REVIEW OF LITERATURE

I. Multiple antibiotic resistant uropathogens - Pathogenesis and Occurrence:

Urinary Tract Infection (UTI) is found to be most prevalent in underdeveloped and developing countries which are mostly caused by opportunistic pathogens, mostly *E. coli*. Theoder Escherich in 1885 has first described *Escherichia coli* as a member of family *Enterobacteriaceae* which is present as normal flora in humans and animals. However, some strains can cause gastrointestinal illness, Upper and lower UTI and may lead to potentially fatal complications. They become most drug resistant microbes i.e. “Superbugs” well described by Kumarswami *et al.* in 2010. This higher drug resistance was needed to be documented by various researchers worldwide. Bauer *et al.* in 1966 has given the method of Antibiotic susceptibility testing as a standardized Single disk diffusion method in American Journal of clinical pathology. This gives the quantitative determination of antimicrobial agent’s efficacy in an invitro condition. Since then the resistotyping pattern of antimicrobial agent was considered to deside the dosage regimen for any tyoe of infection. In the recent thirty yearsa remarkable increase in such drug resistance was observed.

Belkum *et al.* in 2001 have detected the rapid emergence of ciprofloxacin-resistant Enterobacteriaceae containing multiple Gentamycin Resistance- associated integrons in a Douch hospital. Rahman Khan and Malik in 2001 studied antibiotic resistance in bacterial strains of Staphylococci and *Escherichia*
coli isolated from foodstuffs. Manchanda and Singh in 2003 have studied the occurrence and detection of AmpC β-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bhadur Hospital; Delhi. Yamamoto in 2007 has worked on the molecular epidemiology of uropathogenic E.coli.

Sayah R. in 2005 had worked at State University, Michigan; USA had observed the patterns of antimicrobial resistance in E. coli. He studied 1286 E. coli isolates amongst those 21% of isolates exhibited resistance to more than one antimicrobial agent. Farahana et al. in 2004 at GMC, Shrinagar, India, had found that the recurrent UTI (550 cases) in female out patients (562 cases) is more common than male out patients and more than 65% of isolates were resistant to newer Quinolones like Ciprofloxacin and Norfloxacin. Tankhiwale et al. in 2004, worked at GMC, Nagpur had studied the multidrug resistant uropathogenic E. coli. Tembekar et al. in 2006 while working in S. G. B. A. U., Amrawati, India had worked on MARI and antibiotic susceptibility pattern of uropathogenic bacteria and observed that the E. coli is the major pathogen causing UTI and Ampicillin (87%, MARI: 0.041) is the most resistance obtained antibiotic. Umolu et al. in 2006 has analysed the multiple antibiotic resistant index and plasmid of E. coli in beef extract.

In 2007 Arunkumar et al. in Chennai, India had worked with ICU patients and obtained E. coli as the major pathogen. Of these 76 isolates, 48 were resistant to Cefoxitin. Kannan et al. in 2008 at Bharathidasan University, Tamil Nadu had worked on Antibiotic sensitivity pattern for UTI. The study came up with E. coli as major pathogen and only 5.92% organisms were sensitive
to all antibiotics tested. Mashouf et al. in 2009 worked at Dept. of Medical Microbiology, Hamadan University of medical sciences, Iran to analyse the bacteriology and antibiotic resistance pattern of UTI. In his study he found *E. coli* as most common pathogen but the degree of drug resistance was found to be more in *Klebsiella*. Rai et al. in 2008 at Dept. of Paediatric Medicine, K. C. Hosital, Kathmandu; Nepal had studied various causative agents of 538 urinary tract infections in children and find *E. coli* as the most occurring pathogen. They are mostly resistant to Cephalexin, Nalidixic acid, Co-Trimaxazole, Norfloxacin and sensitive to Amikacin, Nitrofurantoin. Taneja et al. in 2008 in Chandigarh, India had studied the 1979 uropathogens (22.1% highly Drug Resistant Uropathogens), their distribution as *Klebsiella sp.* (51.2%), *E. coli* (40.2%), *Enterobacter* (33.4%).

