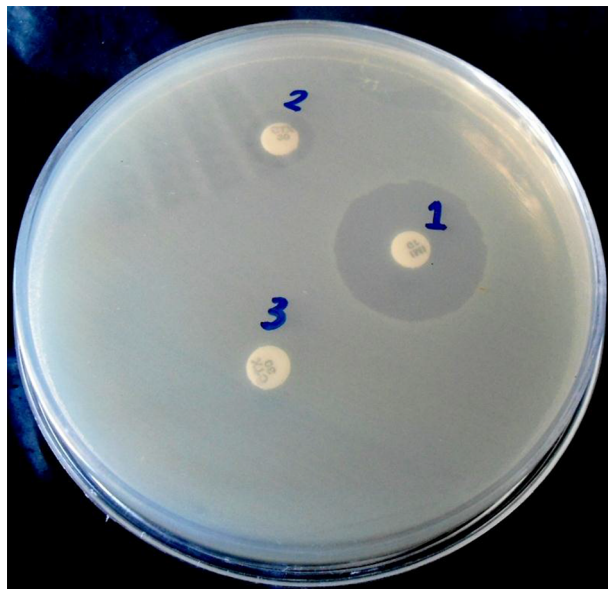
There were several contemplative findings in this study, including the presence of a significant percentage (57.1%) of beta-lactamase production in negative-lactose-fermentation *Enterobacter* spp. Further research should be carried out on the probable relationship between  $\beta$ -

lactamase production and the inability of lactose consumption in bacteria. Furthermore, it was observed that two non-beta-lactamase-producing bacteria of each genus were resistant to all antibiotics, which drew attention to other resistance mechanisms, such as efflux pump activity or the lack of outer membrane proteins (18).

In addition to the findings described above, 26 bacteria of each genus showed resistance against cefotaxime disks (without a growth inhibition zone), which could not be detected by the iAmpC disk method; therefore, it appears this method is not suitable for the detection of the iAmpC enzyme (Figures 3 and 4).

**Figure 3.** Negative Inducible AmpC Producing Isolate

1, imipenem; 2, CTX + boronic acid; 3, CTX.

**Figure 4.** Non Detectable Isolate by Inducible Method

1, imipenem; 2, CTX + boronic acid; 3, CTX.

### 5.1. Conclusions

According to the findings of this study, it is necessary to pay special attention to ESBL production in *Klebsiella* spp., while in *Enterobacter* spp., it is essential to search for AmpC production (chromosomal and plasmid), and there should be evaluation of multiple beta-lactamases produced by a bacterium in order to choose the proper treatment. In addition, the genotypic evaluation of bacteria producing a variety of beta-lactamases will help to prevent the spread of these genes among gram-negative bacteria in certain geographic areas.

### Acknowledgments

The authors would like to thank Babol University of Medical Sciences for financial support, and the personnel of the microbiology laboratory of the affiliated hospitals of Babol University of Medical Sciences for their help in this study. Also, our special thanks go to Dr. Evangeline Foronda for her excellent comments on this article.

