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EFFECTIVENESS OF COMBINATION DRUGS IN EXTENDED SPECTRUM BETA LACTAMASES PRODUCING GRAM NEGATIVE ISOLATES- EXPERIENCE IN A TERTIARY CARE HOSPITAL OF UTTARAKHAND

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ABSTRACT

Resistance by virtue of production of ESBL in gram negative bacteria is an emerging problem leading to therapeutic failure when β-lactam drugs are used. However, their presence may be missed while using routine disc diffusion methods for antibiotic susceptibility testing. All isolates of *E. coli*, *K. pneumoniae, K. oxytoca* and *P. mirabilis* were studied for ESBL production by CLSI recommended Phenotypic confirmatory test (PCT) and Double disk approximation test (DDAT). Out of 10,055 clinical samples,1738 study isolates were obtained of which 1196 were *E. coli* (68.82%), 465 *K. pneumoniae* (26.75%), 17 *K. oxytoca* (0.97%) and 60 *P. mirabilis* (3.46%). 43.10% (749 out of 1738) study isolates were confirmed to be ESBL producers. The best sensitivity was found for Tigecycline (100%) followed by Polymyxin B (91%) and Imipenem (81%). To conclude, we found a very high prevalence of ESBL producers (43.10 %) in our hospital especially from the IPD wards.

KEYWORDS; Extended Spectrum Beta Lactamases, ESBL, Phenotypic confirmatory test, PCT, Double disk approximation test, DDAT

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INTRODUCTION

The discovery of penicillin changed the course of history and forever altered the treatment of bacterial infections. Since then the introduction of every new antimicrobial is soon followed by discovery of its resistance[1]. In Gram negative pathogens, β-lactamase production remains the most important contributing factor to β-lactam resistance which is chiefly due to plasmid mediated Extended Spectrum β-Lactamase (ESBL) production. They can be found in a variety of Enterobacteriaceae species, however, the majority of ESBL producing strains are *K. pneumoniae, K. oxytoca* and *E. coli*[2] .
Resistance by virtue of production of ESBL in gram negative bacteria is an emerging problem leading to therapeutic failure when β-lactam drugs are used. However, their presence may be missed while using routine disc diffusion methods for antibiotic susceptibility testing and special methodology is required to detect their presence. Hence, the following study was conducted to estimate the burden of these highly drug resistant gram negative bacteria in clinical specimens received in the Bacteriology laboratory at our tertiary care center to guide the clinicians in prescribing appropriate antibiotic therapy.

MATERIALS AND METHODS

The present study was carried out from 1st March 2009 to 28th February 2010.
All isolates of *E. coli*, *K. pneumoniae, K. oxytoca* and *P. mirabilis* obtained from clinical samples like blood, pus, CSF, ascitic fluid, pleural fluid, urine etc. were included in the study.
Particulars of patient, relevant clinical history and reports of investigations were recorded after taking written informed consent. The study was approved by the ethics committee of the institution.

Inclusion criteria

- All the culture isolates of *E. coli, K. pneumoniae, K. oxytoca* and *P. mirabilis* obtained from clinical specimens in bacteriology laboratory were included in the study [3].

Ten thousand and fifty five clinical samples were received in our laboratory during the study period and the maximum number were urine (45.66%) followed by blood (18.54%), pus (15.19%), body fluids (06.78%), respiratory secretions (04.23%), sputum (03.24%), foley catheter (01.48%), vascular devices (01.10%), and etc.(03.78%).
Out of 10,055 clinical samples, 1738 study isolates were obtained of which 1196 were identified by colony morphology and biochemical reactions as *E. coli* (68.82%), 465 as *K. pneumoniae* (26.75%), 17 as *K. oxytoca* (0.97%) and 60 as *P. mirabilis* (3.46%) [3] .