II. Functional analysis of Multiple Drug Resistant Pathogens

a. Virulence and atypical properties:

The severity of infection was potentiated by simultaneous expression of various virulence and atypical characters with the drug resistance given towards various antibiotics by MAR pathogens. These factors contribute to the life threatening conditions occur during the disease presentation. They are the traits acquired or the molecules produced by *E. coli* either to survive in altered conditions e.g. capsule production, toxin production, colicin production, serum resistance, altered cell surface hydrophobicity, urease activity, H$_2$S production etc. or to get convert in a fastidious pathogen e.g. formation of aerobactin an iron acquisition system, showing hemolysin and
haemagglutination, atypical sugar utilization etc. Various researchers have studied many of these factors in detail so that to elucidate the disease presentation. Jann et al. in 1988 had worked at Max-Planck-Institute, Freiburg, on the Structure of capsular polysaccharide from uropathogenic *E. coli* and describes the method of precipitation using Cetyltrimethylammonium bromide, extraction using aqueous CaCl$_2$ and structural elucidation with various analytical tools as G. L. C. and N. M. R.

Aono in 1988 at Yamanashi University, Japan and has found that the *E. coli* lysis peptides are slowly processed and the major lipoprotein is matured by modifications with glycerol, O-acylation, processing with signal peptidase and N-acylation. Siegerifed et al. had worked in 1994 at Institute of Medical Microbiology, Kosice and studied 168 strains of *E. coli* from cases pyelonephritis and lower urinary tract infection. Of these 168 *E. coli* strains investigated, 129 were typable with 52 monovalent O antisera. Serogroups O6 (21 strains) were detected most frequently. Mannose resistant haemagglutination of type A human erythrocytes was found in 43% MRSH in 14% and no agglutination was found in 43% of isolates. Colicins were observed in 21%, aerobactin production in 75%, cell surface hydrophobicity 92% and serum resistance in 58% of strains. Further in 1995 he analysed the association between the alpha haemolysin produced (109) and serum resistance amongst the *E. coli* isolated from children with dyspepsia and UTI.

Wanderley Dias da Silveira in Brasil has analysed various pathogenicity characters of 18 uropathogenic *E. coli* strains in 1996 in Brasil by considering the AST pattern,
haemagglutination (10 isolates) and expression of fimbriae (type I-11 isolates, type P-7 isolates), superficial protein extraction, outer membrane protein extraction, colicin production (8 colicin producers) and hemolysin production (10 isolates). Adherence assay revealed all the isolates have the capacity of adhesion to epithelial cells. Otto and Dooren, in 1998 in The Netherlands had characterized the Hemoglobin Protease Secreted by the Pathogenic Escherichia coli Strain EB1 using PCR.

After purification and characterization they concluded that Hbp is a heme-binding protein that obtains its heme from hemoglobin by degradation of this protein. The protein is likely to be the shuttle protein of a hemophore-dependent heme acquisition system in this human pathogenic E. coli strain. Hbp is a member of the “Tsh family" and its structural gene is located on a ColV virulence plasmid. All these characteristics strongly suggest that Hbp is an important virulence factor that may play a significant role in the pathogenesis of E. coli infections.

Torres et al. in Texas (2001) has studied uropathogenic E. coli mutant with multiple iron acquisition systems, including heme and siderophore transporters and found a major role of Ton-B dependent systems in the virulence of uropathogenic E. coli. Emődy et al. has studied various virulence factors viz, surface virulence factors and Exported virulence factors of uropathogenic E. coli (2003) in Hungary. He revealed that the net virulence of individual UPEC strains in a given infection is determined by the presence and actual expression of the virulence factors they have, and also by the environmental conditions present in the host organism.
Antje Burse and his co-workers in 2004 at School of Engineering and Sciences, Germany had worked on the Phytoalexin-Inducible Multidrug Efflux Pump AcrAB Contributes to Virulence in the Fire Blight Pathogen, *Erwinia amylovora* using PCR and analysed that the AcrB transport protein is distributed widely within human- and plant-pathogenic enterobacteria. Moreno *et al.* in 2005 at Hospital Valld’ Hebron, Spain had worked on the comparative study of *E. coli* Virulence Determinants in UTI strains versus strains causing pyelonephritis etc. and found that the Pap gens were more prevalent in uropathogenic and pyelonephritis *E. coli* than in other infections whereas *sfa/focDE* and *cnf1* were more prominent in urinary bacterimeia than in pyelonephritis.

