2.0 History

For centuries, urinary tract infections (UTI) have been recognized as major bacterial infections, affecting humans throughout their life span. During the past three decades, the intensive investigations of these infections have been carried out, in an attempt to define more accurately the epidemiology, pathogenesis (bacterial virulence vs. host defence), natural history, treatment and prevention of these diseases.

History of UTI commenced with the work of Wagner (1882), who was the 1st one to describe the pathological features of pyelonephritic contracted kidneys. Later on Bredier (1902) on the basis of clinical and experimental observations, reported the association of pyelonephritis with pregnancy.

Lohlein (1917) diagnosed accurately the cases of pyelonephritis, which had earlier been reported of chronic glomerulonephritis or nephrosclerosis. Initially, Chown (1927) was able to differentiate clearly between ‘pyelonephritis’ and ‘pyelitis’. However, Wilson and Schloss (1929) stated that ‘pyelitis’ for this pathology is a misnomer, since the conditions always involve a suppurative lesion of interstitial tissue of kidney and corrected the term to more relevant term ‘pyelonephritis’.

Scott (1929) reported \textit{E. coli} as the most frequently isolated organism from the cases of hematogenous infection of kidney. It was Barash in the same year, who first favored the ascending route of infection for pyelonephritis.
Various cases of chronic pyelonephritis with vague symptoms were reported clinically by Braasch in the third decade of twentieth century but the real characteristic of chronic pyelonephritis, its recognition, clinical symptoms and its involvement as a cause of renal failure was emphasized in 1933 by Longscope and Winkerwerder. They also noticed the frequency of disease to be more in young women probably due to shorter urethra than in case of males.

Braasch (1938) reviewed a number of cases of pyelonephritis and found that the disease often was so mild and symptoms were so vague that disease condition could be recognized only by urine analysis with culture and gram staining of urine sediments. Mallory (1940) found the ureter obstruction as one of the important factors in the development of blood born pyelonephritis.

Rashcon (1948) reported that UTI can occur in anatomically normal urinary tract and bacteria may eventually ascend to kidney causing renal damage and chronic renal failure.

It has been reported by Beeson (1955) that about 20% of pregnant women develop pyelonephritis, where probably physiological dilation of the ureter occur in these patients. Guze and Beeson (1956) reported that catheterization or other urethral instrumentation may push the urethral microorganisms into bladder through the thin film of fluid between the catheter and urethral mucosa. This happens because fluid collected around catheter has been shown to be an excellent medium for bacterial growth (Kass and Schneiderman, 1957).
The relationship between pregnancy, bacteriuria and pyelonephritis was determined by Kass (1960) and he also reported that 40% of patients with bacteriuria detected in early pregnancy, developed acute pyelonephritis later in pregnancy.

Ehrenkranz and Carter (1960) found a four fold or greater rise in serum antibody titer in over 75% of their patients with acute urinary tract infection. However Cabe and Jacson (1960) reported that antibodies were present during all the phases of bacterial proliferation and therefore does not have a protective or remedial influence on local renal lesion.

Stanford et al. (1962) noted that antibody against O111 antigen conferred virtually complete resistance to induction of hemolytic E. coli pyelonephritis in rats. He concluded that specific immunity play an important role in the establishment of effective resistance to experimental pyelonephritis. Williamson et al. (1964) reported that acute pyelonephritis resulted in an increased titre of O antibodies against the infecting organism which decreased to preinfection level within six months. At the same time it was suggested that serum O antibodies play no effective role in preventing the inception of pyelonephritis or in modifying its course.

Kalmanson (1964) reported the decrease in serum bactericidal activity in pyelonephritic patients. But Gutman et al. (1967) recognized first the presence of ‘L-forms’ in the urinary tract of chronic pyelonephritic patients and described the role of L-forms of microorganisms in chronic renal infection.
Jones et al. (1974) showed the presence of antibody coated bacteria in the urinary sediment of patients suffering from pyelonephritis and tried to draw a correlation between antibody coated bacteria and pyelonephritis.

Jodal et al. (1974) detected secretory IgA as well as IgG antibodies against O and K-antigens of infecting bacteria in the urine of girls with urinary tract infection. However, protective capacity of antibodies against surface antigens of *E. coli* was initially evaluated in rabbit hematogenous model in which different *E. coli* strains were used for immunization. Antibodies against O and K-antigens showed protection whereas antibody to flagellar antigen failed to protect against hematogenous pyelonephritis (Kaijser and Olling, 1973).

