IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 12 Ver. I (Dec. 2015), PP 19-24
www.iosrjournals.org

The occurrence of AmpC β-lactamase and ESBL producing Gram-negative bacteria by a simple and convenient screening method and its suitability in routine use

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Abstract:
Background: *All clinical samples (e.g. Pus, urine, sputum, blood, tracheal aspirate, peritoneal fluid, catheter tip, ET tip tracheostomy aspirate) etc are sent for culture and antibiotic sensitivity in a clinical microbiology laboratory to achieve etiological diagnosis.*
Aims: *The study was done to detect the AmpC β-lactamase and ESBL producing gram negative bacteria from different clinical samples. This study included AmpC disc screening test and found out that the modified three dimensional tests using whole cell growth gives clearer result.*
Setting and Design: *A 6-month prospective analytical study was done in a tertiary care hospital.*
Materials and Methods: *A total of 141 sample, non-enteric Gram-negative clinical isolates obtained from different clinical samples (e.g. Pus, urine, sputum, blood, tracheal aspirate, peritoneal fluid, catheter tip, ET tip tracheostomy aspirate, etc) The organism included E.coli , Pseudomonas spp , Klebsiella pneomoniae , Klebsiela spp , Acinetobacter spp , Proteus spp ,Citrobacter spp , and Enterobacter spp . Antimicrobial susceptibility of the strains were put according to CLSI guidelines, for ESBL and AmpC enzyme detection source of the discs were HiMedia 19*
Result: *Among all the strains out of 141 isolates were tested 47 are AmpC producer and 94 are AmpC non-producer. Maximal incidence of AmpC producers was found among E. coli (20) followed by Klebsiella pneumonia (10). Isolates were tested for ESBL detection and 91 (64.53%) were found to be ESBL producer and 50 (35.46%) were ESBL non-producer. E. coli was the highest occurrence of ESBL producer (45.07%), followed by Klebsiella pneumonia (29.57%).*
Conclusion: *Modified three dimensional test using whole cell growth in peptone water is well comparable to the modified 3 dimensional test using cell extract method and is better than AmpC disc screening assay at the same time is very cost effective and simple assay to be used for routine reporting of AmpC β-lactamase.*

I. Introduction

The first bacterial enzyme reported to destroy penicillin was the AmpC β-lactamase of *Escherichia coli* [(1)]. Mutation with stepwise-enhanced resistance were termed as *ampA* and *ampB* [(2,3)]. A mutation in an *ampA* strain that resulted in reduced resistance was then designated as *ampC.* In the Ambler structural classification of β-lactamases[(4)], AmpC enzyme belong to class C, while in the functional classification scheme of Bush et al. [(5)], they were designated to group 1.

They are active on penicillins but even more active on cephalosporins and can hydrolyze cephmycins such as cefoxitin and cefotetan; oxyiminocephalosporins such as ceftazidime, cefotaxime, and ceftriaxone; and monobactams such as aztreonam but at slow rate [(6)]. Inhibitor of class A enzyme such as clavulanic acid, sulbactams, and tazobactam have much less effect on AmpC β-lactamase, although some are inhibited by tazobactam and sulbactam[(7,8,9)]. AmpC β-lactamase are poorly inhibited by *p*-chloromercuribenzoate and not at all by EDTA. Cloxacillin, oxacillin, and aztreonam are good inhibitors [(5)].

The predominant mechanism for resistant to β-lactam antibiotics in gram-negative bacteria is the synthesis of β-lactamase. To meet this challenge, β-lactamase with greater β-lactamase stability, including cephalosporins, carbapenems, and monobactams, were introduced in the 1980s.

There is presently no CLSI or other approved criteria for AmpC β-lactamase detection [(11)], however various workers have detected AmpC enzyme by three dimensional assay using cell extract, and AmpC disc screening assay etc. The true rate of occurrence of AmpC β-lactamases in different organisms, including members of *Enterobacteriacae*, remains unknown Coudron et al.[(12)] used the standard disc diffusion breakpoint for cefoxitin (zone diameter <18mm) to screen isolates, and used a three dimensional extract test as a confirmatory test for isolates that harbour AmpC β-lactamases. The disc diffusion test was found to be non specific and there is always a search for newer methods and the aim to make existing methods more user-

friendly to detect these enzymes for use in routine diagnostic laboratories. The main aim is to pass on the benefit to the ultimate beneficiary, the patient, as quickly as possible and, obviously, at lowest possible cost[13].