Martin *et al.* (2006) in Brasil had worked on 100 strains of uropathogenic *E. coli* and their related virulence factors viz. haemagglutination and expression of type 1 and P fimbriae, hemolysin production, Aerobactin production and colicin production. He concluded that the strains isolated at the outpatient clinic exhibited a higher number of virulence factors per strain and seemed to be more aggressive than strains from hospitalized patients. Naveen and Mathai in 2005 had studied the existence of p-fimbriae, type 1 fimbriae and haemolysin in uropathogenic *E. coli* and get the higher occurrence of p-fimbriae and haemolysin production in antenatal and postnatal women than in urologic abnormalities. Rashid *et al.* in 2006 has found the expression of Putative Virulence Factors of *Escherichia coli* O157:H7 differs in bovine and human infections.
Rowbery et al. in 1985 has worked on the envelope protein changes, Autoagglutination Sensitivity to hydrophobic Agents and a conditional division lesion in *Escherichia coli* strains carrying colV plasmid. Yadav et al. while working at department of veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand in 2007 has worked on *E. coli* isolates from meat and detect various virulence factors using PCR and had found the occurrence of VT1 (33.33%), VT2 (40%), LT (26.67%) and LT (6.67).

b. **Extended Spectrum beta lactamases:**

The beta lactamases resistance was observed in terms of MRSA (Mithicillin Resistant *S. aureus*), VRE (Vancomycin Resistant *Enterococci*) and grouped resistance as ESBL (Extended Spectrum Beta Lactamases). Various types of ESBLs were found in many part of world and the recent metallo beta Lactamases i.e. NMD1 (New Delhi Metallo beta lactamases gene 1) was seen to be the burning issue.

NCCLS being the monitoring authority in infectious agents and related treatments has given the guidelines for Antimicrobial sensitivity testings and ESBL detection of all the pathogenic microorganisms and is updated every year. Shubha et al. in 2003 has characterized the Amp C producing *E. coli* and *klebsiella sp.* from UTI using the method of Three Dimensional Extract Method and observed that majority of *E. coli* (100%) and *klebsiella sp.* (77%) were resistant to all 3GC antibiotics and all *E. coli* isolates were resistant to at least one non beta lactam antibiotics tested. Transconjugantion when done, all the
transconjugants show the transfer of Amp C ESBL production. Avison et al. in 2004 at Bristol Center for Antimicrobial Research and Evolution, University of Bristol, Bristol, UK had analysed the Amp C beta lactamases expression and sequencing in biochemically atypical ceftazidime resistant Enterobacteriaceae from paediatric patients and found that the 8% of ceftazidime resistant Enterobacteriaceae isolates were confirmed as *C. murliniae* and 8% as *C. youngie* as a rare specimens.

Tankhiwale et al. in 2004 worked at GMC, Nagpur had studied the multidrug resistant uropathogenic *E. coli* and the ESBL production exhibited by these uropathogens (48.3%). Rattan A. and Singhal S. in 2004 had worked on evolution of methods like MMDDDM and Amp C disk test for Amp C detection. Amongst total 173 (64%) of the isolates were found to be ESBL positive and occurrence of Amp C was found to be 8%. Ghotole et al. (2004) in Dr. V.M. Medical College, Solapur, India, had obtained the correlation between ESBL production (74.6%) and with cephalosporin resistance (32.1%) in *Klebsiella* sp. amongst the gram negative bacilli. She has studied the inducible nature of beta lactamases along with the drug resistance pattern among clinical isolates.