Nicholson and Glynn (1975) found that UTI could be produced in mice model by the inoculation of *E. coli* into the bladder, provided suture had first been inserted into the bladder wall to act as foreign body. The frequency of kidney infection was directly proportional to the amount and activity of K-antigen of injecting strain of *E. coli*.

There was a local immune response in experimental pyelonephritis wherein immunological enhancement involving leukocyte infiltration played a crucial role in pathogenesis of pyelonephritis and renal functions were shown to be impaired during process of pyelonephritis (Miller et al., 1976).

In addition to urinary findings, the patients with pyelonephritis had increased levels of IgG, IgA and IgM antibodies in serum. The serum and urine antibodies were found to be against bacterial surface antigens,
mainly somatic antigens (Kaijser and Ahlstedt, 1977). However antibodies against K-antigen appear less frequently (Kaijser et al., 1983b).

Larsson et al. (1980) established rat as an experimental model for ascending pyelonephritis without any traumatic manipulation of the urinary tract. They found that both *E. coli* and *Proteus mirabilis* were able to cause pyelonephritis in rat model.

Rene and Silverblatt (1982) showed that specific antipilus antibodies of classes G, A and M were increased in pyelonephritic patients. However, few or no antibodies were secreted in the urine of infected or control patients.

Garg et al. (1986) reported for the first time that pyelonephritis alter the reabsorption of nutrients and brush border membrane enzymes of rat kidneys. Furthermore the new sensitive marker for the early detection of experimental ascending pyelonephritis was introduced and studied (Garg et al., 1987a). They found that kinetic parameters of renal brush border membrane enzymes like alkaline phosphatase, maltase, leucine aminopeptidase and glutamyl transpeptidase were altered during pyelonephritis. In the same year Garg et al. (1987d) described the quantitative histopathological method for the evaluation of renal lesion in pyelonephritis.

It has been reported that microorganism may initiate the infection, but progression of the disease may be a result of excessive production of reactive oxygen species (ROS). The ROS, when released within the phagosomes play important role in bacterial and parasitic killing but triggering of excessive ROS production may be deleterious to the
surrounding tissue as well as the phagocyte which produce them (Grishan and Macord, 1987).

Kaur et al. (1988) studied the effect of various oxygen free radical scavengers in preventing tissue injury caused by E. coli in pyelonephritic mice. Treatment with superoxide dismutase and catalase showed a significant decrease in the extent of lipid peroxidation even in the presence of infection. In addition, brush border membrane enzymes were increased in treated groups as compared to infected groups which ultimately resulted into less injury in presence of oxygen free radical scavengers.

Gupta et al. (1992) studied the mechanism of tissue injury at the cellular level by following the chemiluminescence response of various phagocytic cells in E. coli induced experimental pyelonephritis. The chemiluminescence response was increased significantly with increase in days postinfection.

It has been found that antibiotic therapy beginning 72 h after bacterial inoculation in experimental model attenuated the antibody response to pili antigen. The challenge of same monkey with same bacteria three months later produced acute pyelonephritis with prolonged bacteriuria. But animals with untreated infections had an antibody response that lasted for a sufficient period of time to prevent acute pyelonephritis after renal challenge (Neal et al., 1991).

Recently it has been seen that cytokines play an important role in natural host defence against pyelonephritis. The possible role of cytokines was reported by Rugo et al. (1992) in BALB/c mouse model of E. coli pyelonephritis. Cytokines IL-1, IL-6, G-CSF, GM-CSF, TNF-α, H-
400 and IL-10 were produced locally in the kidney. The IL-6 response was seen as early as 12 h after bladder inoculation of *E. coli*.

Miller *et al.* (1992) described the pathobiology of sub-clinical pyelonephritis. An animal model was developed to obtain quantitative information on comparative pathobiology of lesion induced and non-lesion induced infections.

2.1 **Experimental model of pyelonephritis**

Human epidemiological studies can identify associations between certain virulence properties of infecting organisms with their ability to cause UTI, but direct assessment of the contribution to virulence of these pathogens require the use of animal models. Therefore, selection of an appropriate animal model is crucial. Models involving non physiological manipulations of the urinary tract (Renal trauma, ureteral ligation, direct intrarenal injection etc.) or intravenous injections of bacteria do not faithfully reproduce human UTI (Brooks *et al.*, 1979; Larsson *et al.*, 1980; Kaijser and Oiling, 1973; Krohonen *et al.*, 1981).