Susceptibility testing:

The susceptibility of the isolates to antimicrobial agents was performed on Muller Hinton agar (Hi Media, Mumbai, India) by modified Kirby Bauer disc diffusion method. Antibiogram was determined for the following antimicrobials - Amikacin(Ak, 30µg), Gentamicin (G, 10 µg), Netilmicin (Nt, 30 µg), Tetracycline (T,30 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (Cf, 5 µg), Ofloxacin(Of, 5 µg), Cotrimoxazole (CO, 25 µg), Amoxicillin-clavulanic acid (AC, 20/10), Piperacillin-tazobactam (Pt, 30 µg), Cefoperazone-sulbactam (CFS, 30 µg), Ticarcillin-clavulanic acid (TC, 10 µg), Imipenem(I, 10 µg), Polymyxin B (PB, 30 µg), Cefepime (CPM, 30 µg), Tigecycline (T, 30 µg), and Nitrofurantoin (Nf, 300 µg) for urinary isolates [3,4].

I. Detection of ESBL producers

A. Screening test:

Screening test for ESBL production was done by the Clinical Laboratory Standards Institute (CLSI) proposed disk diffusion method. Ceftazidime, Cefotaxime, Ceftriaxone and Aztreonam were used as an indicator drugs [4].

B. Confirmatory test for ESBL:

1. Phenotypic confirmatory test:

ESBL producers were detected by the confirmatory method of Clinical and Laboratory Standards Institute (CLSI) using disks of Ceftazidime (30 µg) and Ceftazidime with Clavulanic acid (30 µg and 10 µg) placed at a distance of 20 mm on a lawn culture (0.5 McFarland inoculum size) of suspected ESBL

producer on Mueller-Hinton Agar (MHA). *E. coli* ATCC 25922 was used as the negative control and *K. pneumoniae* ATCC 700603 as the ESBL positive control.
ESBL production was inferred if the inhibition zone increased ≥ 5 mm for Ceftazidime with Clavulanic acid disk in comparison to the Ceftazidime disk alone [4].

2. Double disk approximation test:

A plate was inoculated as for a standard disk diffusion test. Disk containing Aztreonam and Expanded-spectrum Cephalosporins were placed 30mm (center to center) from an Amoxicillin-Clavulanate or Clavulananic acid (10 µg) disk. After overnight incubation the production of an ESBL by the test organism was inferred by the presence of characteristic distortion/expansion of the inhibition zones towards the Clavulanate disk indicative of Clavulanate potentiation of the activity of the test drug [5].

Statistical analysis:

The results were analyzed using simple statistical tests such as averages and percentages. The significance of the results obtained has been statistically evaluated using appropriate tests i.e., Chi-square test, Fisher's Exact test and mean calculations.

RESULTS

Out of 1738 study isolates were obtained in our laboratory of which 1196 were identified as *E. coli* (68.82%), 465 as *K. pneumoniae* (26.75%), 17 as *K. oxytoca* (0.97%) and 60 as *P. mirabilis* (3.46%).
The number of potential ESBL producers identified by CLSI recommended screening test were 1278 (73.53%). Maximum positivity was found amongst the *K. pneumoniae* isolates, that is out of 465 *K. pneumoniae* 80.22 % were found to be positive. [Table 1]

Table 1
Result of Screening test and comparison of CLSI Phenotypic confirmatory test(PCT) with Double disk approximation test (DDAT)

Study isolate	Total number	Screening test positive	Confirmed ESBL Producer				
			Only PCT positive	Only DDAT positive	PCT and DDAT positive	Total Number	%
E. coli	1196	863	18	0	584	**602**	69.76
K. pneumoniae	465	373	1	0	125	**126**	33.78
K. oxytoca	17	13	0	0	7	**7**	53.85
P. mirabilis	60	29	0	0	14	**14**	48.28
Total	**1738**	**1278**	**19**	**0**	**730**	**749**	**58.61**

All these potential ESBL producers were further tested for confirmation of ESBL production by
CLSI recommended Phenotypic confirmatory test (PCT) with combination disk *and* ***double disk approximation test (DDAT).***
Out of 1278 screening positive isolates 749 (58.61%) were confirmed to be ESBL producers by PCT; 602 *E. coli* (69.76%), 126 *K. pneumoniae* (33.78%), 7 *K. oxytoca* (53.85%) and 14 *P. mirabilis* (48.28%) isolates were confirmed ESBL producers.

Overall, 43.10% (749) out of 1738 study isolates were confirmed to be ESBL producers either by PCT or DDAT. Out of these 19 (2.54%) were positive by PCT alone, and 730 (97.46%) by both by PCT and DDAT. [Table 1]
ESBL production was detected in 50.33% of *E. coli,* 27.10% of *K. pneumoniae,* 41.18% of *K. oxytoca* and 23.33% of *P. mirabilis* isolates.
Out of all ESBL producers; maximum were *E. coli* (80.37%) followed by *K. pneumoniae* (16.82%).

