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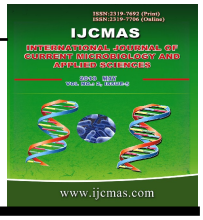

Original Research Article

Prevalence of Extended Spectrum Beta-Lactamase Producing Gram Negative Bacteria in Private Hospital, Tiruchengode, Tamilnadu, India

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Keywords

Enterobacteriaceae; Antibacterial test; Multi drug resistance; ESBL production; Ceftriaxone; Ceftazidime; Cephotaxime; Ceftazidime+ Clavulanic acid.

A B S T R A C T

A total of 120 urine samples were collected Tiruchengode private hospitals, among that 100 isolates were studied for the existence of beta- lactamases. Susceptibility tests were carried out according to the criteria of national committee for clinical laboratory standards. MICs were obtained by agar diffusion method. Existence of extended spectrum of beta-lactamases were assessed by double disc synergy test [DDST].Out of 120 samples,100 isolates showed growth on the nutrient agar medium and 20 showed no growth. Among the 120 isolates, 62 isolates were Gram negative and the remaining 38 showed other than Gram negative organisms. The distribution of Gram negative bacterial isolates, *Klebsiella pneumoniae* was predominate (24.2%) followed by *Escherichia coli* (15%), *Proteus mirabilis* (12.5%). Antibiotic sensitivity pattern showed, out of 100 isolates, (19.4%) isolates resistant and (80.6%) isolates were sensitive. Presence of beta- lactamase enzyme was done by DDST. (17.74%) isolates showed positive results for DDST among the 62 Gram negative isolates. Routine diagnosis of ESBL producing strain should be done in hospital. The most important in avoiding misuse and overuse of antibiotics may reverse the undesired effects of multi drug resistance and ESBL producing Gram negative organism.

Introduction

Urinary Tract Infection (UTI) is the second most common infections present in community practice. It is an acute infection members of Enterobacteriaceae specifically, *Escherichia coli* are the main cause of urinary tract infection (Gupta, 2003). Extended spectrum beta lactamases (ESBL) was first detected in Europe in 1980. Beta lactamases were group of enzymes capable of hydrolyzing the membered beta lactam ring of beta lactam antibiotics such as penicillin, cephalosporins, monobactams and carbapenemases. ESBL were usually plasmid mediated or chromosomal origin. Beta lactamase inhibitors were clavulanic acid, sulbactam and lazobactam. They are generally inhibit ESBL producing strains

Pitout *et al* ., (1998). Extended –spectrum β-lactamases are a rapidly evoling group of β-lactamases which share the ability to hydrolyze third- generation cephalosporins and aztreonam yet are inhibited by clavulainic acid.

Amp C lactamases are group, Beta lactamases that are intrinsically resistant to beta lactamases inhibitors they are mostly coded by chromosomal genes and are inducible. These are mainly found in *Enterobacter* spp.,*Citrobacter* spp., and *Pseudomonas aeruginosa*. Bacteria producing group1 beta lactamases are resistant towards beta lactam/beta lactamases inhibitor combinations such as penicillin,cephamycin and first, second, and third generation cephalosporins health protection agency (2007). They remain susceptible to cefepime and the carbapenems (Bauerfiend, 1989). Extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* have spread rapidly worldwide and cause a serious threat in health care-associated infection (Chaudhary *et al*., 2004).

The CTX-M enzymes are the predominant type of ESBL found in many regions of the world, including Asia, South America, Europe and Africa (Bonnet, 2004) , Paterson *et al*., (2005), (Apisarnthannarak *et al*.,2007). The routine susceptibility tests done by clinical laboratories fail to detect ESBL positive strains and can erroneously detect isolates sometimes to be sensitive to any of the Broad spectrum Cephalosporine like Cefotaxime, Ceftazidime, Ceftriaxone (Fisher *et al*., 2005).

Materials and Methods

Sample collection

The urine samples were collected from Lakshmi lab, Tiruchengode during December 2009 to February 2010. Urine specimens were collected carefully without any urethral contaminations. A wide mouth screw cap bottle was used for urine sample collection. Overfilling was avoided. Clean catch mid stream urine used for the routine microbiological purpose collected samples were brought to the laboratory with the aid of ice pack.