### References

- Mahon CR, Lehman DC, Manuselis Jr G. Textbook of diagnostic microbiology. Elsevier Health Sciences; 2014.
- Jones RN, Flonta M, Gurler N, Cepparulo M, Mendes RE, Castanheira M. Resistance surveillance program report for selected European nations (2011). *Diagn Microbiol Infect Dis*. 2014;**78**(4):429-36. doi: [10.1016/j.diagmicrobio.2013.10.008](https://doi.org/10.1016/j.diagmicrobio.2013.10.008). [PubMed: [24440509](https://pubmed.ncbi.nlm.nih.gov/24440509/)].
- Mohamudha Parveen R, Harish BN, Parija SC. AmpC Beta lactamases among gram negative clinical isolates from a tertiary hospital, South India. *Braz J Microbiol*. 2010;**41**(3):596-602. doi: [10.1590/S1517-83822010000300009](https://doi.org/10.1590/S1517-83822010000300009). [PubMed: [24031534](https://pubmed.ncbi.nlm.nih.gov/24031534/)].
- Ahmad SS, Ali FA. Detection of esbl, AmpC and metallo beta-lactamase mediated resistance in gram-negative bacteria isolated from women with genital tract infection. *Eur Sci J*. 2014;**10**(9).
- Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and Carbapenemase issues. *J Clin Microbiol*. 2010;**48**(4):1019-25. doi: [10.1128/JCM.00219-10](https://doi.org/10.1128/JCM.00219-10). [PubMed: [20181902](https://pubmed.ncbi.nlm.nih.gov/20181902/)].
- Willems E, Cartuyvels R, Magerman K, Raymaekers M, Verhaegen J. Comparison of different phenotypic assays for the detection of extended-spectrum beta-lactamase production by inducible AmpC-producing Gram-negative bacilli. *Eur J Clin Microbiol Infect Dis*. 2013;**32**(4):549-55. doi: [10.1007/s10096-012-1772-x](https://doi.org/10.1007/s10096-012-1772-x). [PubMed: [23135757](https://pubmed.ncbi.nlm.nih.gov/23135757/)].
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 24th ed.; 2014 Jan. M100-S24.
- Fukigai S, Alba J, Kimura S, Iida T, Nishikura N, Ishii Y, et al. Nosocomial outbreak of genetically related IMP-1 beta-lactamase-producing *Klebsiella pneumoniae* in a general hospital in Japan. *Int J Antimicrob Agents*. 2007;**29**(3):306-10. doi: [10.1016/j.ijantimicag.2006.10.011](https://doi.org/10.1016/j.ijantimicag.2006.10.011). [PubMed: [17276038](https://pubmed.ncbi.nlm.nih.gov/17276038/)].
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility test. Approved standard M02-A1; 2012.
- Manoharan A, Chatterjee S, Mathai D, Sari Study Group. Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. *Indian J Med Microbiol*. 2010;**28**(3):241-4. doi: [10.4103/0255-0857.66486](https://doi.org/10.4103/0255-0857.66486). [PubMed: [20644314](https://pubmed.ncbi.nlm.nih.gov/20644314/)].
- Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol*. 2000;**38**(1):40-3. [PubMed: [10618060](https://pubmed.ncbi.nlm.nih.gov/10618060/)].
- Rudresh SM, Nagarathnamma T. Two simple modifications of modified three-dimensional extract test for detection of AmpC beta-lactamases among the members of family Enterobacteriaceae. *Chron Young Sci*. 2011;**2**(1):42.
- Gupta V, Kumarasamy K, Gulati N, Garg R, Krishnan P, Chander J. AmpC beta-lactamases in nosocomial isolates of *Klebsiella pneumoniae* from India. *Indian J Med Res*. 2012;**136**(2):237-41. [PubMed: [22960890](https://pubmed.ncbi.nlm.nih.gov/22960890/)].
- Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D, Khilnani GC, et al. Phenotypic & molecular characterization of AmpC beta-lactamases among *Escherichia coli*, *Klebsiella* spp. & *Enterobacter* spp. from five Indian Medical Centers. *Indian J Med Res*. 2012;**135**:359-64. [PubMed: [22561623](https://pubmed.ncbi.nlm.nih.gov/22561623/)].
- Dunne WJ, Hardin DJ. Use of several inducer and substrate antibiotic combinations in a disk approximation assay format to screen for AmpC induction in patient isolates of *Pseudomonas aeruginosa*, *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp. *J Clin Microbiol*. 2005;**43**(12):5945-9. doi: [10.1128/JCM.43.12.5945-5949.2005](https://doi.org/10.1128/JCM.43.12.5945-5949.2005). [PubMed: [16333080](https://pubmed.ncbi.nlm.nih.gov/16333080/)].
- Mirelis B, Rivera A, Miro E, Mesa RJ, Navarro F, Coll P. A simple phenotypic method for differentiation between acquired and chromosomal AmpC beta-lactamases in *Escherichia coli*. *Enferm Infecc Microbiol Clin*. 2006;**24**(6):370-2. [PubMed: [16792938](https://pubmed.ncbi.nlm.nih.gov/16792938/)].
- Lee W, Jung B, Hong SG, Song W, Jeong SH, Lee K, et al. Comparison of 3 phenotypic-detection methods for identifying plasmid-mediated AmpC beta-lactamase-producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* strains. *Korean J Lab Med*. 2009;**29**(5):448-54. doi: [10.3343/kjlm.2009.29.5.448](https://doi.org/10.3343/kjlm.2009.29.5.448). [PubMed: [19893354](https://pubmed.ncbi.nlm.nih.gov/19893354/)].

