IN VITRO EVALUATION OF A NEW CEFIXIME-CLAVULANIC ACID COMBINATION FOR GRAM-NEGATIVE BACTERIA

Deepti Rawat[1], Azra S Hasan[1], Malini R Capoor[1], Smita Sarma[1], Deepthi Nair[1], Monorama Deb[1], Parukutty Pillai[2] and Pushpa Aggarwal[1]

[1]Department of Microbiology, Vardhaman Mahavir Medical College and Safdarjung Hospital, New Delhi; [2]Department of Microbiology, Majeedia Hospital, New Delhi, India

Abstract. The study was conducted to evaluate a new cefixime-clavulanic acid combination for *in vitro* susceptibility towards gram-negative bacteria. A total of 220 isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa, Acinetobacter* spp, *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Typhimurium were included in the study. The isolates were tested for susceptibility towards the new combination antimicrobial molecule cefixime with clavulanic acid by disk diffusion and Epsilometer strip (E-strip) Minimum Inhibitary Concentration (MIC) method. Of the 101 *E. coli* and *K. pneumoniae* isolates, 62.4% were found to be extended spectrum beta-lactamase (ESBL) producers. Almost half of these were from the community and 55.6% were hospital isolates. Of the ESBL isolates, 19% were AmpC (cephalosporinases that are poorly inhibited by beta lactamase inhibitor) producers while the remaining 81% were non AmpC ESBL producers. The AmpC producers were resistant to both cefixime and the combination, while the non-AmpC producers were sensitive to the combination. The addition of clavulanate to cefixime did not improve the sensitivities of *P. aeruginosa* and *Acinetobacter* isolates. There were no ESBL isolates among the *S.* Typhi isolates, all of which were sensitive to cefixime. Of the *S.* Typhimurium, 88.9% were ESBL producers and all of these were resistant to cefixime but sensitive to the combination. The combination of cefixime with clavulanic acid offers the advantage of oral administration and appears to be a viable option for the treatment of uncomplicated community acquired infections caused by non-AmpC ESBL producing gram-negative bacteria.

INTRODUCTION

Enterobacteriaceae, especially *Klebsiella* spp producing extended-spectrum β-lactamases (ESBLs) such as SHV and TEM types, have been detected since the 1980s as a major cause of hospital-acquired infections. However, since the late 1990s Enterobacteriaceae (mostly *Escherichia coli*) producing novel ESBLs, CTX-M enzymes, have been identified as a cause of community-acquired urinary tract infections (Paterson and Bonomo, 2005; Pitout *et al*, 2005; Doi *et al*, 2007). Several community-acquired pathogens that commonly cause diarrhea have also been found to have ESBLs including *Salmonella*, *Shigella*, *Vibrio cholerae* and *Escherichia coli* (Paterson and Bonomo, 2005).

ESBL enzymes are often encoded by genes located on large plasmids, which also carry genes for resistance to other antimicrobial agents, such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol. Recent studies have dem-

Correspondence: Dr Deepthi Nair, D II/2201, Vasant Kunj, New Delhi-70, India.
E-mail: deepthinair2@gmail.com, deepthinair67@yahoo.co.in

onstrated fluoroquinolone resistance mediated by co-transfer of the *qnr* determinant on ESBL-producing plasmids. Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing isolates (Pitout *et al*, 2005). As a result ESBL producing organisms pose a major problem for clinical therapeutics.

Nonfermenting gram-negative bacteria are niche pathogens that primarily cause opportunistic healthcare-associated infections in patients who are critically ill or immunocompromized. They pose a particular difficulty for the healthcare community because they are often multidrug resistant. Treatment of infections caused by these pathogens is both difficult and expensive (Mc Gowan, 2006).

The present study screened nosocomial and community isolates for ESBL- production and evaluated their sensitivity to cefixime/clavulanic acid.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, Vardhman Mahavir Medical College and Safdarjung Hospital and Majeedia Hospital, New Delhi over a period of 6 months from March to August 2007.

