Summary and Conclusion
A total of 14776 clinical samples (urine 5318, blood 4113, respiratory specimens 2627, pus 2359, and body fluids 359) were processed for bacterial growth. Isolates of *E.coli* (1230) and *K.pneumoniae* (523) recovered from clinical specimens from inpatients and outpatients of PSG Hospitals from January 2002 to June 2004 were included as study material.

ESBL production in these isolates was detected by screening test, double disc approximation test (DDAT) and NCCLS phenotypic confirmatory test. The NCCLS confirmatory test detected a total of 807 (46%) isolates as ESBL producers, which includes 576 *E.coli* and 231 *K.pneumoniae*. The sensitivity and specificity of screen test and DDAT were found to be 100% and 95%, and 96% and 100% respectively.

The prevalence of ESBL production in *E.coli* and *K.pneumoniae* were found to be 46.8% (95% CI; 44.0, 49.6) and 44.2% (95CI; 39.8, 48.5) respectively. Higher prevalence of ESBLs was noted in body fluids (53.3%) followed by pus (52.1%), respiratory specimens (51.7%), urine, (41.4%) and blood samples (37.1%). Among the ESBL producing organism, higher prevalence (78.4%) of ESBL-producing *E.coli* was observed in respiratory specimens. Whereas higher prevalence of ESBL-producing *K.pneumoniae* was noted in pus (46.9%) and urine 46.8% samples.

Resistance to *β*-lactam and non *β*-lactam antibiotics was found to be high among ESBL producers than non-ESBL producers. Among the six *β*-lactam class of drugs included in this investigation, imipenem and meropenem were found to be the most active against all the isolates tested. The overall, rank order of activity of six agents was imipenem, meropenem (100% susceptible) > piperacillin tazobactam (97.4%) > cefoxitin (42.2%) > ceftazidime (12.6%) and cefotaxime (10.2%). Among the five non *β*-lactam antibiotics tested, amikacin was found to be the most active antibiotic against ESBL producers. The overall, rank order of activity of 5 antimicrobial agents tested was found to be amikacin (79.3%) > chloramphenicol (56.2%) > cotrimoxazole (27.4%) > gentamicin (17.2%) > ciprofloxacin (12.1%). More resistance to ciprofloxacin was found in *E.coli* isolates (94.8%) compared to *K.pneumoniae* (70.6%). The difference in antibiotic resistance pattern in ESBL and non-ESBL producers was found to be
statistically significant (P < 0.001). The MIC results revealed that 88% of the isolates showed MIC value of >32μg/ml MIC value to cefotaxime than ceftazidime. The MIC results of ESBL positive isolates revealed that all ESBL producers tested showed a >3 two fold dilution decrease in MIC value in the presence of clavulanic acid, a typical findings of ESBL producers. MIC determination of ceftazidime and ceftazidime plus clavulanic acid by E test (ESBL strip) on selected ESBLs showed 100% correlation with conventional agar dilution technique. Resistance of ESBL producers to cefoxitin was found to be 57.8%. Cefoxitin resistance in ESBL isolates is due to loss of porin in the isolates, as testing of few representative strains revealed that cefoxitin resistance was unaffected by cloxacillin making AmpC β-lactamase production unlikely. Moreover the non-transferability of resistance of cefoxitin in transconjugants indicate that the cefoxitin resistance observed in clinical isolates was likely due to loss of a porin required for cefoxitin entry. Among the urinary antibiotics, nitrofurantoin has the highest activity (83.8%) against ESBL producers from urine samples.

Analysis of the trends of ESBL prevalence during the study period revealed there was no seasonal prevalence except that there was a slight rise in number of ESBLs during September of 2002 and August months of year 2003. Analysis of demographic data of patients infected with ESBL producing organism showed that majority of the patients from whom ESBL isolates recovered were in the age group of 40 – 70 years. A mild male preponderance was observed (55.4%).

ESBL positive isolates were recovered more often from surgery (30.4%), and medicine ward (20.1%), followed by intensive care unit (10.1%). Significantly, 6.8% of patients also harboured these isolates. Risk factor associated with infection with ESBL producing organism was analyzed by case control study by analyzing 165 patients infected with ESBLs producers and 177 patients with non-ESBL producing isolates as cases and controls respectively. The risk factors associated with ESBL production in univariate analysis in our study was found to be, hospitalization, prior antibiotic use, the use of catheter, prior admission, stay in ICU and long duration of hospitalization. Age and gender were not found to be statistically different between the controls and cases (P = 0.072, P=0.327). More number of ESBL positive isolates was obtained from patients admitted in surgery ward among cases than control group. Similarly more ESBL positive
isolates were obtained from ICU (7.9%) in ESBL producers (cases) than controls. More ESBL isolates were obtained from pus samples (33.9%) in cases than controls. Of the comorbid conditions, Diabetes mellitus was found to be more important risk factor for acquisition of ESBL-producing isolates (p-0.008).

