



# Research Journal of **Microbiology**

ISSN 1816-4935



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## Prevalence of ESBL Producing Strains in Tuberculosis Patients

R. Jayapradha, S. Muruges, N. Mahesh and D. Brahatheeswaran  
School of Chemical and biotechnology, SASTRA-University,  
Thanjavur, South India

**Abstract:** The present studies was taken up to evaluate the ESBL production and *in vitro* susceptibility of *Klebsiella pneumoniae* and *E. coli*, isolates collected from Tuberculosis (II phase) patients admitted in a tertiary care center. The bacterial isolates were collected during March 2004 to December 2005. One hundred and twenty Isolates of *Klebsiella pneumoniae* and *E. coli* were collected from urine, Blood, pus, sputum, bronchial wash and aspiration fluid. Antimicrobial susceptibility was carried out by Kirby-bauer's disc diffusion technique using NCCLS criteria. A screening of ESBL production was done by Double Disk Synergy method (DDS) and three dimensional tests. The frequency of  $\beta$ -lactamase activity was found in 22 (12%) isolates out of 120 strains, 12 were *Klebsiella* sp. and 10 *E. coli* demonstrated ESBL activity. The study shows alarming rise in ESBL production among *Klebsiella pneumoniae* and *E. coli* strains on high rate of resistance to a wide range of antibiotics.

**Key words:** *Klebsiella pneumoniae*, *E. coli*, ESBL, tuberculosis

## INTRODUCTION

The production of  $\beta$ -lactamases is an important mechanism of resistance to  $\beta$ -lactam antibiotics among gram-negative bacteria. Expanded-spectrum cephalosporins have been specifically designed to resist degradation by the older broad-spectrum  $\beta$ -lactamases such as TEM-1, TEM-2 and SHV-1. The response to the expanded-spectrum cephalosporins among members of the family *Enterobacteriaceae* lacking inducible  $\beta$ -lactamases has been the production of mutant forms of the older  $\beta$ -lactamases called extended-spectrum  $\beta$ -lactamases (ESBLs). In addition, resistance to the expanded spectrum cephalosporins has also arisen in *Klebsiella pneumoniae* and *Escherichia coli*. Extended (or expanded) spectrum  $\beta$ -lactam antibiotics such as third generation cephalosporins (3GC) form the major component of the empiric antibacterial armamentarium in most clinical setups and especially in tertiary care centers (Chaudary and Aggarwal, 2004). Extensive use of 3GC has contributed to the evolution of ESBL (Extended spectrum  $\beta$ -lactamases). These plasmid mediated groups of enzymes are the products of point mutations at the active site of TEM, SHV and OXA enzymes. The ESBL hydrolyze penicillins, extended spectrum cephalosporins with an oxyimino side chain including ceftazidime, ceftriaxone and cefotaxime and oxyiminomonobactam antibiotics such as aztreonam (Jacoby and Medeiros, 1991). ESBL occur predominantly in *Klebsiella* sp. and *Escherichia coli* but have also been increasingly reported in other genera of the family *Enterobacteriaceae* (Spanu *et al.*, 2002; Thomson and Sanders, 1992).

Treatment for *Mycobacterium tuberculosis* has to be lengthy, since populations of this bacillus differ in metabolic activity and it has to consist of various associated drugs, since spontaneous

---

**Corresponding Author:** Dr. N. Mahesh, School of Chemical and Biotechnology, SASTRA-University,  
Thanjavur-613 402, Tamilnadu, India Tel: 91 4362 264101-108

chromosome mutations can give rise to drug resistance. The multi-resistant phenotype emerges with sequential acquisition of mutations in several loci of separate genes. Knowledge of the mechanisms of resistance permits the development of techniques for the early detection of resistant strains, thereby making proper control possible. Tuberculosis treatment includes isoniazid, rifampicin and pyrazinamide during the first two months and isoniazid and rifampicin to complete six months of treatment (Pere Coll *et al.*, 2003).

The present studies aimed at analyzing the susceptibility pattern of major Enterobacteriaceae members namely *Klebsiella* sp. and *E. coli* and detect presence of ESBLs from Tuberculosis patients, attending a tertiary care center at Thanjavur District, Tamilnadu, South India.