A total of 220 isolates; *E. coli* (52*)*, *K. pneumoniae* (49), *P. aeroginosa* (42), *Acinetobacter* spp (18), *S. enterica* serovar Typhi (50) and *Salmonella enterica* serovar Typhimurium (9) were included by random selection in the study. These include both community and hospital isolates. All the *Acinetobacter, P. aeruginosa* and 47 (46.5%) of the *E. coli* and *K. pneumoniae* isolates were recovered from ICU, burns unit and nursery. The community isolates of *E. coli* and *K. pneumoniae* were from urine of adults and stool samples from children < 2 years old, attending the out patient departments.

Antimicrobial susceptibility testing of the isolates was done by the disk diffusion method (Kirby Bauer) on Mueller Hinton agar using the Clinical and Laboratory Standards Institute technique (CLSI, 2007). The antimicrobial disks used for *E. coli* and *K. pneumoniae* were amikacin (30 μg), ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), cefipime (30 μg), cefoxitin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), trimethoprim/sulphamethoxazole (1.25/23.75 μg), gentamicin (10 μg), imipenem (10 μg), piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), and tetracycline (30 μg). The antimicrobials used for *P. aeroginosa* and *Acinetobacter* spp were amikacin (30 μg), cefipime (30 μg), ciprofloxacin (5 μg), colistin (30 μg), gentamicin (10 μg), imipenem (10 μg), piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), and tobramycin (10 μg). Cefoperazone (30 μg), gatifloxacin (5 μg) and kanamycin (30 μg) were additional discs used for *P. aeroginosa* only.

For *S.* Typhi the antimicrobials used were ampicillin (10 μg), cefipime (30 μg), chloramphencol (30 μg), ciprofloxacin (5 μg), trimethoprim/sulphamethoxazole (1.25/23.75 μg), gatifloxacin (5 μg), levofloxacin (5 μg), nalidixic acid (30 μg), ofloxacin (5 μg), and tetracycline (30 μg). For *S.* Typhimurium isolates the antimicrobials tested were ampicillin (10 μg), amikacin (30 μg), cefipime (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), trimethoprim/sulphamethoxazole (1.25/23.75 μg), imepenem (10 μg), meropenem (10 μg), nalidixic acid (30 μg) and netilmycin (30 μg).

ESBL screening was performed for all the *E coli*, *K. pneumoniae* and *Salmonella* spp by the double disk diffusion method using cefotaxime (30 μg) and ceftazidime (30 μg) disks, with and without clavulanate per CLSI guidelines (CLSI, 2007). A difference of ≥ 5 mm between the zone diameters of either of the cephalosporin disks and their

Table 1
Percent sensitivities of ESBL producing isolates of *Escherichia coli* and *Klebsiella pneumoniae* to various antibiotics.

Antibiotic	Sensitive, %	Intermediately sensitive, %	Resistant, %
Amikacin	52.4	9.5	38.1
Ampicillin	0	0	100
Amoxycillin-clavulanic acid	6.3	11.2	82.5
Cefepime	19	4.8	76.2
Cefoxitin	23.8	0	76.2
Chloramphenicol	46	7.9	46.1
Ciprofloxacin	22.2	9.5	68.3
Trimethoprim/sulphamethoxazone	17.5	0	82.5
Gentamicin	20.6	3.2	76.2
Imipenam	100	0	0
Piperacillin	9.5	3.2	87.3
Piperacillin-tazobactam	69.8	11.1	19.5
Tetracycline	12.7	9.5	77.8

respective combination with clavulanate was taken as a phenotypic confirmation of ESBL production. All the isolates showing resistance to both the cefotaxime and ceftazidime and their corresponding combination disks with clavulanate were phenotypically taken to be AmpC producers. All the AmpC producers were resistant to cefoxitin.