The present study was designed to determine the occurrence of β-lactamases from Barabanki region. In present study used whole cell growth in place of cell extract. The three dimensional test being made more user friendly to be applied as a phenotypic screening method for the detection of AmpC-harbouring Gram negative isolates.

II. Material And Method

A total of 141 sample, non-enteric Gram-negative clinical isolates obtained from different clinical samples (e.g. Pus, urine, sputum, blood, tracheal aspirate, peritoneal fluid, catheter tip, ET tip tracheostomy aspirate, etc) in clinical bacteriology laboratory Department of Microbiology Mayo Medical College and Hospital, during June 2014 to January 2015 were included in this study.

The organism included *E.coli* (51 isolates), *Pseudomonas spp* (32 isolates), *Klebsiella pneomoniae* (23 isolates), *Klebsiela spp* (18 isolates), *Acinetobacter spp* (10 isolates), *Proteus spp* (5 isolates),C*itrobacter spp* (one isolate), and *Enterobacter spp* (one isolate).

The isolates were identified by standard microbiological techniques used in the laboratory.

Antimicrobial susceptibility of the strains were put according to CLSI guidelines, source of the discs were HiMedia (19)

a. ESBL Detection

Irrespective of their antimicrobial susceptibility profile all isolate of *E.coli* and *Klebsiella spp* and *Proteus spp* were tested for ESBL production using Ceftazidime (30µg) discs and Ceftazidime / Clavulanic acid (30/10 µg) discs were used as, recommended by CLSI Guideline. *Eschericia coli* ATCC 25922 was included in the study for ensuring quality control. *Klebsiella pneumoniae* 700603 ATCC were used as an ESBL Positive control. Increase in zone diameter of ≥5 mm for Ceftazidime/Clavulanic acid versus its zone when tested alone was a positive test for ESBL producer.

b. AmpC enzyme Detection : three-dimensional extract test

AmpC enzyme production was detected by a modified three-dimensional extract test

Fresh overnight growth from Mueller-Hinton agar was transferred in peptone water and incubated it for 2-4 hours at 37ºC.

Lawn culture of *E. coli* ATCC 25922 were prepared on Mueller-Hinton Agar plate and Cefoxitin (30 µg) disc were placed on the plate.

Linear slits (3cm) were cut using a sterile lancet 3mm away from the periphery of Cefoxitin disc.

Small circular wells were made on the slits at 5mm distance, inside the outer edge of the slit, by stabbing the cut end of micropipette tip.

The wells were loaded slowly with peptone water growth in 10 µL increments until the well was filled to the top, taking care to not overflow.

The plates were kept upright for 5-10 minutes until the solution dried, and the plates were incubated at 37ºC. overnight.

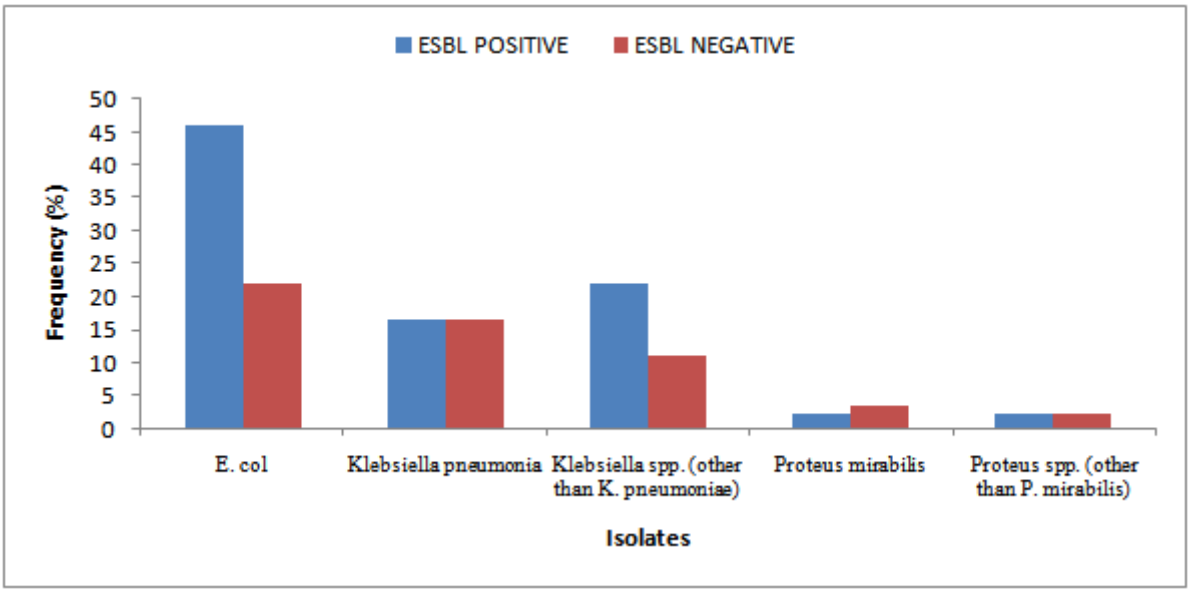
The isolates showing clear distortion of zone of inhibition of cefoxitin disc were taken as AmpC producers. The isolate with no distortion were taken as AmpC non-producers.