## MATERIALS AND METHODS

### Collection of Samples

One hundred and twenty clinical isolates of *Enterobacteriaceae* from Tuberculosis (II phase) patients were admitted in TB sanatorium hospital, a tertiary care center at Thanjavur district, South India from March 2004 to December 2005 were studied. The test strains were collected from urine, Blood, pus, sputum, bronchial wash and aspiration fluid. The case sheets were maintained for all the patients based on therapy. The isolates were collected in Brain heart infusion agar slants. Identification of isolates was done based on cultural characteristics and reactions in standard biochemical tests.

### Sensitivity Pattern Test

The test strains were performed for the third generation's cephalosporin namely isoniazid, rifampicin, pyrazinamide, ceftriaxone, ceftizoxime, ceftazidime, gentamicin, streptomycin, imipenem, isepamicin, thiaacetazone, aztreonam, cefaperazonesulbactam and amoxycyclav using Disk diffusion susceptibility test.

### Tests for Determination of ESBL Activity

#### Double Disk Synergy Method (DDS) (Jarlier *et al.*, 1998)

Test strains were pre-incubated in brain heart infusion broth (BHIB) at 37°C to an optical density matching that of 0.5 McFarland turbidity standards. This suspension was then used to inoculate Mueller Hinton Agar (MHA) plates by swabbing them with a sterile cotton swab. Thirty microgram discs of aztreonam, ceftazidime, ceftriaxone and 10 µg of cefpodoxime were placed 30 mm from an amoxicillin-clavulanate (20 and 10 µg) disc, respectively. Inoculated plates were incubated overnight at 37°C. Enhancement of the zone of inhibition between the clavulanate disc and any one of the β-lactam discs indicated the presence of an ESBL (Thomson, 1992; Coudron *et al.*, 1997; Vercauteren, *et al.*, 1997).

#### Three Dimensional Tests (Thomson, 1992)

MHA plates were inoculated with test strains matching 0.5 McFarland turbidity standards as described for DDS and a disc of ceftazidime, ceftriaxone, cefotaxime or aztreonam was placed in the center of the plate. A well of 4 mm (diameter) was punched at a distance of 2 mm from the antibiotic disc. The inoculum (30 µL) of the test strain in BHIB pre-adjusted to 5.0 McFarland standard was seeded into the well. Plates were then incubated at 37°C for 24 h. Heart shaped distortion of zone of inhibition with growth of test organism appearing behind the well and reaching the well was indicative of a positive TDT as shown in Fig. 1.

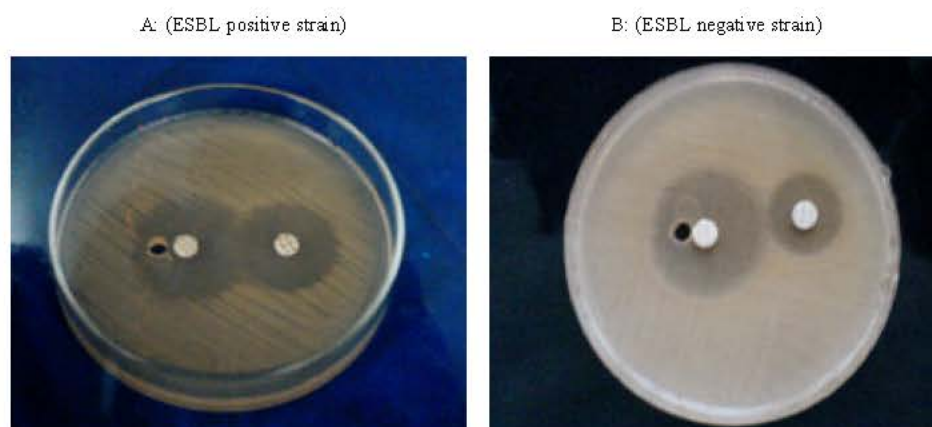


Fig. 1: A shows the enhancement of the zone of inhibition between the clavulanate disc and any one of the  $\beta$ -lactam discs indicated the presence of an ESBL

## RESULTS

Among the 120 isolates of family *Enterobacteriaceae* analysed 6% were susceptible to all antibiotics tested. 77.3% were resistant to three or more antibiotics tested and 17% were intermediate. In the present study, TDTs were found to be better than DDS in the detection of ESBLs. The combined positivity of both the TDTs was 78.6% against the 15.2% by the DDS.