Isolation and preservation

The urine samples were directly streaked in to different media such as Nutrient agar, MacConkey agar and Eosin-methylene blue agar (Himedia Mumbai) for the isolation of organisms. The selected colony was once again streaked on the selective media for the pure-culture isolation. The suspected colonies were streaked on nutrient agar slants and incubated at 37ºC for 24 hrs. The obtained cultures were maintained at 4ºC.

Morphological and Biochemical Tests

The experimental isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* was isolated from above method were Subjected to morphological studies and biochemical tests as recommended by using standard biochemical tests(National committee for clinical laboratory standards).

Kirby Bauer Disc Diffusion Technique

Antimicrobial susceptibility was determined by Kirby-Bauer disk Diffusion method. (CLSI recommendations, 2004). Antimicrobial disks used were Amikacin (30mcg), Lomefloxacin (10mcg), cephadroxil (30mcg), sparfloxacin(5mcg), Netillin (30mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg), ciprofloxacin (5mcg), cephotaxime(30mcg), Gentamicin (10mcg), Cefaperazone (75mcg), Ampicillin (10mcg). Muller Hinton agar plates were prepared and sterilized at 121ºC for 15 minutes and swabbed the cultures on the plates with sterile swab. Plate was left at room temperature to remove excess of moisture. With sterile forceps, different antibiotics were placed and kept at refrigerator for 30 minutes for pre- diffusion of the disc. Then the plates were incubated at 37ºC for 24 hours. Following incubation, The zone of inhibition in diameter was noted and the results were interpreted using standard chart (Wayne, PA).

Double Disc Diffusion Synergy Test (DDST)

Muller – Hinton agar plates were prepared and sterilized at 121ºC for 15 minutes and swapped the cultures on to the plates with sterile swabs. With sterile forceps Imepenem was placed at the centre of the plate. Other discs such as Ceftriazone, Ceftazidime, Cephodoxime, Ceftazidime + Clavulanic acid was placed at a distance of 30mm from the centre of the plate. Then the plates were incubated at 37ºC for 24 hours.

Positive

Zone size around the test antibiotic disc increased towards the Imepenem. This increase occurs because of the inactivation of extended spectrum β-lactamase produced by the test organism (Palucha *et al*., 1999, Subha and Ananthan, 2002).

Results and Discussion

Morphology and biochemical characteristics of bacterial isolates

The data regarding this study and other background variables, such as age, resistance and enzyme production, etc., were elicited from the isolates over the area. The collected samples and information were put into several statistical analyses to extensively explicate the prevalence of extended β- lactamase products among the isolates over the study area. The study is based on the sample of 120 subjects.

As previously stated, there is no doubt that the ESBLs will become increasingly complex and diverse in the future. This will create increasing challenges for those creating guidelines for detection of ESBLs in the Clinical microbiology laboratory. Alteration of antibiotic susceptibility breakpoints may become necessary but need to be carefully considered in combination with pharmacokinetic, pharmacodynamic and Clinical data (David *et al*., 2005).

The patients those who are suffereing from various type of diseases.The samples were processed in various media such as Nutrient and MacConkey Agar medium, Eosin Methylene Bule Agar medium. Clinical specimen processed were, urine samples 120 (Table 1).

The present study (Table 2) shows that the preliminary examination of Gram negative Enterobactericeae in urine sample. The identification of isolates *Escherichia coli*.,

Klebsiella pneumoniae, Proteus mirabilis were done by special biochemical test with reference to authentic culture. All these isolates were also confirmed by using selective medium like Eosin methylene Blue agar, MacConkey agar, Cystine-Lactose-Electrolyte-Deficient Agar. The Enterobactericeae organisms are identified based on the biochemical analysis (Table 3).

Table.1 Type of sample used for the study

S.No	Type Of Sample	Bacterial Isolates	
		Number	Percent
1.	Urine	120	100

Incidence of growth of organism among the study subjects

The present study (Table 4) shows that the growth of organism on agar medium and the identification of organisms done by preliminary test.The incidence of growth of organism is as more than half 62 (51.7%) samples were found to be gram negative organisms. Nearly one third 38 (31.7%) were evidenced to be other than gram negative and rest of the samples 20(16.6%) were found to be no growth.