III. Result

a. ESBL Profile

Among all the strains tested out of 141 isolates were tested for ESBL detection and 91 (64.53%) were found to be ESBL producer and 50 (35.46%) were ESBL non-producer. *E. coli* was the highest occurrence of ESBL producer (45.07%), followed by *Klebsiella pneumoniae* (29.57%).

ISOLATES	ESBL POSITIVE N (%)	ESBL NEGATIVE N (%)
E. col	42(46.15)	20(21.97)
Klebsiella pneumonia	25(16.48)	15(16.48)
Klebsiella spp. (other than *K. pneumoniae*)	20(21.97)	10(10.98)
Proteus mirabilis	2(2.19)	3(3.29)
Proteus spp. (other than *P. mirabilis*)	2(2.19)	2(2.19)



b. AmpC β-lactamase Profile

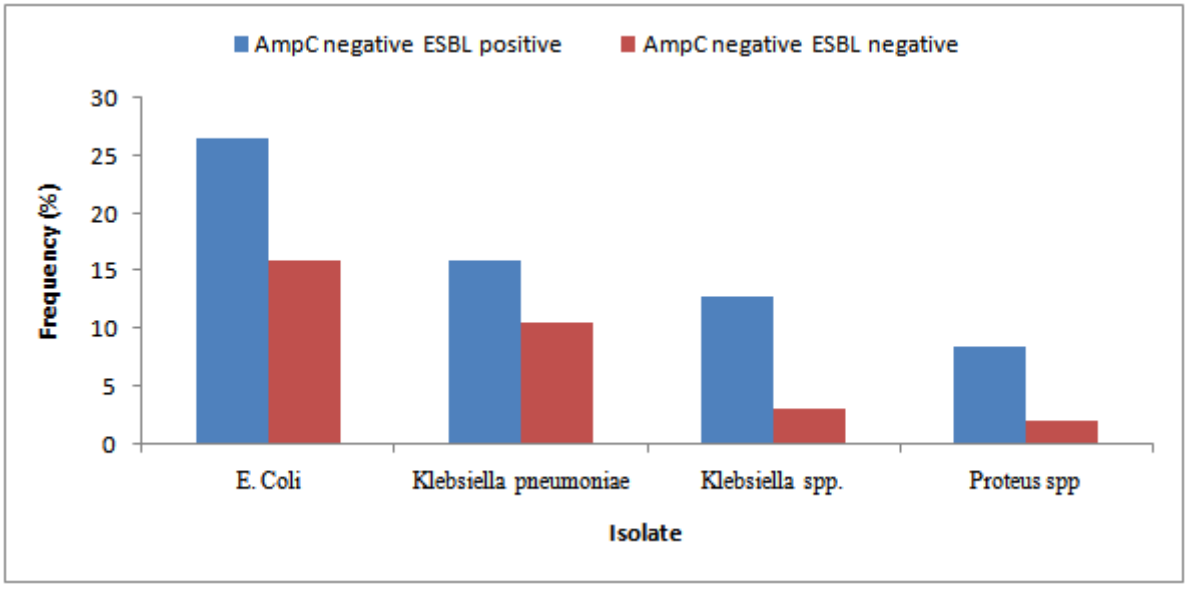
Among all the strains out of 141 isolates were tested 47 are AmpC producer and 94 are AmpC non-producer. Maximal incidence of AmpC producers was found among *E. coli* (20) followed by *Klebsiella pneumoniae* (10).