The criteria for sensitivity to the combination of cefexime and clavulanic acid was extrapolated from the CLSI criteria for sensitivity to combinations of ceftazidime or cefotaxime and clavulanic acid.These were confirmed by the E-strip method.

The cefixime-clavulanate disks were prepared in-house per CLSI guidelines to achieve a final concentration of clavulanate of 10 μg/ml (NCCLS, 1999). The combination E-strips were prepared by adding a fixed concentration of 4 μg/ml of clavulanate throughout the E-strip. These were then placed on the plate for testing. Both the disks and E-strips were prepared in-house on a daily basis. The ESBL control strain used was *K. pneumoniae* ATCC 700603 and the negative control strain used was *E. coli* ATCC 25922 (CLSI, 2007).

RESULTS

Of 101 isolates of *E. coli* and *K. pneumoniae* included in the study, 53.5% were from the community and the remaining 46.5% were from hospital isolates. Eighty (79.2%) of these isolates were found to be multidrug resistant (*ie*, resistance to ≥ 3 drugs). Only 7 (6.9%) were sensitive to all antibiotics tested.

Sixty-three (62.4%) isolates were found to be ESBL producers, 28 (44.4%) were from the community and 35 (55.6%) were hospital isolates. Twelve (19%) of the ESBL isolates were AmpC producers while the remaining 51 (81%) were non-AmpC ESBL producing isolates. Amongst the 12 AmpC producers, 2 (16.6%) were community acquired and 10 (83.4%) were nosocomial isolates. Of the 51 non-AmpC ESBL, 26 (50.9%) were from the community and 25 (49.1%) were from the hos-

Table 2
Percent sensitivities of *Pseudomonas* isolates to various antibiotics.

Antibiotic	Sensitive, %	Intermediately sensitive, %	Resistant, %
Amikacin	19	4.8	76.2
Cefepime	23.8	2.4	73.8
Cefoperazone	21.5	2.3	76.2
Ciprofloxacin	23.8	7.1	69.1
Colistin	78.6	7.1	14.3
Gatifloxacin	23.8	9.5	66.7
Gentamicin	21.5	0	78.5
Imipenam	78.6	4.8	16.6
Kanamycin	16.7	0	83.3
Piperacillin	26.2	4.8	69
Piperacillin-tazobactam	54.7	4.8	40.5
Tobramycin	21.5	2.3	76.2

Table 3
Percent sensitivities of *Acinetobacter* isolates to various antibiotics.

Antibiotic	Sensitive, %	Intermediately sensitive, %	Resistant, %
Amikacin	22.2	5.6	72.2
Cefipime	16.7	5.5	77.8
Ciprofloxacin	44.4	0	55.6
Colistin	33.3	0	66.7
Gentamicin	16.7	0	83.3
Imipenam	11.1	5.6	83.3
Piperacillin	11.1	0	88.9
Piperacillin-tazobactam	77.8	11.1	11.1
Tobramycin	22.2	0	77.8

Table 4
Percent sensitivities of *S.* Typhi isolates to various antibiotics.

Antibiotic	Sensitive, %	Intermediately sensitive, %	Resistant, %
Ampicillin	80	0	20
Cefipime	100	0	0
Chloramphenicol	96	0	4
Ciprofloxacin	98	2	0
Trimethoprim/sulphamethoxazone	90	0	10
Gatifloxacin	100	0	0
Levofloxacin	100	0	0
Nalidixic -Acid	4	0	96
Ofloxacin	100	0	0
Tetracycline	58	10	32

Table 5
Percent sensitivities of *S.* Typhimurium isolates to various antibiotics.

Antibiotic	Sensitive, %	Intermediately sensitive, %	Resistant, %
Ampicilin	22.2	0	77.8
Amikacin	66.7	0	33.3
Cefipime	11.1	0	88.9
Ceftriaxone	11.1	0	88.9
Chloramphenicol	77.8	0	22.2
Trimethoprim/sulfamethoxazole	33.3	0	66.7
Ciprofloxacin	100	0	0
Imipenam	100	0	0
Meropenam	100	0	0
Nalidixic -acid	55.6	0	44.4
Netilmycin	44.4	0	55.6

Table 6
Percentage of multidrug resistant isolates and percentage sensitivities to cefixime and the combination of cefixime with clavulanate.