Risk factors identified in multivariate analysis were found to be the previous use of antibiotics, use of catheters, stay in ICU and prolonged hospital stay (hospital stay >7 days). More deaths were observed among patients with ESBL producers than control but the difference was not found to be statistically significant.

Results of PFGE investigation of selected ESBL positives isolates revealed considerable diversity in strains having ESBL phenotype. Dendrogram pattern of ESBL positive isolates revealed that these were genetically unrelated and high level of genetic heterogeneity was found among the isolates. Molecular characterization on 23 ESBL positive isolates was carried out to establish the type of ESBLs prevalent in this region. The cefotaxime resistance was transferred to E.coli in Transconjugation study. Only 13 isolates yielded transconjugants. These isolate showed varied diverse resistance pattern to non β-lactam antibiotics.

In isoelectric focusing majority of the ESBL producers showed bands with PI of approximately 8.2 to 8.5 suggesting the presence of SHV type and CTX-M type ESBLs. Results of PCR done with specific primers for SHV gene on 3 transconjugants showed the presence of SHV type ESBLs in only one isolate, and remaining 2 transconjugants were found to be negative. Out of 23 isolates tested with CTX-M primers, 19 isolates gave a positive amplicon and CTX-M type ESBL was detected by PCR in 19 isolates. Sequencing results of 4 amplicons revealed that they belong to CTX-M-15 type ESBLs. Among 19 CTX-M type ESBL positive isolates, 15 were obtained from inpatients and 4 were from out patients. Our results documented the spread of CTX-M type ESBLs in the community setting.

The present study showed the prevalence of ESBL production among clinical isolates of E.coli and K.pneumoniae in a Tertiary Care Hospital, Tamilnadu, and South India. This is the first documented report of prevalence of CTX-M type ESBLs in South India. Their presence among inpatients and community shows that dissemination of
CTX- M type ESBL occur on both resistance plasmids harbouring the CTX M gene and isolates themselves. CTX- M producing \textit{E.coli} is an emerging pathogen in the community setting. Antibiotic resistance is one of the most serious global threats to the treatment of infectious diseases. The detection of ESBL producing-organism in laboratories is a critical requirement for appropriate management of patients, infection prevention and control efforts, as well for tracing these organisms in surveillance systems. Surveillance of resistance strain in both hospital and community setting provide key information for effective management patient care and prescription practices. Knowledge about the type of ESBLs present in hospital and community setting can serve to guide the infectious disease physician towards choosing appropriate therapy without need for secondary testing. Identification of specific \(\beta\)-lactamases can play an important role in terms of epidemiology or long-term effect of antibiotic use.

A high prevalence rate of ESBL phenotype with dissemination has been observed in Indian hospitals. A combination of cefotaxime and ceftazidime should be included in the test panel for routine susceptibility testing to detect ESBLs effectively. All resistant strains should be checked for ESBL production by a confirmatory test to avoid false positives. It is important for the clinical labs to implement one or two methods to detect ESBLs. Most of studies from abroad have documented that, the ceftazidime resistance is a useful indicator in detecting ESBLs and this is found to be the better substrate for TEM and SHV type ESBLs. Since our study documented the presence of CTX-M type ESBLs, susceptibility to cefotaxime should also be tested to reduce the risk of overlooking CTX-M production in addition to susceptibility to ceftazidime. Nation wide surveillance programs are essential to detect antimicrobial resistance among hospital and community acquired pathogens. Data from multicentric study using standardized quantitative and comprehensive testing are needed to plan for appropriate antibiotic policies for hospitalized patients. The information obtained on mechanism of resistance among ESBL producers in hospital setting will help in designing strategies for maximizing the therapeutic usefulness of drugs and minimizing the emergence of resistance. Finally restriction of 3\textsuperscript{rd} generation cephalosporin and antibiotic cycling would arrest any further transfer of resistant among organism with plasmid encoded ESBLs.