Out of the 749 ESBL producers, 521 (69.56%) were obtained from IPD patients and 228(30.44%) were obtained from OPD patients. In IPD patients, the highest isolation rate was from surgical wards.

Maximum isolation rate of ESBL producers was from Body fluids (58.62%), followed by Sputum (49.44%), Pus (44.91%), Urine (44.86%) and Blood (40.63%).

E.coli was found to be the major ESBL producer and was the predominant isolate in all clinical specimens except respiratory secretions where *K.pneumoniae* was found to be predominant. [Table 2]

Table 2
Distribution of ESBL producers from various clinical samples (n = 749)

Clinical samples	Total number of isolates (%)	ESBL producers (n= 749)					
		E. coli (n=602)	*K. pneumoniae* (n=126)	*K. oxytoca* (n=7)	*P. mirabilis* (n=14)	Total	
						No.	%
Urine	963	381	44	3	4	432	44.86
Blood	64	15	11	0	0	26	40.63
Pus	334	115	25	2	8	150	44.91
Body fluids	29	13	4	0	0	17	58.62
Respiratory secretions	126	10	20	0	2	32	25.40
Sputum	89	30	14	0	0	44	49.44
Foley catheter	66	14	5	0	0	19	28.79
Vascular devices	17	2	2	0	0	4	23.53
Others	50	22	1	2	0	25	50.00
Total	**1738**	**602 (34.58%)**	**126 (7.25%)**	**7 (0.4%)**	**14 (0.80%)**	**749**	

In *E. coli* least resistance was seen to Imipenem(02.16%), Piperacillin-tazobactam (08.47%) and Cefoperazone-sulbactam(13.79%), in *K. pneumoniae* and *K. oxytoca* least resistance was seen to Cefoperazone-sulbactam(02.38% and 0%), Imipenem (03.17% and14.29%) and Piperacillin-tazobactam (19.05% and 28.57%) and in *P. mirabilis* least resistance was seen to Piperacillin-tazobactam(0%) and Imipenem (07.14%). [Table 3]

Table 3
Sensitivity pattern of ESBL producers to β-lactam / β-lactamase inhibitors combination and Imipenem. (n=749)

Antimicrobials	ESBL producers (n=749). Number of resistant isolates (%)				
	E. coli (%) (n=602)	*K. pneumoniae* (%) (n=126)	*K. oxytoca* (%) (n=7)	*P. mirabilis* (%) (n=14)	Total resistant isolates (%)
Amoxicillin-clavulanic acid	598 (99.34)	125 (99.20)	7 (100.00)	14 (100.00)	744 (99.33)
Piperacillin-tazobactam	51 (08.47)	24 (19.05)	2 (28.57)	0 (00.00)	77 (10.28)
Cefoperazone-sulbactam	83 (13.79)	31 (02.38)	0 (00)	2 (14.29)	116 (15.49)
Ticarcillin- clavulanic acid	590 (98.00)	124 (98.41)	7 (100.0)	8 (57.14)	729 (97.33)
Imipenem	13 (02.16)	4 (03.17)	1 (14.29)	1 (07.14)	19 (02.54)

In our study, we found out that the sensitivity pattern of ESBL screening test negative isolates was better than ESBL producers (this difference was found to be statistically significant for all classes of antimicrobials; p value < 0.05, Fisher's Exact Test); good sensitivity was seen to Aminoglycosides, Chloramphenicol and Ciprofloxacin.

Among the ESBL producers maximum sensitivity was seen to Tigecycline (100%) followed by Polymyxin B (90.50%, 89.89% respectively).

Among the non β- lactam drugs sensitivity pattern of ESBL producers to Aminoglycosides revealed maximum sensitivity to Amikacin (83.31%) and Netilmicin (73.03%). All urinary isolates including ESBL producers (89.35%) show good sensitivity to Nitrofurantoin. [Table 4]

Table 4

Comparison of sensitivity pattern to non- β-lactam drugs amongst ESBL producers and ESBL screening negative isolates.