Extended spectrum of  $\beta$ -lactamase activity was found in 22 (12%) isolates out of 120 strains, 12 were *Klebsiella* sp. and 10 *E. coli* demonstrated ESBL activity. The resistance was 69.5% against aztreonam and rifampicin followed by 63.1% resistance against ceftriaxone, ceftizoxime, ceftazidime and 78% with pyrazinamide, thiaacetazone and gentamicin. 82.4% with streptomycin, isoniazid, isepamicin and 75.5% with cefaperazonesulbactam. The sensitivity was 100% with imipenem, (Fig. 2).

Where Is-isoniazid, R-rifampicin, Py-pyrazinamide, Ci-ceftriaxone, Ca-ceftazidime, G-gentamicin, S-streptomycin, Ck-imipenem, Ip-isepamicin, Th-thiaacetazone, Ao-aztreonam, Cs-cefaperazonesulbactam.

All isolates were screened by a combination of the disk diffusion and three dimensional tests on agar plates with cephalosporin and amoxycylav.

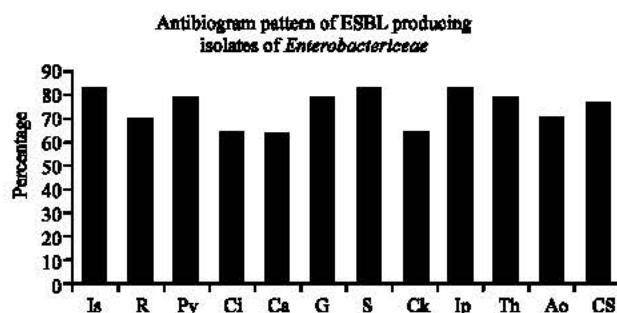


Fig. 2: Anti-biogram pattern of ESBL producing isolates of *Enterobacteriaceae*

## DISCUSSION

Antibiotic resistance surveillance has a central role among all strategies to manage the problem of antibiotic resistance. Since their first description in the mid 1970s, ESBLs have been isolated worldwide and form a major contributor of drug resistance in many genera of *Enterobacteriaceae*.

Previous studies from India have reported prevalence of ESBL producers to be 6.6 to 68% (Shukla *et al.*, 2004). ESBL production (68%) was reported among gram negative bacteria from a tertiary care hospital by Mathur *et al.* (2002), Tankhiwale *et al.*, (2004) reported that 48.3% of urinary isolates tested were ESBL producers. Jain *et al.* (2003) reported 86.6% of *Klebsiella* sp., 73.4% of *Enterobacter* spp and 63.6% of *Escherichia coli* strains from cases of neonatal septicaemia to be ESBL producers (Jain *et al.*, 2004). In South India, Subha and Ananthan (2002), have reported 6.6% ESBL prevalence among *Klebsiella pneumoniae* from children, whereas Babypadmini and Appalaraju (2004), have shown 40 and 41% ESBL positivity among *K. pneumoniae* and *E. coli*, respectively in their study cohort. Another study reported an incidence of 58.06% for ESBL producing *E. coli* and 57.14% for ESBL producing *Enterobacter* sp. (Ananthakrishnan *et al.*, 2004).

The overall objectives of tuberculosis control are to reduced morbidity, mortality and prevent development of drug resistance. This widespread occurrence of ESBL-producing *Enterobacteriaceae* suggests that the community could act as a reservoir and that food could contribute to the spread of these strains. An ESBL-producing *Enterobacteriaceae* prevalence of 28% was observed in *Mycobacterium tuberculosis*. The occurrence of ESBL producers among the *Enterobacteriaceae* isolates in the present study was 12% while 54% *Klebsiella* and 45.2% *E. coli* were found to elaborate ESBL.

High use of antibiotics in winter coincided with a lower prevalence in carriers. ESBL-producing *Enterobacteriaceae* were detected in the samples. The detail from the patient's case history shows that ESBL positive strain yielded patient was on therapy of various antibiotics. TDTs were combined with DDS in the detection of ESBLs. Vercauteren *et al.* (1997) observed 93% positivity by DDS whereas, 79% positivity was reported by Thomson *et al.* (1992).