18. Shahandeh Z, Sadighian F, Rekabpou KB. Phenotypic study of Extended-spectrum beta-lactamase, AmpC and Carbapenemase among *E. coli* clinical isolates in affiliated hospitals of Babol University of Medical Sciences. *Int J Health System Disaster Manag.* 2015;**3**(2):74.
19. Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC beta-lactamases among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates at a veterans medical center. *J Clin Microbiol.* 2000;**38**(5):1791-6. [PubMed: [10790101](#)].
20. Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev.* 2009;**22**(1):161-82. doi: [10.1128/CMR.00036-08](#). [PubMed: [19136439](#)] Table of Contents.
21. Shoorashetty RM, Nagarathnamma T, Prathibha J. Comparison of the boronic acid disk potentiation test and cefepime-clavulanic acid method for the detection of ESBL among AmpC-producing Enterobacteriaceae. *Indian J Med Microbiol.* 2011;**29**(3):297-301. doi: [10.4103/0255-0857.83917](#). [PubMed: [21860114](#)].
22. Nagdeo NV, Kaore NM, Thombare VR. Phenotypic methods for detection of various  $\beta$ -lactamases in Gram-negative clinical isolates: Need of the hour. *Chron Young Sci.* 2012;**3**(4):292.
23. Akbari M, Amir Mozaffari N, Peeri Dogah H. Prevalence of Extended-Spectrum Beta Lactamase in Enterobacteriaceae Isolated from Urinary Tract Infections in Ardabil, Iran. *J Ardabil Univ Med Sci.* 2014;**14**(3):285-91.
24. Fatemeh A, Emran A, Elnaz K, Mohammad JGS, Mahboubeh N. The frequency of extended spectrum beta lactamase (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: a report from Mashhad. *Iran J Med Bacteriol.* 2012;**1**(3):12-9.
25. Yusuf I, Haruna M, Yahaya H. Prevalence and antibiotic susceptibility of AmpC and ESBLs producing clinical isolates at a tertiary health care center in Kano, north-west Nigeria. *Afr J Clin Exp Microbiol.* 2013;**14**(2):109-19.
26. Khosravi AD, Hoveizavi H, Mehdinejad M. Prevalence of *Klebsiella pneumoniae* Encoding Genes for Ctx-M-1, Tem-1 and Shv-1 Extended-Spectrum Beta Lactamases (ESBL) Enzymes in Clinical Specimens. *Jundishapur J Microbiol.* 2013;**6**(10).
27. Saeidi S, Shayan NR. S. Antimicrobial Susceptibility and Distribution of TEM and CTX-M Genes among ESBL-producing *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Causing Urinary Tract Infections. *Zahedan J Res Med Sci.* 2013;**16**(4):1-5.
28. Paterson DL. Resistance in gram-negative bacteria: enterobacteriaceae. *Am J Med.* 2006;**119**(6 Suppl 1):S20-8. doi: [10.1016/j.amjmed.2006.03.013](#). [PubMed: [16735147](#)] discussion S62-70.
29. Miossec C, Claudon M, Levasseur P, Black MT. The beta-lactamase inhibitor avibactam (NXL104) does not induce ampC beta-lactamase in *Enterobacter cloacae*. *Infect Drug Resist.* 2013;**6**:235-40. doi: [10.2147/IDR.S53874](#). [PubMed: [24348054](#)].
30. Nicolle LE. Antimicrobial Resistance. *Eur Infect Dis.* 2011;**5**(2):92-7.
31. Shrestha S, Chaudhari R, Karmacharya S, Mishra SK, Bam N, Banjade N, et al. Prevalence of nosocomial lower respiratory tract infections caused by Multi-drug resistance pathogens. *J Inst Med.* 2012.