Poeril et al. in 1999 have studied the Molecular and biochemical characterization of VEB-1, a novel class A Extended Spectrum Beta Lactamases encoded by an *Escherichia coli* integron gene and have analysed MIC for antibiotics, Plasmid isolation and chatracterization from the selected isolate using PCR, Matting out assay.
UK National Guidelines for laboratories for detection and characterization of Beta Lactamases, resistance in gram negative bacteria of veterinary significance were released by SGDIA which are written by Teale C. as author in charge in 2005. It includes the classification, confirmatory tests and significance of ESBL and its reporting. Arunkumar A. (2007) had worked on inhibitor based method for detection of Amp C in ICU patients. Of the 76 isolates, 48 were 14 were resistant to Cefoxitin. 36 isolates show zone enhancement with boronic acid and confirmed as Amp C. Taneja N. in 2008 in Chandigarh, India had studied the distribution of uropathogens and their ESBL occurrence (36.5%). He also correlates drug resistance with AmpC beta lactamases production.

Kannan V. in 2008 at Dept. of Microbiology, Bharathidasan University, Tamil Nadu had worked on Antibiotic sensitivity pattern for UTI and ESBL (60.7%) pattern exhibited. The study came up with *E. coli* as major pathogen and only 5.92% organisms were sensitive to all antibiotics tested.

c. Enzyme beta lactamases:

The higher degree of drug resistance was exhibited by these pathogenic *E. coli* was found to be in terms of production of the degradative enzyme, beta lactamases, enzyme group including *Penicillinase*, *Cephalosporinase* etc. Various scientists have studied this enzyme for its therapeutic significance. Both in positive as well as negative role the enzyme were studied.
As described by Smith and Hamilton in 1979, the enzyme though has the very serious disadvantage of degradation of antibiotic, can be used as therapeutic enzyme in cases of penicillin toxicities during the second world war period when the antibiotic resistance era was about to began. Various experiments were done to know more about the structural and molecular aspects of various beta lactamases. Ambler in 1980 had described the structure of enzyme Beta Lactamases and suggests that the enzymes have polyphyletic origin. Waley et al. in 1984 at Sir William Dunn School of Pathology, University of Oxford, U.K. has purified Beta Lactamases extracted from *Pseudomonas maltophilia* using affinity chromatography on phenyl boronic acid-agarose in high yield as an efficient process.

In India, Khan and Malik in 2001 had worked ESBL detection in *E. coli* and *staphalococci* isolated from foodstuffs. He determines the detection method using starch agar and iodine reagent to revel the enzyme production by isolate where out of thirteen *E. coli* isolates only three were found to be positive. Mallard et al. in 2001 in University of Licester, UK had done the kinetic and spectroscopic studies of a broad spectrum inhibitor of Zinc beta lactamases. A series of 35 analogues were synthesized and studied in all and the Ki values and SAR values.

### III. Molecular characterization of ESBL producing pathogens:

The modification of genetic makeup of pathogenic *E. coli* thus expresses altered functional products. The grouped drug resistance as observed in ESBL production. The horizontal and vertical gene transfer confers this alteration. The origin of multiple antibiotic resistances was observed to be plasmid in
many studies. Many researchers have observed the gene transfer by means of transformation.

Rasool and Wahab in 2003 at University of Karachi had worked on plasmid born antibiotic resistance among *Klebsiella sp.* by using the method of Acridine orange plasmid curing. He analysed the in vivo gene transfer of MDR isolates to recipients and obtained the Ampicillin, Ofloxacillin and Streptomycin resistant transformants. Pérez-Pérez and Hanson had worked at Center of Research in Antiinfectives and Biotechnology on detection of plasmid mediated Amp C beta lactamases and the PCR based method of differentiating 6 different families using primer amplicon ranging from 109 bp-520bp in 2002.