The susceptibility data collected in the present study demonstrates the high degree of resistance among the major members of *Enterobacteriaceae*. Continued monitoring of their susceptibility pattern is necessary in clinical settings to detect the true burden of antibiotic resistance for proper disease management.

## REFERENCES

- Ananthakrishnan, A.N., R. Kanungo, A. Kumar and S. Badrinath, 2004. Detection of extended spectrum  $\beta$ -lactamase producers among surgical wound infections and burn patients in JIPMER. Indian J. Med. Microbiol., 18: 160-165.
- Babypadmini, S. and B. Appalaraju, 2004. Extended-spectrum  $\beta$ -lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*-prevalence and susceptibility pattern in a tertiary care hospital. Indian J. Med. Microbiol., 22: 172-174.
- Chaudary, U. and R. Aggarwal, 2004. Extended-spectrum  $\beta$ -lactamases (ESBL)-An emerging threat to clinical therapeutics. Indian J. Med. Microbiol., 22: 75-80.
- Coudron, P.E., S.E. Moland and C.C. Sanders, 1997. Occurrence and detection of extended-spectrum  $\beta$ -lactamases in members of the family *Enterobacteriaceae* at a veterans medical center: Seek and you may find. J. Clin. Microbiol., 35: 2593-2597.
- Jacoby, G.A. and A.A. Medeiros, 1991. More extended-spectrum  $\beta$ -lactamases. Antimicrob. Agents Chemothe., 35: 1697-704.

- Jain, A., I. Roy, M.K. Gupta, M. Kumar and S.K. Agarwal, 2003. Prevalence of Extended spectrum- $\beta$ -lactamase producing gram negative bacteria in septicemic neonates in a tertiary care hospital. J. Med. Microbiol., 52: 421-425.
- Jarlier, V., M.H. Nicolas, G. Fournier and A. Philippon, 1998. Extended broad spectrum  $\beta$ -lactamases conferring transferable resistance to new  $\beta$ -lactam agents in *Enterobacteriaceae*: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis., 10: 867-878.
- Mathur, P., A. Tatman, B. Das and B. Dhawan, 2002. Prevalence of extended beta lactamase producing gram negative bacteria in a tertiary care hospital. Ind. J. Med. Res., 115: 153-157.
- Pere Coll, Beatriz Mirelis, Ferran Navarro, Elisenda Miró, Raul Jesús Mesa, Guillem Prats, 2003. Drugs with activity against Mycobacterium tuberculosis. Emerg. Infect. Dis., 9: 1024-1025.
- Shukla, I., R. Tiwari and M. Agrawal, 2004. Prevalence of extended-spectrum  $\beta$ -lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital. Indian J. Med. Microbiol., 22: 87-91.
- Spanu, T., F. Luzzaro, M. Perilli, G. Anicosante, A. Tonioto and G. Fadda *et al.*, 2002. Occurrence of extended-spectrum  $\beta$ -lactamases in members of the family *Enterobacteriaceae* in Italy: Implications for resistance to  $\beta$ -lactamases and other antimicrobial drugs. Antimicrob Agents Chemother., 46: 196-202.
- Subha, A. and S. Ananthan, 2002. Extended-spectrum  $\beta$ -lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. Indian J. Med. Microbiol., 20: 92-95.
- Tankhiwale, S.S., S.V. Jalgaonkar, S. Ahamad and U. Hassani, 2004. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J. Med. Res., 120: 553-556.
- Thomson, K.S. and C.C. Sanders, 1992. Detection of Extended-spectrum  $\beta$ -lactamases in members of the family *Enterobacteriaceae*: Comparison of the double-disk and the three-dimensional tests. Antimicrob. Agents Chemother., 36: 1877-1882.
- Vercauteren, E., P. Descheemaeker, M. Ieven, C.C. Sanders and H. Goossens, 1997. Comparison of screening methods for detection of Extended-spectrum  $\beta$ -lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* sp. in a Belgian teaching hospital. J. Clin. Microbiol., 35: 2191-2197.