Human infections most commonly occur in patients with anatomically and functionally normal urinary tracts and involves spontaneous ascent of bacteria from the urethra to the bladder and to the kidney and blood stream. The animal species used must have in common with humans those aspects of urinary tract that are important in the functions of the virulence factors being studied. Out of the different animal models that have been employed, the primate and murine models are the most acceptable ones.
2.1.1 Rat Model

Rat had been the most extensively used animal for experimental work of pyelonephritis. Experimental pyelonephritis is relatively easy to establish in rats because vesico-ureteral reflux (VUR) occurs spontaneously. This allows easy passage of the bacteria up to the renal pelvis (Sommer, 1961; Heptinstall, 1964; Tuan et al., 1970).

Guze and Beeson (1956, 1958) produced renal infection in rats with one of the ureter ligated. The organism used was hemolytic and coagulase negative strain of *Staphylococcus aureus*. They further observed the aggravating effect of partial ureteric obstruction on induction of renal infection and found that partial obstruction cause increased susceptibility to infection.

Sanford et al. (1962) produced acute hematogenous pyelonephritis in male rats by intracardiac infection of *E. coli*, followed by high kidney massage through the intact abdominal wall.

Kyriakas and Ikari (1969) ligated ureters with silk suture and produced acute pyelonephritis in rats following intracardiac injection of the *E. coli*.

Kaijser et al. (1983) was able to produce successfully ascending pyelonephritis in rats. They used this model to study the protective effect of *E. coli* K13 antigens conjugated to bovine serum albumin against acute pyelonephritis by *E. coli* O6:K13:H1.

Reid et al. (1985) established chronic pyelonephritis in female rats with bacteria incorporated into agar beads injected periuretherally into urinary bladder using French ureteral catheter No.3.
Topley et al. (1989) used female rats to establish the role of type-I pili in the pathogenesis of renal scarring. They used intra-renal route for induction of chronic pyelonephritis. They reported that degree of renal scarring developing in rats after direct intrarenal injection of *E. coli* was greatest among type-I fimbriated strains.

Miller et al. (1992) established the rat model for studying the pathobiology of subclinical pyelonephritis. In this model, bacterial invasion was not associated with gross or histopathological changes within the renal parenchyma, but minor foci of inflammatory cells were seen beneath the epithelial lining of calyces.

However, Korhonen et al. (1981) questioned the value of rats as an experimental model for evaluating the pathogenic role of P-fimbriae in UTI. They reported that rat uroepithelial cells do not express receptors for P-fimbriae and the form of globoside isolated from rat kidneys contains a Gal (1-3) Gal and linkage in place of the critical Gal (1-4) Gal linkage present in human globoside (Naiki & Marcus, 1975).

### 2.1.2 Primate Model

Monkeys had been widely used in the study of pyelonephritis. Roberts (1975) successfully produced experimental pyelonephritis in female stump tailed monkeys. Different strains of *E. coli* were inoculated unilaterally in one of the ureter under pressure using catheter. Monkeys were sacrificed eight weeks after infection. The renal culture was found to be negative, but histology revealed establishment of chronic pyelonephritis.
Smith and Roberts (1978) produced pyelonephritis in monkey to study ultrastructure of affected kidneys. They noted that the most severe kidney damage occurred in those animals in whom the inflammation was greatest. Tubular cells presented swollen mitochondria with ruptured inner membranes, dilated endoplasmic reticulum and irregular basal lamellae. No recognizable bacterial structures were however seen.

Roberts et al. (1981, 1985) suggested the non human primate model as the ideal model for studying chronic pyelonephritis. In these species of animals, mannose resistant adherence of certain bacteria to the uroepithelial cells is a predisposing factor for the initiation of pyelonephritis. Therefore, vaccination of monkeys with purified P-fimbriae (mediating MR adhesins) gave protection against ascending pyelonephritis. They also observed that ascending pyelonephritis can be established in monkeys in the absence of vesico-ureteral reflux.

2.1.3 Mouse Model

Gorill (1951) produced hematogenous pyelonephritis in mice following intravenous injection of \textit{Staphylococci} without any manipulation of the urinary tract. Fredman and Beson (1958) produced pyelonephritis by direct inoculation of bacteria into the kidney and found that medulla was more susceptible to infection than the cortex.

Hagberg et al. (1981, 1983) were successful in producing ascending model of pyelonephritis in mice without any mechanical manipulations of the urinary tract. The various strains of mice like CBA/C9, BALB/c, CBH/HeN and C3H/HeJ were used.