Organism	No.of strains	MDR, No. (%)	Cfx, %s	Cfx/CA, %s
S. Typhi	50	2 (4)	100	100
S. Typhimurium	9	2 (22.2)	0.2	88.9
Acinetobacter	18	16 (88.9)	22.2	27.8
Pseudomonas aeroginosa	42	36 (85.7)	13.95	13.95
E. coli	52	41 (78.84)	40	86.5
Klebsiella	49	39 (79.6)	34.7	89.8

MDR, multidrug resistance; Cfx, cefixime; Cfx/CA, cefixime and clavulanic acid.
%s, percent sensitive.

pital. All the ESBL producers were multidrug resistant. Thirteen (20.6%) were resistant to amikacin, ciprofloxacin, tetracycline, chloramphenicol and trimethoprim/sulphamethoxazole. The percentage sensitivity of the ESBL isolates to various antibiotics tested in this study are given in Table 1.

Tables 2, 3, 4 and 5 give the percent sensitivities to the various antibiotics for *P. aeruginosa, Acinetobacter* spp, *S.* Typhi and *S.* Typhimurium isolates respectively.

All the isolates were tested for sensitivity to cefixime and its combination with clavulanate (Table 6).

Among the *E.coli* and *K.pneumoniae* isolates, all the AmpC producers were found to be resistant to both the cefixime and cefixime/clavulanate combination, while all the non-AmpC producing isolates were 100% sensitive to the combination. All the AmpC producers were resistant to cefoxitin.

Multidrug resistance was seen in most of the isolates of *Acinetobacter* spp and *P. aeruginosa.* The sensitivities of *P. aeruginosa* and *Acinetobacter* did not improve with the addition of clavulanate to cefixime (Table 6).

For *S.* Typhi multidrug resistance, which is defined as resistance to ampicillin, chloramphenicol, and Co-trimoxazole (trimethoprim/sulphamethoxazole) (ACCo) was found in 4%, and 96% of the isolates were nalidixic acid resistant *S.* Typhi (NARST). There were no ESBL isolates amongst the *S.* Typhi, all of which were sensitive to both cefixime and the combination. Multidrug resistance (ACCo) was seen in 22.2% and nalidixic acid resistance was seen in 44.4% of *S.* Typhimurium isolates. Eight out of 9 (88.9%) *S.* Typhimurium isolates were positive for ESBL and all of these were resistant to cefixime but sensitive to the combination.

DISCUSSION

ESBL producing organisms have become a common problem for patients in hospitals and other health care facilities (Rodriguez-Bano *et al*, 2004; Paterson and Bonomo, 2005). Several studies indicate that infections with ESBL producing organisms are a serious emerging problem in various parts of the world (Paterson and Bonomo, 2005; Pitout *et al*, 2005). Initially restricted to hospital, they are now being frequently isolated from the community as well.

In the present study, 62.4% of the isolates of *E. coli* and *K. pneumoniae* were found to be ESBL producers. This is similar to the prevalence reported by other authors from India (Mathur *et al*, 2002; Mohanty *et al*, 2004, 2005; Singhal *et al*, 2005). Mathai *et al* (2002) conducted a study covering 10 hospitals from different regions in India and found the prevalence of ESBL phenotype to be over 60%. Community acquired ESBL was found in 44.4% of isolates. This is similar to other report from India (Supriya *et al*, 2004; Akram *et al*, 2007). Although community acquired ESBL infections are on the rise in other countries the prevalence reported are much lower than those in India (Colodner *et al*, 2001; Hryniewicz *et al*, 2001; Calbo *et al*, 2006).