	Number of sensitive isolates (%)	
Antimicrobials	**ESBL producers (n=749)**	**ESBL Screening negative (n=460).**
Amikacin	624 (83.31)	418 (90.87)
Gentamicin	205 (27.37)	350 (76.09)
Netilmicin	547 (73.03)	407 (88.48)
Tetracycline	129 (17.22)	235 (51.09)
Chloramphenicol	378 (50.47)	331 (71.96)
Ciprofloxacin	61 (08.14)	294 (63.91)
Ofloxacin	61 (08.14)	271(58.91)
Nitrofurantoin	386 (89.35)	309 (88.82)
Co-trimoxazole	42 (05.61)	226 (49.13)
Polymyxin B	678 (90.50)	-
Tigecycline	749 (100.00)	-

DISCUSSION

There has been sporadic reporting on ESBL producers from different hospitals all over India, but the sample numbers have been low, furthermore no such kind of study has ever been conducted in the state of Uttarakhand. The current study conducted on 1738 isolates from various ward and samples demonstrates the high prevalence of ESBL producers .

***Detection of ESBL producers*:**

Out of 1738 study isolates screened, 1278 were presumptively identified to be ESBL producers on the basis of their resistance to any four screening agents (Ceftazidime, Cephotaxime, Ceftriaxone and Aztreonam). All screening test positive isolates were uniformly resistant to all cephalosporins and Aztreonam. Hence any of these disks can be used for screening potential ESBL producers. Though, any third generation cephalosporin can be used, some workers have recommended Cefpodoxime as a good screening agent for *E. coli* and *K pneumoniae* but not for *K.oxytoca* [6].

Not all 1278 screen positive isolates were confirmed to be ESBL producers, only 58.61% were found to be ESBL producers using CLSI recommended PCT. Similarly other studies have also demonstrated that not all screen positive isolates were ESBL producers (61.7%) [7]. Therefore, in such non ESBL producers there can be some other mechanism of resistance besides ESBL production in place like; AmpC production, efflux mechanisms, change in porin channels etc.[8] . Some workers have also demonstrated that certain β-lactamases like inhibitor

resistant TEMs, may give a false negative results by phenotypic confirmatory test [9] .

Results from SENTRY Asia-Pacific surveillance programme published in 2007, suggests that majority of non confirming *E. coli* and *K. pneumoniae* from Asia-Pacific region harbor important β-lactamases and a positive screening test alone should be sufficient ground to report resistance to extended spectrum cephalosporins in this region [10] .

Prevalence of ESBL varies across continent, countries and hospitals as demonstrated by large scale studies like SENTRY, SMART, MYSTIC. In Indian studies prevalence varied in different institutions from 28-84 % [11] .

As per the SMART study conducted in Asian-Pacific in 2007, the prevalence of ESBL production in Enterobacteriaceae was reported to be highest from India. Maximum ESBL production was found in *K. oxytoca* (100%) followed by *E. coli* (79.0%) and *K. pneumoniae* (69.4%) [12] . Several other studies conducted over various periods of time and in different countries and regions have demonstrated that all Enterobacteriaceae members are capable of ESBL production; *E.coli* and *Klebsiella species* being the major producers [10, 13-15] .

Different Indian studies conducted over the last 5-6 years, demonstrate regional differences in prevalence rates ranging from 24.80 % to 63.80 % in *E. coli* and 20 % to 92.5 % in *K. pneumoniae*. Comparatively ESBL production was found to be higher in *E.coli* as compared to Klebsiella spp.. However in SMART, a large scale study ESBL production was found to be marginally higher in Klebsiella spp. (84.7%) as compared to *E. coli* (789.0%) [12] . [Table 5]

The overall ESBL detection rate in our study was 43.10%, in *E.coli* isolates detection rate was the highest (50.33%) followed by Klebsiella species (34.14%).

