CHAPTER 1
GENERAL INTRODUCTION

1. INTRODUCTION:

Bacterial genomes are extremely dynamic and flexible in nature. A substantial amount of antibiotic resistance is frequently caused by the acquisition or deletion of new genes rather than by mutation (Dutta and Pan, 2002). Through the introduction of novel physiological traits from distinctly related organisms, horizontal gene transfer often cause drastic changes in the pathogenic character of bacterial population and promote microbial diversification and specification (Casjens, 1998). The origins of these traits, and the way in which they are acquired and spread among different bacterial species, are of interest not only to understanding the emergence and dissemination of resistance but also to understanding the mechanisms by which microorganisms acquired new genes (Recchia and Hall, 1997). Recently most of the antibiotic resistant genes in gram negative bacteria are found in gene cassettes, one or more of which can be integrated at a specific position in an integron.

An integron is found as a genetic unit that includes the determinants of the components of a site-specific recombination system capable of capturing and mobilizing genes that are contained resistance gene cassettes (Hall and Collis, 1995). The gene cassettes which contain the resistance genes have been shown to be mobile and can exists in a free circular form (Hall and Collis, 1992). Many of the antibiotic resistance genes in Gram-negative bacteria are found in gene cassettes, one or more of which can be integrated at a specific position of integron (Hall and Recchia, 1995).Inter-species transfer of genetic information occurs quite readily, and the fact that integrons and gene cassettes are found in a

range of bacterial pathogens, particularly in the Enterobacteriaceae, indicates that inter-species transfer has occurred (Huovinen et al., 1995).

A major source of multidrug resistance integrons and their resistance gene cassettes observed in clinical isolates. The acquisition of exogenous genes represents a rapid adaptation against antimicrobial compounds in bacterial population. For instance, pathogens developed resistance to sulphonamides and penicillin in the late 1930s, streptomycin resistance was also developed in the late of 1940s against mycobacteria (Mazel, 2004). The genes identified to date determine resistance to a range of antibiotics (aninoglycosides, trimethoprim, chloramphenicol and penicillins and cephalosporins), although the prevalence of antibiotic resistance genes in the pool of genes identified to date presumably reflects the fact that the genes studied were all derived from antibiotic-resistant organisms (Hall et al., 1991). For each antibiotic family several distinct genes have been found (*aad, dfr, aac, oxa, cml*) (Hall et al, 1991 and Sundstrom and Skold, 1990, Recchia et al, 1995). The emergence of multiple resistant strains could not be attributed to mutation alone, the encoded enzymes confer resistance by distinct mechanisms, antibiotic modification (aminoglycosides, chloramphenicol and beta- lactams), metabolic by-pass (trimethoprim) and efflux of the antibiotic (Chloramphenicol) (Hall and Collis, 1995, Julian Davies, 1994, Mazel, 2004). In addition, several open reading frames (ORFs) with no known function have been found (Hall et al, 1991). These genes not only occurred in the same location but many arrays containing more than one gene have been found in the wild.

The most common structure found associated with the antibiotic-resistance genes is *sul*1 gene (conferring resistance to sulphonamides) is located

adjacent to the variable region. The inserted resistance genes in this group of integrons are oriented at the same direction with respect to the flanking sequences of 5´- and 3´- conserved segments (5´-CS and 3´-CS) (Collis and Hall, 1992a). However, integrons which do not contain inserted gene cassettes are also found with intact 5´-CS and 3´-CS, those are called as “Cassette-free” integron configuration (Bissonnette and Roy, 1992).

The orientation specificity of the integrated resistance genes allows them to be transcribed from a common promoter. In *sul*1-associated inegrons, a promoter (P_{ant}) is located in the 5´-CS and is responsible for transcription of the antibiotic-resistance genes (Levesque et al., 1994 and Collis and Hall, 1995). The promoter is one of the locations where minor variations in the sequence of 5´-CS are found and these alterations have recently been shown to effect promoter strength over at least a 20-fold range (Schmidt et al., 1998). A second promoter, *P2*, has arisen by the insertion of three G residues to increase the spacing between potential-10 and -35 sequences (Levesque et al., 1994).