The activity of enzymes, which lead to ESBL production, may be chromosomal or plasmid mediated. Plasmids responsible for ESBL production tend to be large (80 kb or more in size) and carry resistance to several agents, an important limitation in the designing of treatment alternatives. The most frequently found co-resistance patterns in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and trimethoprim/sulphamethoxazole (Chaudhary *et al*, 2004).

In the present study, 79.2% of *E. coli*, *K. pneumoniae* isolates were multidrug resistant. This is similar to the percentages reported earlier (Manchanda and Singh, 2003). Among the ESBL producers all were found to be multidrug resistant. Other authors have also found similarly high rates of multidrug resistance in ESBL producing organisms (Subha and Ananthan, 2002; Mohanty *et al*, 2004).

Thirteen of the 63 ESBL isolates were resistant to gentamicin, amikacin, ciprofloxacin, tetracycline, chloramphenicol and trimethoprim/sulphamethoxazole. Of these, 4 isolates were from the community and the remaining 9 were from the hospital. Recent surveys from Canada, Italy, Spain, Greece and the UK have shown an alarming increase in multidrug resistant ESBL producing organisms in the community (Pitout *et al*, 2005). Table 1 shows the percentages of sensitivity of the ESBL isolates to various antibiotics tested in this study.

Resistance to commonly used antimicrobial agents presents a problem for the treatment of these infections. There are few oral antimicrobials active against ESBL producing bacteria. In the present study only 6.3% of ESBL isolates were sensitive to amoxicillin-clavulanic acid and 34.9% were sensitive to oral antibiotics. Similar findings

have been previously reported (Rodriguez-Bano *et al*, 2004).

Cefixime is an orally active third generation cephalosporins. There was 100% resistance to it among ESBL isolates but 100% sensitivity to the cefixime/clavulanate combination in non-AmpC ESBL isolates. There was total resistance in AmpC producers.

All the *S.* Typhi isolates were sensitive to both cefixime and the combination. There are few reports of ESBL producing *S.* Typhi (Pokhare *et al*, 2006). There was no ESBL detected in any of the *S.* Typhi isolates in our study. ESBL was detected in *S.* Typhimurium, which has been previously described (Miriagou *et al*, 2004; Weill *et al*, 2006). All ESBL producing *S.* Typhimurium strains were sensitive to the combination.

Multidrug resistance is increasing among gram-negative nonfermenters and a number of strains have now been identified that exhibit resistance to essentially all commonly used antibiotics, including antipseudomonal penicillins and cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim/sulphamethoxazole and carbapenems (McGowan, 2006). In the present study we found the majority (>85%) of isolates of *Acinetobacter* spp and *P. aeruginosa* to be multidrug resistant.

Beta lactam – beta lactamase inhibitor combinations are not considered optimal therapy for serious infections due to ESBL producing organisms, carbapenems being preferred in these conditions. However, they are useful for less serious infections, such as uncomplicated non-bacteremic lower urinary tract infections and diarrhea, because in these cases the antibiotic is excreted in large amounts in the urine (Chaudhary *et al*, 2004; Bhattacharya, 2006). There is also a concern that misuse of carbapenems in uncomplicated cases will result in resistance.

The present study demonstrates the superiority of the cefixime-clavulanic acid over amoxycillin-clavulanic acid in the treatment of infections due to ESBL producing *E. coli* and *K. pneumoniae*. Cefixime-clavulanic acid could prove effective in the treatment of various community-acquired infections, such as middle ear infections, sinusitis, upper and lower respiratory tract infections, urinary tract infections and skin and soft tissue infections. These infections with ESBL producing organisms are common in immunocompromized host being treated at the community level. However, serious infections with potential for septicemia must be treated with carbapenems.

Given the rising trend in commonly acquired infections due to ESBL producing bacteria, there is a need for oral out-patient treatment options.

The combination of cefixime and clavulanate had the advantage of oral administration and is an excellent option for the treatment of uncomplicated community acquired infections caused by non-ampC ESBL producing gram-negative bacteria.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Panacea Biotech, India, for providing the antibiotic powders, disks and E-strips used in the study.

