1. Introduction

Infectious diseases have always been threat to human health and the most common reason for human deaths at the beginning of the 20th century. About one third of these were due to bacterial infections. Introduction of antibacterial agents brought out a breakthrough in treatment of bacterial infections but heavy use and misuse of various groups of antibiotics in clinical and community environment has results in strong selection pressures for emergence of diverse resistance mechanisms in pathogenic organisms. Among them production of inactivating enzymes is the most prevalent mechanisms by which microorganisms acquire resistance especially against β-lactam antibiotics (Paterson and Bonomo 2005). Evolution of resistance to β-lactam antibiotics particularly in gram negative rods commonly results in the production of Extended spectrum β-lactamases (ESBLs) which confers resistance to expanded spectrum cephalosporins as well as monobactam, and are mainly inhibited by clavulanic acid (Bradford 2001). Global episode of infections caused by gram negative pathogens is increasing at an alarming rate and distribution of ESBLs in gram negative rods has been documented as a most severe threat to the management of infectious diseases (Boucher et al., 2007). The ever increasing use of antibiotics has driven with the evolution of intrinsic and acquired resistance mechanism in gram negative rods contributes expansion of multidrug resistance (MDR) epidemics in hospital environment (Poirel et al., 2010a; Poirel et al., 2010b).

Genes encoding ESBLs have been documented frequently in Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, where they are generally either TEM or SHV derivatives (Bradford 2001; Breidenstein et al., 2011). In addition to these ESBLs; CTX-M, VEB, GES, and PER enzymes are also disseminated worldwide (Paterson and Bonomo 2005; Bradford 2001). Off them CTX-M type enzymes have been confirmed to be the most booming in requisites of promiscuity and diffusion in diverse epidemiological settings, where they have mostly replaced and outnumbered other types of ESBLs (Canton 2012). There are mainly two chief strategies of ESBL evolution which

have been developed by *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis*, *Salmonella enterica* and other members of the family Enterobacteriaceae: (A) variety of mutants with expanded substrate specificity from the plasmid mediated TEM and SHV type β-lactamases, which were previously widespread among Enterobacteriaceae; as well as (B) capture of novel β-lactamase genes from the environmental metagenome that encoding enzymes which are naturally endowed with ESBL activity (Andrea et al.,2013).

The mechanisms responsible for their acquisition are very diverse and mainly associated with insertion sequences (ISs), transposons and class 1 integrons, as well as integron containing the ISCR1 element (Canton 2012; Partridge 2011). This type of multiplicity of genetic elements responsible for their origin, mobilization/acquisition processes facilitates their rapid expansion (Poirel et al., 2012a). In most of the cases, spread of the ESBL genes has been restricted to enterobacterial species and being identified in *P. aeruginosa* and *A. baumannii* (Poirel et al., 2012a; Boucher et al., 2007).

Now a day's, ESBL producing isolates from community and hospital are often found to be responsible for outbreaks, and their prevalence varies from centre to centre; or geographical locations. Thus, local approximations are possibly more useful for clinical decision making in the same region, rather than global judgments. While observing the global scenario of prevalence data and genetic background of ESBL producers, we have observed that, paucity of data in this region creates a missing link in their phenotypic behavior with respect to genetic background and resistance mechanisms which can predict a future course of antimicrobial chemotherapy.

The global emergence of plasmid mediated ESBLs are complicated by the fact that, bacteria as well as their resistance genes are spreading faster and further due to irrational use of β-lactam antibiotics. The dissemination of these resistant determinants among gram negative pathogens has been principally recognized due to inter and intra specific DNA exchanges. The horizontal transfer of plasmid mediated genes are the prevalent mechanism of origin and acquisition of multidrug resistance among these pathogenic organisms (Rojas et al., 2013). Plasmids may acquire the insertion sequences and transposons thus facilitate the horizontal transfer of resistance determinants among

bacteria of different species depending on their narrow or broad host range, conjugative properties and competences of conjugation (Carattoli, 2013). The frequency of ascent dissemination of antibiotic resistance in bacterial populations are anticipated directly to the volume of antibiotic use and inversely related to the cost of resistance, imposed on the fitness of bacteria. It is clear that the evolution and spread of resistance can be attributed to the use and overuse of antibiotics (Andersson, 2003; Andersson and Levin, 1999). These features may lead to a multidrug resistance to antibiotics while others are linked with just a single resistance marker which makes the analysis of the genetic processes very complex gradually. The diversity of genetic vehicles linked with ESBL genes make them perfect tools for analyzing the spread of antibiotic resistance genes. It may be further predicted that global incidence of plasmid borne ESBLs will rise significantly in coming decade.

There are some of the reports on prevalence rate, phenotypic and genotypic characterization of ESBLs has been documented from India but not much report on their transferability and genetic context. Among them some of the report has been documented on the occurrence of OXA-2, VEB-1, OXA-10, CTX-M-15 and SHV genes (Bhattacharjee et al., 2010; Shahid et al., 2009; Bhattacharjee et al., 2007; Aubert et al., 2004). However, in this geographical location of this country, there are few reports on ESBL. So it may be the essential step to investigate the background of these ESBL producing organisms in hospital environment in this geographical location with a multicentric approach.

The knowledge of molecular characterization of $bla_{\text{ESBL,}}$ epidemiology, genetic context, transmission dynamics, origin and acquisition of ESBLs will facilitate the search for a phenotypic marker in clinical microbiology laboratories which will help to clinicians to implement appropriate antimicrobial chemotherapy and also enable to formulate proper hospital infection control policy. It will also help pharmaceutical industry to search for newer molecules in anti infective research.

If the function of β-lactamase is to hydrolyse β-lactam antibiotics, then what is the role of multiple β-lactamases harbored by a single organism? If they are expressed against a

specific antibiotic stress or they are responsible for survival of bacteria during a course of antimicrobial chemotherapy. There must be factors responsible for their arrangement, selection of host, maintenance within a host even when antibiotic pressure is withdrawn and initiation of their expression against specific inducing condition.

With this background, the present study was designed with the following objectives:

- To screen and confirm ESBL producers by phenotypic and genotypic methods.
- To find out the clonal dissemination or diversity of ESBL gene in the study area and clonal types of ESBL producers
- To assess the genetic environment, gene location, transferability and stability of ESBL genes
- To map bla_{ESBLs} in the organisms harbouring multiple ESBL genes
- To detect transcription level of multiple ESBL genes with inducing and non inducing conditions.