When more than one cassette is present, the cassette-encoded genes are co-transcribed from P_{ant}. However expression of distal genes is reduced by the presence of the upstream cassette and this effect appears to be due to premature termination of transcripts at one or more ends of the cassettes (Hall and Vockler, 1987). The integron associated recombination site, *attI*; that acts as a receptor site for the insertion of cassettes (Stokes and Hall, 1998). The integrative and excisive modes of *IntI*-dependent recombination can also lead to the co-integration of two plasmid molecules present in the same cell (Martinez and de la Cruz, 1988; 1990). However, when two different plasmids containing integrons with different integrated cassettes are involved, the transfer of

cassettes from one plasmid to the other can result in transfer of a particular resistance gene to a new location (Nucken et al., 1991 and Bennett et al., 2004). Because of the conserve sequences in the flanking region of integron, the exchange of resistance gene cassettes in different replicons takes place through homologous and site specific recombination (Martinez and de la Cruz, 1988).

This process is likely to be important factor in the spread of genes during an outbreak of antibiotic resistance in clinical situation. However, once cassettes have become associated with an integron, alternative routes for the movement of cassettes from one integron to another are possible.

As for antibiotic resistance genes that are carried in cassettes, key questions about their origins and how they were disseminated, remained unanswered. Some of these questions are fundamental, i.e; the cassette creation. However, evidence suggests that horizontal gene transfer among bacteria is a continuous activity (Dutta and Paul, 2002). So the process of determining a definitive source for any particular resistance marker will surely prove difficult unless it is explored. In 1992, Raoul Benveniste and Julian Davies proposed that intrinsically resistant or antibiotic-producing organisms routinely supply resistance genes to clinical isolates, and some recent findings substantiate their hypothesis. Their proposal was based in part on the structural relationship between the aminoglycoside phosphotransferase (APH), which protects the actinomycetes that produce aminoglycosides, and the APH encoded in transposons such as *Tn*5 (Mazel, 2004). The best example is certainly the strong similarity in sequence and genetic organization observed by Gerard Wright of McMaster University, Hamilton, Ontario, Canada, between the vancomycin resistance gene clusters found in enterococci and those of the glycopeptide-

producing actinomycetes (Mazel, 2004). While no other direct evidence is presently available, many antibiotic producing strains other than those used industrially exist in nature and could be a source of resistance genes. Since, its impact not just on the existing mobile pool but of the potential for static genes to be mobilized. The recruitment of general housekeeping genes provides an alternate route by which resistance determinants might evolve. For instance, several examples of chromosomally encoded homolog's whose functions are not related to antibiotic resistance also have been identified.

The members of the integron family utilize site specific recombinase for the integration and excision of small gene, while it may be interacting to know the integration and excision of resistance gene cassettes into the integron system. Because the basic feature of integron system differs from the other systems in two fundamental ways. **First,** integration or excision by integrase invariably encoded in the integron, not in the mobile units (cassettes). **Second,** many of distinct mobile units are recognized by the integrase and allowing the integration of different cassettes at the same site. Therefore, multiple integrations of different resistant gene cassettes may be simultaneously resided in an integron system. Other gene capture mechanisms do not provide a useful framework for knowing about the cassette arrangement and acquiring system

So, the marked differences in antibiotic resistance gene among cassettes within the same integrons indicate that these several antibiotic resistance determinants are of diverse origins. However, evolutionary rates and the passing of genes through intermediates undoubtedly created lineage that are appreciably divergent from the original loci. Several question of importance about this system are still without satisfactory answers, including; the original source of

resistance genes, cassette formation and exchange within a complex bacterial population and their diversity with different arrangement in cassette will remain mysterious.

However, no such study from India is conducted so far describing the details of gene cassettes and cassette arrays present in this region. It is also unknown whether cassette arrays are specific to any geographical location or whether they are uniform across the globe.

The gene capture mechanisms of mobile gene cassettes in the integrons system are purely on functional basis. If it so then there must be some phenotypic markers which can used for diagnostic purpose, that can track, identify and locate these gene cassettes, which can further minimise their clonal expansion in hospital and community settings and thereby preventing the spread of multidrug resistant bacteria.

Therefore the present work was undertaken with the following objectives:

1. To screen and characterize integrons among multidrug resistant enterobacterial isolates.
2. To analyze the gene cassettes and their association with multidrug resistance determinants and mobile gene elements.
3. To determine the genetic arrangement of drug resistance determinant in cassette among Enterobacteriaceae.
4. To correlate the genetic information with *in vitro* cotrimoxazole resistance.