The mouse pyelonephritis model was designed by O-Hanley et al. (1985) in the anatomically normal urinary tract. The BALB/c mouse was
chosen for this study because globoseries glycolipids are present in human and mouse kidney and contain α-D-Gal (1-4) β-D-Gal, the receptor moiety for Gal-Gal binding.

Hagberg et al (1984, 1985) while using mouse as model, related natural resistance of *E. coli* urinary tract infection to lipopolysaccharide responsiveness. It was seen that LPS non-responder mouse strain C3H/HeJ had about 1000 fold higher *E. coli* counts than did strain C3H/HeN (responder to LPS) from kidneys within 24 h of infection.

Schaeffer et al. (1987) also developed ascending mouse model for pyelonephritis in BALB/c mice. Sinha et al. (1988) assessed the suitability of LACA strain of Swiss Webster mouse for establishment of acute ascending pyelonephritis.

Domingue et al (1988) analyzed colonization and invasive properties of wild type bacteriuric *E. coli* in experimental non-obstructive pyelonephritic model in female BALB/c mice. Phenotypic characters studied were P and type 1 fimbriae, hemolysin, presence of K. capsules, flagella, serotype, biotype and human and mouse serum-cidal resistance. Findings emphasized that these phenotypic characters may simply represent associated serologiccal markers with host, serving as the dominant determinant of susceptibility of urinary infection.

O’Hanley (1990) studied the distribution and density of mannose and Gal-Gal receptor compounds in urogenital tissue of BALB/c mice and human volunteers. He reported that mannose and Gal-Gal receptor analogues were detected in both species on the epithelial surface of vagina, bladder, ureter, renal pelvis, proximal tubules and collecting ducts. Mannose sensitive pili and Gal-Gal binding pili receptor-
carbohydrate were also sought in the urine of humans and BALB/c mice. Further, urine of both species contained a glycoprotein Tamm-Horsfall protein that inhibited binding of mannose sensitive pili to its receptor analogue. This study establishes the suitability of BALB/c mice as an ideal model for establishment of experimental pyelonephritis.

Mice have also been employed for protection studies. Pecha et al. (1989) used BALB/c mice to evaluate the protective potential of Gal-Gal pili vaccine against *E. coli* strain expressing homologous or heterologous pili. Kruze et al (1989) showed protection against urinary tract infection by immunizing mice with Solco Urovac (Solco Basle Ltd., Switzerland) vaccine.

Rugo et al. (1992) used the BALB/c mice model of *E. coli* pyelonephritis to study the role of cytokines in pyelonephritis. They found that most of cytokines which have some protective role against infection were produced locally in the kidney very early in the course of infection.

However, no mouse model has yet been reported for establishment of chronic pyelonephritis employing ascending route of infection.

### 2.2 Causative Organisms

Table-I shows the causative organisms associated with urinary tract infections.

<table>
<thead>
<tr>
<th>Common G-ve</th>
<th>Common G + ve</th>
<th>Unusual and Rare</th>
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17
In more than 85% of the urinary tract infections, specially uncomplicated ones, a single bacterial species is responsible for infection. Since gastrointestinal tract provides the reservoir from which bacteria originate for this infection, thus the enterobacteriaceae organisms are the most frequent causative agents for the urinary tract infections.

*Escherichia coli* is the most common pathogen for UTI and has been extensively studied. Helmaltz and Beder as early as in 1917 produced hematogenous pyelonephritis in rats by injecting different *E. coli* strains isolated from various sources. However, all the strains could not produce pyelonephritis.

Gillespie *et al.* (1971) observed that 90% of the urinary tract infections in healthy, unhospitalized patients were caused by *E. coli*. Turck and Peterdorf (1962) reported that since, *E. coli* is present as the predominant aerobic flora of the gut in all warm blooded animals, therefore, it is an important mediator of a variety of endogenous infections of the urinary tract. They further proposed that probably, *E. coli* differentiate to act as pathogens in the bowel and so characterization of various strains could give important information.

Vosti *et al.* (1964) reported that strains of *E. coli* isolated from patients with pyelonephritis appear to fall more frequently into certain O and K serotypes. However, Kimball *et al.* (1964) demonstrated that *E. coli* serological groups most frequently isolated from UTI are O1, O4, O6 and O75, but they did not have an increased potential to produce experimental renal lesions in rats.
Vanden Bosch et al. (1979) reported that bacteria found in the urine of patients commonly originate from the patient’s own fecal flora and colonize periurethral area from which further infection occurs.