Distribution of gram negative organisms among the study subjects

The frequency of presence and absence of various organisms in urine samples, is as; Gram negative organisms shows the highest frequency (62). Among the 62 (51.6%). G-ve organisms *Klebsiella pneumoniae* comes the highest 29 (24.2%) followed by *Escherichia coli* 18(15%), and *Proteus mirabilis* 15 (12.5%). Gram negatives are followed by other organisms 38 (31.6%) and 20 (16.7%) shows no growth (Table 5). It is more or less very close to figures reported by Souli *et al.*, (2008).

Distribution of gram negative organisms isolated over the age groups

The present study envisaged that, the distribution of Gram negative organisms among different age groups of individuals.Among the 120 samples, 62 shows Gram negative organisms. More number of organisms were found in age group between30-40 (16) followed by <20, 50-60, 40-50, >60, 20-30 and i.e. (14), 10, 8, 8, 6 respectively. The age group more than 20 shows a growth of 14 and the least was shown by the age group 20-30 (Table 6 and Figure 1).

Antibacterial test by agar disc diffusion method

Beta – lactam antibiotics account for approximately 50% of global antibiotic consumption and this heavy usage was exerted considerable selection for resistance. They are act on bacteria through the inhibition of cell wall synthesis. The sensitive and resistant pattern of gram negative organisms is shown in (Table 7). Out of 62 isolates, 12(19.4%) isolates were resistant and 50 (80.6%) isolates were sensitive.

Multi drug resistance pattern of gram negative organisms

In the present study, the multi drug resistance pattern of gram negative organisms, out of the 62 Gram negative organisms 12 shows resistance and 50 shows sensitive. Among the sensitive (2), 3.2% shows resistant to 1 drugs, (3) 4.8% shows resistant to 2 drugs, (4) 6.4% shows resistant to 3 drugs, (4) 6.4% shows resistance to 4 drugs, (7) 11.4 % shows resistant to 5 drugs, (10) 16.1% shows

Table.2 Preliminary tests of the isolates

Test	Isolate-I	Isolate -2	Isolate -3
Gram's Staining	Gram Negative	Gram Negative	Gram Negative
Motility	Non- motile	Motile	Motile
Oxidase	No purple colour Negative	No purple colour Negative	No purple colour Negative
Catalase	Production of effervescences Positive	Production of effervescences Positive	Production of effervescences Positive

Table.3 Biochemical analysis of isolates

S.No.	Biochemical Tests	*E.coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*
1	Indole	+	-	+
2	MR	+	-	+
3	VP	-	+	-
4	Citrate Utilization	-	+	+
5	Triple sugar Iron Test	A/A, G-, H_2S-	A/A G++ H2S-	A/A G++ H2S+
6	Nitrate	+	+	+
7	Urease	-	_	+
Carbohydrate fermentation test				
1.	Glucose	Acid G+	+G+	+
2.	Sucrose	Weakly +ve	+	+
3.	Lactose	+	+	-
4.	Maltose	+	+	-
5.	Mannitol	+	+	-

Table.4 Incidence of growth of organism among the study subjects

S.No	Growth of organism	Bacterial isolates	
		Number	Percent
1.	Gram negative organisms	62	51.7
2.	Other than gram negative organisms	38	31.7
3.	No growth	20	16.6
Total		120	100.0

Table.5 Distribution of gram negative organisms among the study subjects

S.No	Types of organisms	Bacterial isolates	
		Number	Percent
1.	*Klebsiella pneumonia*	29	24.2
2.	*Escherichia coli*	18	15.0
3.	*Proteus mirabilis*	15	12.5
4.	Other than Gram negative organisms	38	31.6
5.	No growth	20	16.7
	Total	**120**	**100**

Table.6 Distribution of organisms isolated over the age groups

S.No	Age Group	Gram negative organisms			Total No of G-ve Bacteria	Other than G-ve Bacteria
		Klebsiella pneumoniae	*Escherichia coli*	*Proteus mirabilis*		
1.	<20	5	6	3	14	8
2.	20-30	3	2	1	6	5
3.	30-40	10	4	2	16	6
4.	40-50	5	1	2	8	4
5.	50-60	3	3	4	10	7
6.	>60	3	2	3	8	8
	Total	29	18	15	62	38