ISOLATE n = 141	AmpC producer n = 47	AmpC non-producer n = 94	Total
E. coli	20	42	62
Klebsiella pneumonia	10	25	35
Klebsiella spp.	52	20	25
Pseudomonas spp.	5	2	7
Acinetobacter spp.	3	1	4
Proteus spp.	2	4	6
Citrobacter spp.	1	0	1
Enterobacter spp.	1	0	1

c. AmpC negative ESBL profile

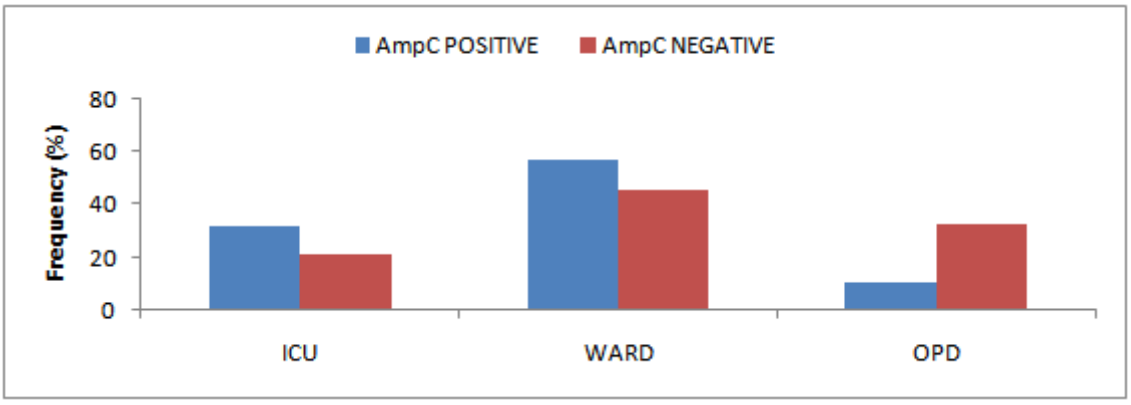
A total of 94 AmpC non producer were tested for ESBL production and out of them 64 (68.08%) were found to be ESBL producer while 30 (31.91%) were ESBL non producer.

Isolate n = 94	AmpC negative ESBL positive n = 64 (68.08%)	AmpC negative ESBL negative n = 30 (31.91%)
E. Coli	25 (26.59%)	15 (15.95%)
Klebsiella pneumoniae	15 (15.95%)	10 (10.63%)
Klebsiella spp.	12 (12.76%)	3 (3.19%)
Proteus spp	8 (8.51%)	2 (2.12%)



d. Ward wise distribution

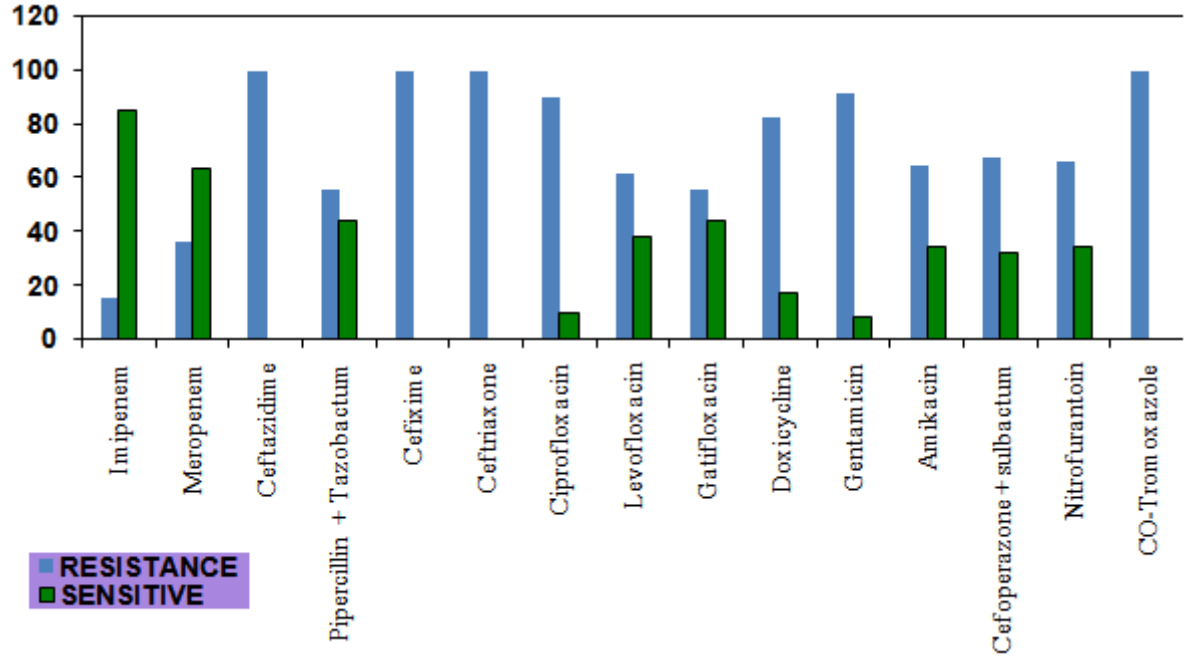
WARD / OPD / ICU N = 141	AmpC POSITIVE n = 47 (%)	AmpC NEGATIVE n = 94 (%)
ICU	15 (31.91%)	20 (21.27%)
WARD	27 (57.44%)	43 (45.74%)
OPD	5 (10.63%)	31 (32.97%)