Table 5
ESBL detection rates in different Indian studies

Author	Year & Place	*E. coli* %	*K. pneumoniae* %	*K. oxytoca* %	*P.mirabilis* %
Varaiya et al [16]	2010 Mumbai	27.77	20		
Bhattacharjee et al [17]	2010 Varanasi		63		
Wani et al [18]	2009 Srinagar	52.94			
Goyal et al [19]	2009 Lucknow	63.6	66.7		
Tsering et al [11]	2009 Sikkim	26.15	57.14		42.85
Rao et al [7]	2010 Davangere	62.9	62.2		70.5
Jain & Mondal [20]	2008 Lucknow		58*		
Agarwal et al [21]	2008 Pune	30	16		
Varsh gupta [22]	2007 Chandigarh	63.8	76.2		
SMART study [12]	2007Asia Pacific	79	69.4	100	
Kumar et al [23]	2006 Hyderabad	24.8	10.1		14.4
Babypadmini et al [24]	2004 Coimbatore	41	40		
Manchanda & Singh [25]	2003 New Delhi	55	92.5		

**K. pneumoniae* and *K. oxytoca* combined*

Method of ESBL detection:

In our study all DDAT positive isolates were also detected by PCT. However, 02.5% of isolates identified by PCT were not detected by DDAT. Although the specificity of DDAT has been well documented, its sensitivity has been variably reported as 76.5%, 3%, 87% and 79 % in various studies [26-29] . DDAT can

lack sensitivity because of the problems of optimal disc spacing, correct storage of clavulanate containing disk, the inability of clavulanate to inhibit all ESBLs and the inability of the test to detect ESBL in strains producing chromosomal cephalosporinases [30] .

PCT are highly sensitive and specific when compared to genotypic confirmatory methods, however there are a number of instances when PCT may be both falsely positive and falsely negative. *K. pneumoniae* or *E. coli* isolates which lack ESBLs but which hyperproduce SHV-1 or have a decrease in the quantity of a minor 45-kDa outer membrane protein may give false-positive confirmatory tests. It has been reported that the use of Cefepime increases the sensitivity of DDAT with extended spectrum cephalosporins for the detection of ESBL [31]. However, CLSI makes no such recommendations.

Susceptibility profile:

In vitro, the carbapenems (including Imipenem, Meropenem, and Ertapenem) have the most consistent activity against ESBL-producing organisms, given their stability to hydrolysis by ESBLs [32] which was also seen in our study with 97.46% ESBL producers sensitive to Imipenem.

In vitro resistance to β-Lactam/β-lactamase inhibitor combinations has previously been noted [32] . In our study out of 749 ESBL positive isolates, 89.72% were sensitive to Piperacillin-tazobactam and 84.51% were sensitive to Cefoperazone-sulbactam. Ticarcillin-clavulanic acid and Amoxicillin-clavulanic acid were least sensitive with 2.67% and 0.67% sensitive isolates respectively. Similar kind of resistance pattern was also reported by both Indian and in various international studies, SMART study (Asia-Pacific) and MYSTIC study group (Europe) [11,12,33,34].

Susceptibility profile: non β-lactams

ESBL producers have shown good sensitivity to Tigecycline and Polymyxin B. Nitrofurantoin has also shown good sensitivity among urinary isolates and is a good choice for urinary tract infection, being available orally and cheaper than its alternatives.

Many workers (table no. 6) have found that resistance to third generation cephalosporins coexists with resistance to other antibiotics like, Cotrimoxazole, Tetracycline, Ciprofloxacin, Amikacin etc. indicating multidrug resistance pattern. One of the possible mechanism for co-resistance is the co-transmission of ESBL and resistance to other antimicrobials with in the same conjugative plasmids [35].

Table no. 6
Sensitivity profile for various antibiotics across India.

Study	Ak	G	Nt	T	C	Cf	Of	Nf	CO
Mohanty et al* [36]	52.8	15.7	52.8			27.1			
Rajni et al*** [37]	80							92	
Agarwal et al *[21]	44	31	50	44	60	54			70
Wani et al*[18]	78.2	34.8				6.9	3.8	91.5	69.1
Babypadmini et al * [24]	86	25				9		89	26
Tsering et al*[11]		45.57	21.52	25.32		48.11			
Khan et al** [38]	7	4				18			

*ESBL producers ** in *P. mirabilis*** AmpC producers

CONCLUSION

Keeping in mind that Imipenem/Meropenem are not only exorbitantly expensive but like Polymyxin B exhibit systemic toxicity and Tigecycline not being the drug of choice in blood stream infection, combination drugs like Piperacillin Tazobactum and Cefoperazone and Sulbactum may be considered as effective and economical alternative to more toxic and expensive though effective drugs. The out-patient presence of ESBL is of

concern as it is now come to the alert that ESBL is spreading fast in the community.