Naohiro and Arakawa *et al.* in 2003 at Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, Tokyo, Japan had worked on PCR Typing of Genetic Determinants for Metallo-beta-Lactamases and Integrases Carried by gram-negative Bacteria Isolated in Japan, with Focus on the Class 3 Integron and found that out of four *P. putida* strains carrying both the *intI1* and *intI3* genes, three were isolated at hospitals located in Mie Prefecture. The three isolated strains (NCB 01-121, NCB 02-182, and NCB 02-204) demonstrated very similar PFGE patterns, suggesting a clonal lineage. In 2005 Paterson and Bonomo at Division of Infectious diseases, USA had worked on plasmid mediated Class C beta Lactamases using three dimensional test and inhibitor based methods. Hamad at University of Suliaemani Iraq in 2008 has worked on distribution of conjugative plasmids amongst 32 MDR pathogenic *E. coli*. He determines existence of drug resistance on plasmids using ETBR. The transformation in
recipient revealed the Chloramphinicol and nalidixic acid resistance.

Aladağ et al. in 2009 at College of health care University of Selçuk, Konya, Turkey had worked on total 125 UTI isolates for their AST, plasmid profiling, ESBL characterization (36%) of *Klebsiella pneumoniae*. The plasmid profiling was done and the transconjugants were obtained by taking Lac⁺*salmonella* sp. as recipient and they show the transfer of drug resistance and Lac⁺ character. Rammazanzadeh et al. in 2010 at Kurdistan University of medical sciences, Sanandaj, Iran worked on the etiological agents and ESBL production in UTI considering the population and specimen type, AST, ESBL production by DDS and by PCR for total 188 samples. *E. coli* was found to be most occurring pathogen and maximum resistance was observed for Co-trimaxazole (51%) whereas lowest is for amikacin (14.4%). ESBL was exhibited by 27 isolates and CTX-M was the most prevalent type among the pathogenic *E. coli*.

**IV. Remedial approach towards the problem: Putative medicinal plants and phytochemical analysis:**

Cleidson, et al in 2007 had worked on various screening methods to determine antibacterial activity of natural products. Alam et al. had worked at Biotechnology and microbiology Laboratory, Dept. of Botany, University of Rajashahi, Bangladesh in 2009 had worked on leaf juice and extracts of *Moringa olifera* against some human pathogenic bacteria and observed that the ethanol extract was found to be effective against Gram negative bacilli. Rao et al. in 2006 had worked at Dept. of Biochemistry, College of Engineering, Gandhi Institute of Technology and Management, Visakhapatnam on
Antibacterial activity of some Indian plants viz. *Artimesia nilagirica*, *Arachynthes aspara*, *Amomum subulatum*, *Aristilochia indica*, *Andrographis Paniculata* (Tiwari et al., 2010) and found that *Artimesia nilagirica* and *Aristilochia indica* were effective against test *E. coli* strain. Iqbal et al. in 2007 had worked at dept. of Agricultural Microbiology, faculty of Agricultural Sciences, Aligarh Muslim University on in vitro efficacy of bioactive extracts of medicinal plants against ESBL producing MDR enteric bacteria. He found *Hemidesmus indicus* (MIC: 2.13 mg/ml), *Terminilia belerica* (MIC: 3.84 mg/ml) and *Terminilia chebula* (MIC: 6.82 mg/ml) as the most potent antimicrobial sources against the test organism.

Prusti et al. in 2007 had worked at dept. of Agricultur in 2008 had worked at P. N. College Orissa on Antibacterial activity of some Indian plants viz. *Listia glutinosa*, *Vitex peduncularis*, *Elephantopus scaber* on urinary tract pathogens and found that 500 and 250 µg/ml of various extracts were effective against the pathogens. Chattopadhyay et al. in 2007 had worked at dept. of Agricultur in 2009 at Agricultural and ecological Research Unit, Indian statistical Institute; Kolkata had evaluated the antibacterial properties of *Chebulic myrobalan* against MDR uropathogenic *E. coli* and found that the ethanoic and water extracts were found to be effective against the test culture.