Svanborg Eden et al. (1978) observed that *E. coli* causing acute pyelonephritis efficiently attached to the epithelial cells from urinary tract while those from patients with asymptomatic bacteriuria either showed poor or no attachment at all.

Korhonen et al. (1980) found that binding of uropathogenic *E. coli* was mediated by proteinaceous appendages termed pili (fimbriae) which attach to receptor carbohydrate. It has further been reported that 91% of the isolated uropathogenic *E. coli* expressed mannose resistant binding (Vaisnen et al., 1981) but both mannose sensitive and mannose resistant pili were simultaneously expressed by most of the pyelonephritic strains (Klemm et al., 1982).

Hagberg et al. (1983) showed that mannose sensitive pili may promote bladder bacteriuria in animals and renal colonization only in patients with VUR.

The relationship of fecal and urinary tract flora was deduced by Caugant et al (1985). Organisms differ depending upon the type of UTI and strains causing acute pyelonephritis in the non-compromised host are a selected subsamples of the fecal *E. coli* flora.

The identification of UTI isolates is based on the coexpression by clones of markers such as O:K:H serotype, biotype or electrophoretic type detected by multilocus enzyme electrophoresis and DNA analysis (Svanborg et al, 1988).
2.3 Factors Affecting Pathogenesis

Although normal urinary tract is resistant to colonization by bacteria through various natural defense mechanism, yet certain host factors in addition to the virulence factors of the causative agent are now recognized to play an important role in the etiology and establishment of the renal infection. Thus the outcome of the infection is an interplay between the host and pathogen (Lomberg et al, 1989).

2.3.1 Virulence Factors of *E. coli*

![Schematic representation of an E. coli cell interacting with host tissue](image-url)

**FIGURE 1a:** Schematic representation of an *E. coli* cell interacting with host tissue
Virulence is defined as the ability of an organism to cause disease in a particular host. In virulence *E. coli* results from the cumulative impact of one or several special properties, or virulence factors (VFs) which serve to distinguish potential pathogen from harmless intestinal strains. These include hemolysin, siderophore, O-antigen, K-antigen and fimbriae (Eisenstein *et al.*, 1988). Fig. I gives the schematic representation of an *E. coli* cell interacting with host tissue, features relevant to bacterial pathogenicity.

### 2.3.1.1 Bacterial Adhesins

The term adhesin describes a specific interaction of bacterial recognition proteins (adhesins) with the carbohydrate moiety of glycoproteins or glycolipids on mammalian cells.

For simple *in vitro* test for measurement of adhesive ability the agglutination of erythrocytes by adhesive bacteria or isolated adhesins can be used. Some adhesions/agglutinations can be inhibited by L-Mannose (mannose sensitive, MS) and others cannot (Mannose resistant, MR) (Duguid and Old, 1980). MS adhesion is due to the interaction of bacterial adhesins with glycoproteins which contain branched oligosaccharides, rich in L-mannose. Some carbohydrate specificities could be attributed to adhesins mediating MR ahesion: a Gal-(1,4)-ß Gal.(P specificity) is recognised by uropathogenic *E. coli* (Leffler and Svanborg-Eden, 1980), a Neu NAc- (2,3)-ß Gal (S specificity) is recognized by *E. coli* causing, septicemia or neonatal meningitis (Korhonen *et al.*, 1984) and some uropathogenic *E. coli* attach to cells exhibiting a yet unknown structural arrangement of galactose, neuraminic acid and serine (M specificity) (Vaisinen-Rhen *et al.*, 1982).
2.3.1.1 Mannose Resistant Adhesins

MRHA are diverse, as indicated by the variety of patterns in which they agglutinate the erythrocytes of different species and blood groups. However the most important MR adhesins in the pathogenicity of UTI are P pili or P fimbriae.

Kallenias et al (1980) studied the adherence of urinary bacteria in the presence of mannose and led to the discovery that most adhering strains agglutinate human erythrocytes of the P1, P2 and P1k but not the p-blood group, suggesting that most of these strains bind to P-blood group antigens. Purified fimbriae from these strains agglutinate human erythrocytes with the same binding specificity as the bacteria from which they are derived leading to the designation P-fimbriae.

Leffler et al (1981) described the nature of MR adhesins present on the surface of E. coli. These fimbriae were termed as P-fimbriae because of their ability to recognize antigen, determinant of the human P-blood group system. The receptors for these fimbriae were found to be globo-series of glycolipids with the common disaccharide Gal-α-(1-4) Gal-β.