To reduce its prevalence effective infection control measures like hand washing and barrier precautions are required. Monitoring the judicious use of cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with these pathogens.

REFERENCES

1. Washington C. Winn, Jr., Stephen D Allen, William M. Janda, Elmer W. Koneman, Gary W. Procop, Paul C. Schreckenberger and Gail L. Woods,Ed. Color atlas and textbook of diagnostic microbiology, 6th Edn, Lippincott William and Wilkins publisher:946-1021, (2006).
2. Medeiros AA, Evolution and dissemination of β -lactamase accelerated by generations of β -lactam antibiotics, Clin Infect Dis, 24:S19-45, (1997).
3. JG Collee, AG Fraser, BP Marmion and A Simmons, Ed. Mackie and Mc Cartney Practical Medical Microbiology, 14th Edn, Churchill Livingstone publisher: (2007).
4. Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing, 17th informational supplement,27(1):M100-S17,(2007).
5 Jarlier V, Nicolas MH, Fournier G, Philippon A, Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns, Rev Infect Dis,10: 867-78, (1988).
6. Thomson KS, Sanders CC, A simple and reliable method to screen isolates of Escherichia coli and Klebsiella pneumoniae for the production of TEM- and SHV-derived extended-spectrum β-lactamases, Clin Microbiol Infect,3:549-54,(1997).
7. SridharRao PN, Basavarajappa KG, Krishna GL, Detection of extended spectrum beta-lactamase from clinical isolates in Davangere, Indian J Pathol Microbiol,51: 497-9, (2008).
8. Jacoby GA, AmpC {beta}-Lactamases, Clin Microbiol Rev,22:161-82, (2009).
9 .Bradford PA, Extended-Spectrum {beta}-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat, Clin Microbiol Rev,14 (4):933-51, (2001).
10. Bell JM, Chitsaz M, Turnidge JD, Barton M, Walters LJ, Jones RN, Prevalence and Significance of a Negative Extended-Spectrum {beta}-Lactamase (ESBL) Confirmation Test Result after a Positive ESBL Screening Test Result for Isolates of Escherichia coli and Klebsiella pneumoniae: Results from the SENTRY Asia-Pacific Surveillance Program, J Clin Microbiol, 45(5): 1478-82, (2007).
11. Tsering DC, Das S, Adhiakari L, Pal R, Singh TS, Extended spectrum beta-lactamase detection in gram-negative bacilli of nosocomial origin, J Glob Infect Dis, 1(2): 87-92, (2009).
12. Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Hsueh PR, Paterson DL, Emergence of High Levels of Extended-Spectrum-{beta}-Lactamase-Producing Gram-Negative Bacilli in the Asia-Pacific Region: Data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) Program, 2007, Antimicrob Agents Chemother,53(8):3280-4, (2009).
13. Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN, Prevalence of extended spectrum β-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998–99), Diagnostic microbiology and infectious disease,42(3):193-8, (2002).
14. Mendes C, Hsiung A, Kiffer C, Oplustil C, Sinto S, Mimica I, Zoccoli C; Mystic Study