e. **Sample wise distribution**

SAMPLE n = 141 (%)	AmpC positive n = 47 (33.33%)	AmpC negative n = 94 (66.66%)
PUS	20	40
URINE	13	34
BLOOD	3	6
SPUTUM	2	4
PERITONEAL FLUID	1	0
TRACHEAL ASPIRATE	6	2
ET TUBE	1	3
CATHETER TIP	1	3
VAGINAL SAWB	0	2

f. Antibiotic Sensitivity Profile



Total (N=141)	Imi	Mrp	Cef	Pip	Cef	Ctx	Cip	Levo	Gati	Doxi	Gen	Amk	Cpz	Nit	Cot
Resistant	15.38	36.58	100	55.81	100	100	90	61.9	55.82	82.92	91.89	64.7	68	66	100
Sensitive	84.61	63.42	0	44.19	0	0	10	38.1	44.18	17.08	8.1	34.3	32	34	0

IV. Keywords:

Ak- Amikacin, Cef- Ceftazidime, Cef- Cefixime, Ctx- Ceftriaxone, Cip- Ciprofloxacin, Cpz- Cefoperazone, Cot- Co-Tromoxazole, Dox- Doxicycline , Gati- Gatifloxacin, Gen- Gentamicin, Imi- Imipenem, Levo- Levofloxacin, Mrp- Meropenem, Nit- Nitrofurantoin, , Pip- Pipercillin

V. DISCUSSION

AmpC and ESBL producing strains all over the world , it is necessary to know the prevalence of these strains in hospitals.The occurrence of AmpC beta lactamase (33.33) in our isolate to be quite high. Also high occorrence of ESBL (64.53%) is seen in our hospital. Maximal incidence of AmpC producers was found among *E. coli* (20) followed by *Klebsiella pneumoniae* (10). Maximal incidence of ESBL producers was found among *E. coli* (42) followed by *Klebsiella pneumoniae* (25). The highest incidence was found in the sample Pus **20** (42.55%), then in Urine **13** (27.65%), followed by Tracheal aspirate **6** (12.76%), then in blood **3** (6.3%). The highest resistance rate was found in cephalosporins like Ceftazidime, Ceftriaxone, & CO-Trimoxazole (100%).

The lowest resistance rate was found in Imipenem 16.39% followed by Meropenem 36.68%. Highest incidence was found in patient those who was admitted in ward i.e, **27**(57.44%) followed by ICUs **15**(31.91%), then in OPD **5**(10.63%). Out of all **AmpC negative** strains 94were tested for ESBL detection and from them 64 (68.08%) were **ESBL positive** and **30 (31.91%)**were **ESBL negative.**

Among the ICUs the highest rate was found in SICU, followed by NICU and MICU.

VI. Conclusion

Various researchers have tried the three dimensional test as well as AmpC disc test for screening of AmpC β lactamases but till date no satisfactory technique has been found for routine use. This study included AmpC disc screening test and found out that the modified three dimensional test using whole cell growth gives clearer result. Modified three dimensional test using whole cell growth in peptone water is well comparable to the modified 3 dimensional test using cell extract method and is better than AmpC disc screening assay at the same time is very cost effective and simple assay to be used for routine reporting of AmpC β-lactamase.