Figure. 1 Distribution of organisms isolated over the Age groups

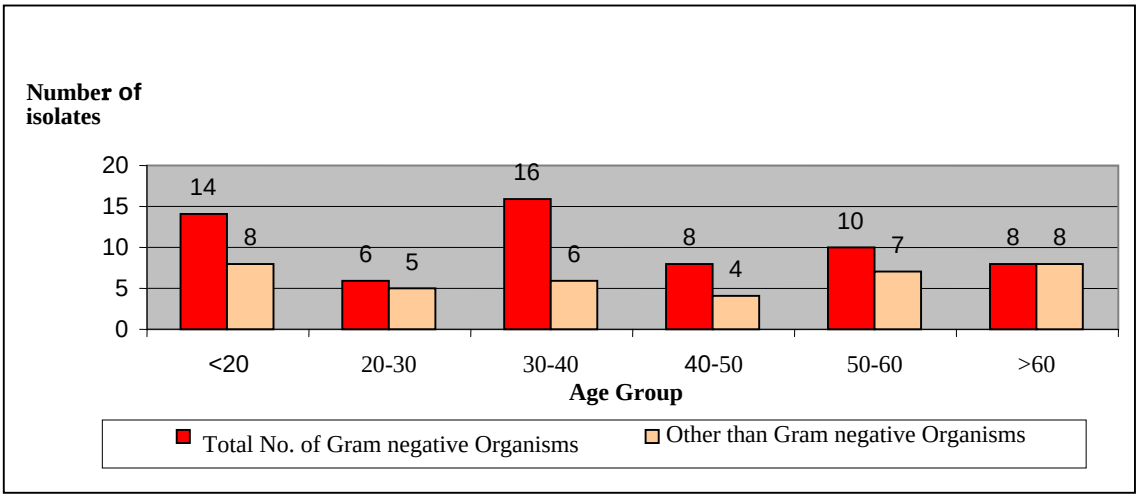


Table.7 Sensitivity and resistant pattern of gram negative organisms in urine sample

S.No	Type of Sample	`Resistant		Sensitive		Total	
		NO	%	NO	%	NO	%
1.	Urine	12	19.36	50	80.64	62	100

Table.8 Hierarchy of Multiple Drug Resistance of Gram Negative Organisms

S.No	Number of drugs resistance	Bacterial isolates	
		Number	Percent
1	12	2	3.2
2.	11	4	6.4
3.	10	6	9.8
4.	9	5	8.1
5.	8	5	8.1
6.	7	10	16.1
7.	6	10	16.1
8.	5	7	11.4
9.	4	4	6.4
10.	3	4	6.4
11.	2	3	4.8
12.	1	2	3.2
	Total	**62**	**100**

resistant to 6 drugs, (10) 16.1% shows resistant to 7drugs, (5) 8.1% shows resistant to 8 drugs, (5) 8.1% shows resistant to 9 drugs, Out of the resistant organisms (6) 9.8%shows resistant to 10 drugs, (4) 6.4% shows resistant to 11 drugs,(2) 3.2 % shows resistant to 12 drugs used(Table 8, Figure 2).

β-lactamase enzyme production by gram negative bacterial isolates

Double Disc Diffusion Synergy Test (DDST) is a confirmatory test for the detection of β-Lactamases from bacterial isolates. In our study, out of 62 gram negative isolates, the Extended – spectrum β-Lactamases production against Ceftazidime, cefodoxime, Ceftriaxone was detected by double diffusion synergy test (DDST). Centers for Disease Prevention and control (2009). The occurrence of Extended – spectrum β –Lactamases producer in Clinical isolates of gram negative organisms in our study was found to be 11 (17.7%).(Table 9, Figure 3). This was relatively slightly lower when compared to the previous study (Hawser *et al.*, 2007) and increasing rate of present study (17.7%) compare to previous study.

The above Table 4, depicts that more than half 62 (51.7%) sample were found to be Gram negative organisms, (such as *Klebsiella* spp., *E.coli* and *Proteus* spp.). Nearly one third 38 (31.7%) were evidenced to be other than gram negative and rest of the samples 20 (16.6%) were shown no growth.

The results are presented in a Table 7. All of the 62 samples, the resistance shows 12(19.36%), from urine samples respectively. The sensitive pattern shows 50(80.64%) from urine samples respectively. The above Table 9 and

Figure 3 depicts that, of all the gram negative isolates, nearly one third ,11 (17.7%) of them produced β –Lactamase enzyme and rest of the major proportion 51 (82.3%) were shown the absence of enzyme production.