Vaisanen et al (1981) demonstrated that mannose resistant hemagglutinating ability of E. coli strain was associated with human pyelonephritis. The receptor structure for P-fimbriae was confirmed to be α-D-galactosyl-(1-4) β-D-galactose by them. Vaisnen et al (1982) also reported that some of pyelonephritogenic strains of E. coli could bring about M-blood group specific mannose resistant haemagglutination. A membrane glycoprotein, glycophorin A isolated from MM-erythrocytes was found to strongly inhibited the hemagglutination.
Svanborg Eden et al (1984) studied the receptors for P-fimbriae on the surface of hPMNLs. They found that receptors for P-fimbriae were not found on hPMNLs and hence P-fimbriated strains of *E. coli* avoid host defense mechanism of phagocytosis by phagocytic cells.

Lamberg et al (1986) reported that human erythrocytes and uroepithelial cells from individual of the P1 and P2 blood groups do not differ in P-fimbrial receptor density. They further reported that uroepithelial cells from men and women have a similar receptor density for P-fimbriated strains as do squamous and transitional uroepithelial cells.

Two viewpoints exist regarding the importance of expression of fimbriation *in vivo*. Harber et al (1986) concluded that fimbriation and adherence were not relevant *in vivo*. Since fimbriation, hemagglutination and epithelial cell adherence properties were absent from organisms collected directly from urine of injected patients. On the other hand, using more sensitive methods and more careful patient selection, Pere et al (1987) concluded that fimbriae are expressed *in vivo* and are important adhesins for mediating adherence during infection.

Lindberg et al (1987) studied in detail the structure of P-fimbriae. They reported that P-pili is composed of approximately 10 helically polymerized subunits, with one major subunit species (Pap A) constituting the bulk of fimbria. Three minor adherence related fimbrial subunits (Pap E, Pap F, Pap G) are present in minute amounts at the fimbrial tips.

Lund et al (1987) reported that Pap G is the actual adhesin molecule responsible for Gal-Gal specificity. Trans complementation of a Pap G mutant with the gene for an alternative adhesin (Prs G) yields
fimbriated cells with Prs binding specificity. They further reported that primary and secondary structures of Pap G (35 KDa) are not similar to those of Pap A (19.5 KDa) and the structural subunits of other *E. coli* fimbriae.

Epidemiological studies reveal that the proportion of strains expressing P-fimbriae declines progressively from as high as of 70% among isolates from patients with pyelonephritis to 36% among cystitis patient isolates, 24% among ABU patient isolates and 19% among fecal strains (Merild *et al* 1988). These observations suggest that P-fimbriae contribute to the ability of *E. coli* strains to cause UTI, especially the more clinically severe forms and the strains lacking P-fimbriae are at a disadvantage in the urinary tract.

Karr *et al* (1989) reported that receptor for P-fimbriae are present in the kidney (bowmen’s capsules, glomeruli, proximal tubules, distal tubules etc), bladder (epithelial and muscular layers) and even uroepithelial cells, contributing towards the colonization of human urinary tract.

### 2.3.1.1.2 Mannose Sensitive Adhesins

With rare exceptions, mannose sensitive adherence mediated by *E. coli* strains is due to type-1 fimbrial (Duguid & Gillies, 1957). In clinical studies, mannose sensitive hemagglutination of guinea pig erythrocytes is generally interpreted to indicate the presence of type-1 fimbriae.

Latham and Stamm (1984) studied the role of type-1 pili in the pathogenesis of UTI in relation to site in the urinary tract. They found that type-1 fimbrial expression is slightly more common in episodes of UTI localized to the lower (65%) as compared to the upper (73%) urinary tract.
Kisselius et al. (1989) studied in-vivo expression and phase variation of type-1 fimbriae. They found that 31 of 41 voided urine specimen from non-compromised patients with acute UTI have type-1 fimbriated organism and type-1 fimbrial phase variants were identified in specimens collected from different sites within the urinary tract.

Type-1 fimbriae are known to bind Tamm Horsefall proteins (THP) when present in high concentration. Since, THP often coats uroepithelial cells and prevent the bacteria from adhering to the urinary mucosa and allow them to be expelled in voided urine, thus act as non-specific defense mechanism in the urinary tract (Orskov et al., 1980).