References

[1]. **Abraham, E. P., and E. Chain.** 1940. an enzyme of bacteria able to destroy penicillin. Nature **146:**837.
[2]. **Eriksson-Grenberg, K. G.** 1968. Resistance of *Escherichia coli* to penicillins. II. An improve mapping of the AmpA gene. Genet. Res. **12:**147-156.
[3]. **Eriksson-Grenberg, K. G., H. G. Boman, J. A. Jansson, and B. C. Herold.** 2006. Resistance of *Escherichia coli* to penicillins. I. Genetic study of some ampicillin-resistant mutants. J. Bacteriol. **90:**54-62.
[4]. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. Lond. B. **289:**321-331.
[5]. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211-1233
[6]. **Galleni, M., and J. M. Frere.** 1988. A survey of the kinetic parameters of class C β-lactamases. Cephalosporins and other β-lactam compounds. Biochem. J. **255:**123-129.
[7]. **Bush, K., C. Macalintal, B. A. Rasumussen, V. J. Lee, and Y. Yang.** 1993. Kinetic interactions of tazobactam with β-lactamases from all major structural classes. Antimicrib. Agents Chemother. **37:**851-858.
[8]. **Kazmierczak, A., X. Cordin., J. M. Duez, E. Siebor, A. Pechinot, and J. Sirot.** 1990. Differences between clavulanic acid sulbactam in induction and inhibition of cephalosporinases in enterobacteria. J. Int. Med. Res. **18**(Suppl. 4)**:**67D-77D.
[9]. **Monnaie, D., and J. M. Frere.** 1993. Interaction of clavulanate with class C β-lactamases. FEBS Lett. **334:**269-271.
[10]. **Alain Philippon., Guillaume Arlet., George A. Jacoby.** 2002. Plasmid-determined AmpC-Type β-lactamases. Antimicrob. Agents Chemother. **46:**1-11.
[11]. **Doi, Y., and D. L. Paterson.** 2007. Detection of plasmid-mediated class C β-lactamases. Int. J. Infect. Dis. **11:**191-197.
[12]. **Coudron, P. E., Moland, E. S. and Thomson, K. S.** 2000. Occurrence and detection of AmpC β-lactamases among *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabillis* isolate at a Veterans Medical Center. J. of Clinical Microbiol. **38:**1791-1796.
[13]. **Manchanda, V. and Singh, N. P.** 2003. Occurrence and detection of AmpC β-lactamases among Gram-negative isolates using a modified three-dimensional test. J of Antimicrob. Chemother. **51:**415-418.
[14]. **J. D. D. Pitout, P. G. Le, K. L. Moore, D. L. Church and D. B. Gregson.** 2010. Detection of AmpC β-lactamases among *Escherichia coli, Klebsiella spp., Salmonella spp.* and *Proteus mirabilis* in a regional clinical microbiology laboratory. Clin. Microbiol. Infect.**16:**165-170.
[15]. **Upadhyay at. el.** 2010. Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries. **4(4):**239-242.
[16]. **Hemlata, M. Padma, T.M. Vinodh and A.S. Arunkumar.** 2007. Detection of AmpC beta lactamases production in *Escherichia coli* and *Klebsiella* by an inhibitor based method. Indian J Med. Res. **126:**220-223.
[17]. **Rand KH, Turner B, Seifert H, Hansen C, Jhonson JA, and Zimmer A.** 2011. Clinical laboratory detection of AmpC β-lactamase: does it affect patient outcome?. Am. J. Clin. Pathol. **135(4):**572-576.
[18]. **Perez-Perez F J and Hanson ND.** 2002. Detection of plasmid mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40(6):**2153-2162.
[19]. **Polsfuss, S., Bloemberg, G. V., Giger, J., Meyer, V., Bottger, E. C. and Hombach, M.** 2011. A practical approach for reliable detection of AmpC beta-lactamse producing *Enterobacteriaceae.* J. Clin. Micribiol. **49(8):**2798-2803.
[20]. **Rajni E, Rawat U, Malhotra VL, Mehta G.** 2008. Occurrence and detection of AmpC beta lactamases among clinical isolates of E. coli and K. pneumoniae causing UTI. J. Commun. Dis. **40(1):**21-25.
[21]. **S. Ananthan and A Subha**. 2005. Cefoxitin resistance mediated by loss of a porin in clinical strains of *Klebsiella pneumoniae* and *Eschrrichia coli*. Indian J. Med. Microbiol. **23(1):**20-23.
[22]. **S Singhal, T Mathur, S Khan, DJ Upadhyay, S Chugh, R Gaind, A Ratttan.** 2005. Evaluation of methods for AmpC beta-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian J. Med. Microbiol. **23:**120-124.
[23]. **Steven M. Opal and Aurora Pop-Vicas.** Molecular mechanisms of Antibiotic Resistance in Bacteria. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases 7th ed. Mandell, G. L., Bennett, J. E., Dolin, R.(editors). Churchill Livingstone, 2010.
[24]. **George A. Jacoby.** 2009. AmpC β-lactamases. Clin. Microbiol. Reviews. **22(1):**161-182.