Extended spectrum β – lactamases have evolved greatly over the last 20 years. Their presence, plus the potential for plasmid mediated quinolone and carbapanem resistance, will be sure to create significant therapeutic problems in the future. It is unlikely that many new antibiotic options will be available in the next 5 to 10 years to tackle such multi resistant infections. Enhanced infection control, coupled with antibiotic stewardship programs, therefore plays an important role in limiting the spread of ESBL- producing organisms.

There seems to be a wide disparity of ESBL positive gram negative infection in different age group. The incidence of ESBL producing strains of *Klebsiella spp.* And other than members of Enterobacteriaceae needs to be carefully monitored in patients. The spread of ESBL producing strains in hospitals all over the world, it is necessary to know the prevalence of ESBL positive strains in a hospital so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms is much higher. To prevent unnecessary use of 3rd and 4th and aminoglycoside antibiotics, Hence it is suggested – spectrum β –Lactamases producing strains should be carried out in patients in order to avoid undesired effect of multi- drug resistant and ESBL producing Gram negative organism by strict control of antibiotic usage.

Figure.2 Hierarchy of Multiple drug resistance of Gram negative organisms

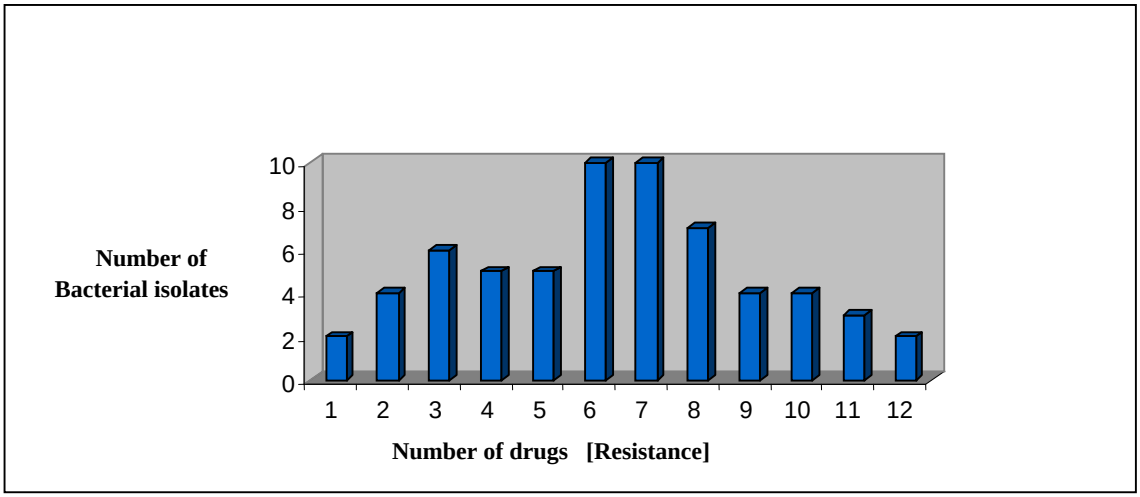
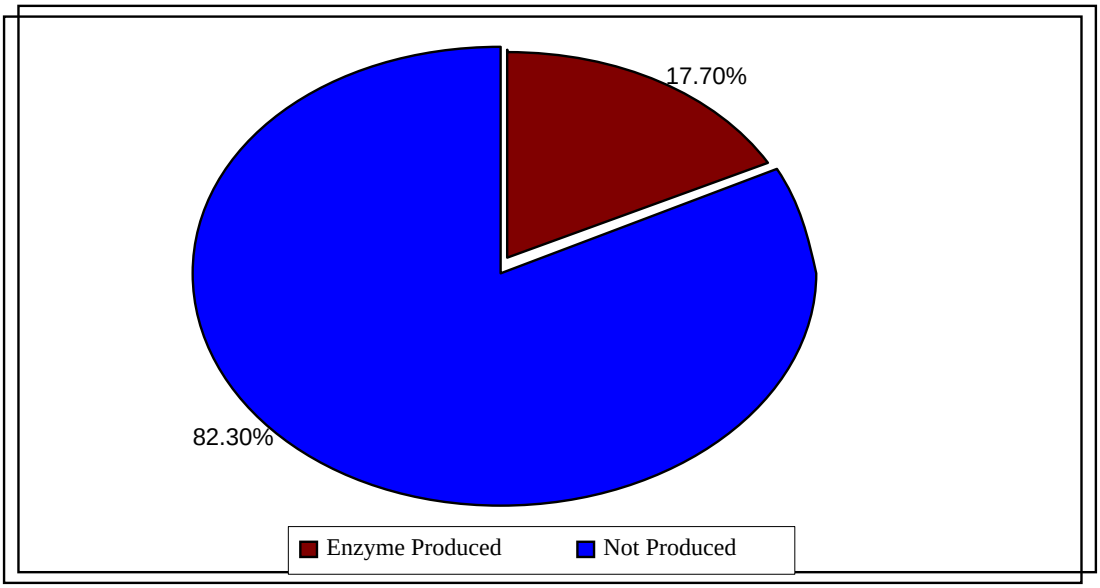