REFRENCES

Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Ann Clin Microbiol Antimicrob* 2007; 23: 4.

Bhattacharya S. ESBL-From petri dish to the patient. *Indian J Med Microbiol* 2006; 24: 20-4.

Calbo E, Romani V, Xercavins M, *et al*. Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. *J*

Antimicrob Chemother 2006; 57: 780-3.

Chaudhary U, Aggarwal R. Extended spectrum -lactamases (ESBL) - An emerging threat to clinical therapeutics. *Indian J Med Microbiol* 2004; 22: 75-80.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testings. 17th Information Supplement. CLSI/ NCCLS Document M100-S16. Wayne, PA: Clinical and Laboratory Standards Institute, 2007.

Colodner R, Keness Y, Chazan B, Raz R. Antimicrobial susceptibility of community-acquired uropathogens in northern Israel. *Int J Antimicrobial Agents* 2001; 18: 189-92.

Doi Y, Adams J, O'Keefe A, Quereshi Z, Ewan L, Paterson DL. Community-acquired extended-spectrum beta-lactamase producers, United States [Letter]. *Emerg Infect Dis* [serial on the Internet] 2007. [Cited 2008 Mar 16]. Available from: URL: http://www.cdc.gov/EID/content/13/7/1121.htm

Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother* 2001; 47: 773-80.

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 Antimicrobial Chemother* 2003; 51: 415-8.

Mathai D, Rhomberg PR, Biedenbach DJ, Jones RN. India Antimicrobial Resistance Study Group: Evaluation of the *in vitro* activity of six broad-spectrum beta-lactam antimicrobial agents tested against recent clinical isolates from India: a survey of ten medical center laboratories. *Diagn Microbiol Infect Dis* 2002; 44: 367-77.

Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 2002; 115: 153-7.

Mc Gowen. Resistance in nonfermenting gram-negative bacteria: Multidrug resistance to the maximum. *Am J Infect Countr* 2006; 34: S29-37.

Miriagou V, Tassios NT, Legakis NJ, Tzouvelekis LS. Expanded spectrum cephalosporin resistance in non-typhoid *Salmonella. Int J Antimicrob Agents* 2004; 23: 547-55.

Mohanty S, Kapil A, Dhawan B, Das BK. Bacteriological and antimicrobial susceptibility profile of soft tissue infections from Northern India. *Indian J Med Sci* 2004; 8: 10-5.

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* 2005; 122: 425-8.

National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing. NCCLS approved standard M100-S9. Wayne, PA: National Committee for Clinical Laboratory Standards, 1999.

Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005; 18: 657-86.

Pitout JDD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *J Antimicrob Chemother* 2005; 56: 52-9.

Pokhare BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar NR. Multidrug-resistant and extended spectrum beta lactamase (ESBL) producing *Salmonella enterica* (serotypes typhi and paratyphi A) from blood isolates in Nepal: surveillance for resistance and a search for newer alternatives. *Int J Infect Dis* 2006; 10: 434-8.

Rodriguez-Bano JMD, Navarro L, Romero L, *et al*. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. *J Clin Microbiol* 2004; 42: 1089-94.

Singhal S, Mathur T, Khan S, *et al*. Evaluation of methods for AmpC beta-lactamase in gram

negative clinical isolates from tertiary care hospitals. *Indian J Med Microbiol* 2005; 23: 120-4.

Supriya S, Tankhiwale S, Jalgaonkar V, Sarfraz Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J Med Res* 2004; 20: 553-6.

Subha A, Ananthan S. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. *Indian J Med Microbiol* 2002; 20: 92-5.

Weill FX, Guesnier F, Guibert V, *et al.* Multidrug resistance in *Salmonella enterica* serotype Typhimurium from humans in France (1993-2003). *J Clin Microbiol* 2006; 44: 700-8.