Receptors for type-1 fimbriae are reported to be present on hPMNLs as well and hence type-1 fimbriae promote adherence to hPMNLs and phagocytosis by hPMNLs (Weinstein & Silverblatt, 1983). The fate of bacteria adhering to hPMNLs via type-1 fimbriae is variable depending upon bacterial hydrophobicity and state of opsonization. Some strains escape phagocytosis, but are killed all the same, presumably by the contents of phagocytic granules that are released following bacterial binding or by toxic products of respiratory burst triggered by bacterial binding (Stenqvist et al., 1982). Further, type-1 fimbriated bacteria that are phagocytosed can survive within lysosomal vacuoles as long as they are unopsonized possibly because of subnormal level of myeloperoxidase activity in lysosomes containing unopsonized bacteria (Goetz et al., 1987).

Ishikawa (1991) found that incidence of P-fimbriated E. coli with pyelonephritis, cystitis and asymptomatic bacteruria was 78.6%, 31.9% and 22.2% respectively. However, almost all the P-fimbriated E. coli
have also type-1 fimbriae. Hence, simultaneous presence of P- and type-1 fimbriae is one of the significant virulence factors in UTI.

2.3.1.2 Hemolysin

Two distinct types of hemolysins (α and β) are found to be produced by *Escherichia coli*. Third kind of hemolysin (Γ-hemolysin) produced by *E. coli* mutants resistant to nalidixic acid was reported by Walton and Smith (1969). Most hemolytic *E. coli* secrete alpha hemolysin which is a cytolytic toxic proteins with some evidence of minor phospholipid or lipopolysaccharide component. Its molecular weight ranges between 2 x 10^6 to 8 x 10^5 and largest protein found by gel electrophoresis was of 110 KD (Rennie et al, 1974; Cavaliesi et al, 1984).

In addition to lysing erythrocytes, hemolysin is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses. Exposure of hPMNLs to hemolysin stimulates chemiluminescence, degranulation and release of leukotrienes and ATP, causing marked morphological alternation and impairs chemotoxis and phagocytosis (Bhakdi et al, 1988, 1989).

As early as 1921, Dudgeon et al suggested that hemolytic activity might play a part in virulence of *E. coli* in human urinary tract. It was Brooks et al (1980,1981) who carried out survey to find out the importance of hemolysin as a VF in upper as well as lower UTI. They observed that hemolysin strains were more prevalent in UTI episodes localized in the upper (51%) than the lower (30%) urinary tract infections.
Lomberg et al. (1989) studied the virulence associated traits of the \textit{E. coli} strains isolated from patients with renal scarring. They found that hemolysin production among isolates from patients with pyelonephritis is less prevalent in strains from patients with compromising urological or medical condition (32%), children with renal scarring (21%) or pregnant women (40%).

Relationship between site of infection in the urinary tract and frequency of hemolysin producing \textit{E. coli} has been studied by Arthus et al. (1990). They found that in human UTIs, hemolysin production is most common among strains from patients with pyelonephritis (49%) followed by those from patients with cystitis (40%) and ABU (20%).

2.3.1.3 Siderophore

Iron is needed by all living organisms; Weinberg, (1978) reported that though the total iron concentration in biological fluid is $>20$ uM, almost all of this iron is complexed with host iron proteins. Part of the host response to infection is to further reduce the amount of iron available to the invading pathogen by decreasing intestinal iron absorption, synthesizing additional iron proteins, and shifting iron from the plasma pool into the intracellular storage.

To procure, iron required by microorganism for their metabolic activities, organisms are now known to elaborate iron sequestring substances called siderophores. It has been reported that most of the gram negative organisms including \textit{E. coli} produce two type of siderophores i.e. Enterochelin and Aerobactin.

Warner et al. (1981) reported that in \textit{E. coli} the hydroxamate siderophore is the most effective of the several iron chelation systems.
employed by enteric bacteria for iron acquisition. They further observed that enterobactin deferrates transfer iron more rapidly than aerobactin in aqueous solutions; however the reverse is true in serum or in the presence of albumin, presumably because enterobactin binds to and is inactivated by proteins. Enterobactin is less soluble and less stable than aerobactin. Release of iron from enterobactin requires hydrolysis of siderophore.

Staurt *et al.* (1982) concluded that ability to carry out hydroxamate mediated transport of iron was widely distributed among natural isolates of *E. coli* but the distribution of hyd + *E. coli* was not random. It seemed that production of hydroxamate was an important virulence factor against the active host defense based on restricted availability of iron.