Group , Evaluation of the in vitro activity of 9 antimicrobials against bacterial strains isolated from patients in intensive care units in brazil: MYSTIC Antimicrobial Surveillance Program, Braz J Infect Dis,4(5):236-44, (2000).
15. Turner PJ, MYSTIC (Meropenem Yearly Susceptibility Test Information Collection): a global overview, Journal of Antimicrobial Chemotherapy,46 (suppl T2):9-23, (2000).
16 Varaiya AY, Dogra JD, Kulkarni MH, Bhalekar PN, Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infections, Indian J Pathol Microbiol,51(3):370-2, (2008).
17. Bhattacharjee A, Sen MR, Prakash P, Gaur A, Anupurba S, Nath G, Observation on integron carriage among clinical isolates of Klebsiella pneumoniae producing extended-spectrum b-lactamases, Indian J Med Microbiol, 28(3):207-10, (2010).
18. Wani KA, Thakur MA, Siraj Fayaz A, Fomdia B, Gulnaz B, Maroof P, Extended Spectrum -Lactamase Mediated Resistance in *Escherichia Coli* in a Tertiary Care, International Journal of Health Sciences,3(2):155-63, (2009).
19. Goyal A, Prasad KN, Prasad A, Gupta S, Ghoshal U, Ayyagari A, Extended spectrum beta-lactamases in Escherichia coli & Klebsiella pneumoniae & associated risk factors, Indian J Med Res,129(6):695-700, (2009).
20. Jain A, Mondal R, Detection of extended spectrum beta-lactamase production in clinical isolates of Klebsiella spp., Indian J Med Res,127(4):344-6, (2008).
21. Agrawal P, Ghosh AN, Kumar S, Basu B, Kapila K, Prevalence of extended-spectrum b-lactamases among Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital, Indian J Pathol Microbiol,51(1):139-42, (2008).
22 .Gupta V, Singla N, Chander J, Detection of ESBLs using third and fourth generation cephalosporins in double disc synergy test, Indian J Med Res, 126:486-7, (2007).
23. Kumar MS, lakshmi V, Rajagopalan R, Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute, Indian J Med Microbiol,24(3):208-11, (2006).
24. Babypadmini S, Appalaraju B, Extended spectrum -lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae - prevalence and susceptibility pattern in a tertiary care hospital, Indian J Med Microbiol, 22(3):172-4, (2004).
25. Manchanda V, Singh NP, Occurrence and detection of AmpC {beta}-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India, J Antimicrob Chemother, 51(2):415-8, (2003).
26. Thomson KS, Sanders CC, Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests, Antimicrob Agents Chemother, 36(9):1877-82, (1992).
27. Hansotia JB, Agarwal V, Pathak AA, Saoji AM, Extended spectrum beta-lactamase mediated resistance to third generation cephalosporins in Klebsiella pneumoniae in Nagpur, central India, Indian J Med Res, 105:158-61,(1997).
28. Abigail S, Mathai E, Jesudason MV, John TJ, Ceftazidime resistance among Klebsiella pneumoniae in south India, Indian J Med Res, 102:53-5, (1995).
29. Cormican MG, Marshall SA, Jones RN, Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen, J Clin Microbiol,34(8):1880-4, (1996).
30. Moland ES, Thomson KS, Extended-spectrum β-lactamases of Enterobacteriaceae, Journal of Antimicrobial Chemotherapy,33(3):666-8, (1994).
31. Thomson KS, Controversies about extended-spectrum and AmpC β-

lactamases, Emerging Infectious Diseases, 7(2):333-6, (2001).

32. Paterson DL, Bonomo RA, Extended-spectrum beta-lactamases: a clinical update, Clin Microbiol Rev,18(4):657-86, (2005).
33. Mohanty S, Singhal R, Sood S, Dhawan B, Das BK, Kapil A, Comparative in vitro activity of beta-lactam/beta-lactamase inhibitor combinations against gram negative bacteria, Indian J Med Res,122(5):425-8, (2005).
34 .Jones RN, Pfaller MA; MYSTIC Study Group (Europe), Antimicrobial activity against strains of Escherichia coli and Klebsiella spp. with resistance phenotypes consistent with an extended-spectrum β-lactamase in Europe, Clinical Microbiology and Infection, 9(7):708-12, (2003).
35. Martínez-Martínez L, Pascual A, Jacoby GA, Quinolone resistance from a transferable plasmid, Lancet, 351(9105):797-9, (1998).
36. Mohanty S, Gaind R, Ranjan R, Deb M, Use of the cefepime-clavulanate ESBL Etest for detection of extended-spectrum beta-lactamases in AmpC co-producing bacteria, J Infect Dev Ctries,4(1):24-9, (2009).
37. Rajni E, Rawat U, Malhotra VL, Mehta G, Occurrence and detection of AmpC beta lactamases among clinical isolates of E. coli and K. pneumoniae causing UTI, J Commun Dis,40:21-5, (2008).
38. Khan MK, Thukral SS, Gaind R, Evaluation of a modified double-disc synergy test for detection of extended spectrum beta-lactamases in AMPC beta-lactamase-producing Proteus mirabilis, Indian J Med Microbiol,26(1):58-61, (2008).