Table. 9 β-lactamase enzyme production by gram negative bacterial isolates

S.NO	Enzyme production	Bacterial isolates	
		Number	Percent
1	Enzyme produced	11	17.7
2.	Not produced	51	82.3
Total		**62**	**100.0**

Figure.3 β - Lactamase enzyme production by Gram negative bacterial isolates



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References

Apisarnthannarak, A., P. Kiratisin, P. Saifon, R. Kitphati, S. Dejsirilert and Mundy,L.M. 2007. Risk factors for and outcomes of healthcare – associated infection due to extended – spectrum beta – lactamase – producing *Escherichia coli* or *Klebsiella pneumoniae* in Thailand.Infect. Control. Hosp. Epidemiol**.** 28: 873-879**.**

Bonnet, R., 2004. Growing group of extended spectrum β - Lactamases the CTX-M enzymes. Antibiocrob. Agents. Chemother. 48(1):1-14.

Bauerfiend, A.,1989. Schweighart. Extended broad spectrum – lactamase in *K.pneumoniae* including resistance to cephamycins. Infection. 316-321.

Chaudhary, U., and Aggarwal R. 2004. Extended – spectrum – β-lactamases(ESBL). An emerging Threat to clinical therapeutics. Indian .J. Med. Microbiol. 22(2): 75-80.

David, L., Paterson and Robert, A., 2005. Extended-spectrum β-lactamases: a Clinical update *E.Coli* . Microbiol. 18(4): 657-686.

Fisher, J.F., S.O. Meroruch and Mobashery.2005. Bacterial resistance to beta – lactam Antibiotics. Compelling opportunism, compelling opportunity. Chem. Rev. 105; 395-424.

Gupta, K., 2003. Addressing antibiotic resistance. Dis .Mon. 49:99-110.

Hawser, S.P., S.K. Bouchillon, D.J. Hoban, R.E. Badal, P.R. Hsueh and

Paterson, D. 2009. Emergence of high levels of extended specterum beta-lactamase producing Gram negative bacilli in Asia ipacific: Data from SMART 2007. Antimicrob.A.Gent. Chemother.34:56-62.

Health protection Agency. 2007. Surveillance of healthcare associated infections Report.

Palucha, A., B. W. Mikiewz, Hryniewiez, and Griadkowski.1999. Concurrent outbreaks of extended spectrum – lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. J Antimicrob Chemother 44:489-499.

Pitout, J.D.D., K.S. Thomson, N.D. Hanson, A.F. Ehrhardt, E.S. Moland and Sanders, C.C.1998. β lactamases responsible for resistance to expanded – spectrum cephalosporins in *K.pneumoniae*, *E.coli* and *P.mirabilis* isolates recovered in South Africa. Antimicrob.Agents. Chemother.43: 1350-1354.

Paterson , D.L. and Bonomo,A.Extended spectrum Beta Lactamases:A clinical update clinical microbiology reviews:2005;18;657-686.

Subha, A., and Ananthan, S.2002. Extended spectrum beta lactamase mediated resistance to third generation cephalosporins among *K.pneumoniae* in Chennai. Ind. J. Med. Microbiol. 20(2): 92-95.

Wayne, P.A., National committee for clinical laboratory standards. Performance standards for Antimicrobial susceptibility testing. NCCLS approved standard M 100-S9.