Montgomerie *et al.* (1984) examined the clinical isolates of *E. coli* for the presence of hydroxamatic siderophore. The incidence of aerobactin positive strains of *E. coli* from the blood was significantly higher (75%) than the incidence of these strains from other sites (38-47%). They further found that in the murine ascending model of UTI, the main proportion of death in aerobactin positive strains were significantly greater than the proportion of death in aerobactin -ve strains. There was a positive correlation of virulence and aerobactin. However, no correlation between aerobactin production and resistance to phagocytosis, dulcitol fermentations, adherence to uroepithelial cells and the presence of K-antigen was found.

Carbonetti *et al.*(1986) screened 516 strains of *E. coli* for the presence and expression of the aerobactin iron uptake system. Incidence was markedly higher among clinical isolates from patients with
septicemia (68.8%), pyelonephritis (74.6%) and symptomatic (59.8%) and asymptomatic (63.2%) lower UTIs than among normal human fecal isolates (34.3%).

James et al (1988) correlated the presence of the aerobactin system with antimicrobial agent resistance, presence of P-fimbriae, hemolysin production and presence of type-I fimbriae. Colony and southern hybridization of total and plasmid DNA with DNA probes for each virulence factor showed that aerobactin determinant was present in 78% of the strains and were plasmid associated in 21% isolates, whereas P-fimbriae, hemolysin and type-I fimbriae determinants were present in 74%, 43% and 98% of strains respectively and were always chromosomal.

2.3.1.4 Lipopolysaccharide O-antigen

Serologically, lipopolysaccharides (LPS) are known as O-antigens. LPS form a part of cell envelop and consists of three parts viz lipid A, core oligosaccharides and O-specific polysaccharides (Orskov et al, 1977). Organisms, in which LPS moiety is complete are turned as smooth and found to be virulent. Loss of O-specific polysaccharides leads to reduction of virulence of the mutant strains which are known as rough strains.

As early as in 1945, Vahlne studied the serotyping of uroisolates of *E. coli*. He found UTI isolates to be less serologically diverse than fecals strains with majority of urinary strains carrying small number of O-groups. The same O-groups predominate among both urinary and fecal strains, but certain of the common O-groups are significantly more prevalent among urinary than among fecal strains and these include O1, O2, O4, O6, O8, O16, O18, O75 (McGeachie, 1965).
Lindberg et al. (1975a) studied the relationship between the prevalence of UTI associated O-groups and the site of infection in urinary tract. They observed that prevalence of the UTI associated O-groups are greatest (80%) among pyelonephritis patient isolates, lower (59%) among cystitis patient isolates and lowest (31%) among ABU patient and fecal isolates.

Lindberg (1975b) reported that O-antigen of strains from acute pyelonephritis and asymptomatic bacteriuria (ABU) belonging to same O-groups behaved differently, like strains from acute pyelonephritis absorbed O-antibodies in rabbit O-antisera better than did strain from cases of ABU.

It was suggested that intact O-antigen may be of the virulence factor of *E. coli* by Sohl-Akerlund et al. (1977). They found that strains from asymptomatic patients are less immunogenic than those recovered from acute UTI cases.

Stendahl et al. (1979) reported that surface lipopolysaccharides can have enormous influences on the interaction between bacteria and host cells. O-antigens prevent direct association between bacteria and phagocytic leukocytes owing to their hydrophilic nature.

Wallwijk and de Groot (1983) showed a clear association of the UTI associated O-groups with more severe forms of UTI and little apparent difference between fecal and ABU patient isolates. They reported that 91%, 75%, 68% and 40% of the *E. coli* from pyelonephritis, cystitis ABU and fecal isolates respectively belonged to UTI associated O-groups.
Hagberg *et al* (1984) compared *E. coli* O75*:K5* with O75*:K5 isolates and found that the presence of O-antigen contribute to the ability to persist in the mouse kidney and bladders.

Linder *et al* (1988) tested the ability of pure lipid A to initiate the inflammatory response. They found that intravesical instillation of lipid A resulted in a significant, dose dependent influx of PMNs into the urinary tract. The role of lipid A in triggering the inflammatory response was further supported by studies in mice with a genetic defect in this response. The LPS responders cleared the infection within a few days however LPS non responders had about 1000 fold higher bacterial counts in the kidney by 24h after infection and remained chronically infected.

### 2.3.1.5 Capsular Polysaccharide K-antigen

In 1945, Kauffmann and Vahlne introduced the term K-antigen (from the German word for capsule, 'Kapsel') as a symbol to denote either envelop or capsule antigens.

**Structure**

Capsular antigen of *E. coli* which has 103 types (Orskov, 1977) are acidic polysaccharides of different chemical composition. Several features have been used for the general classification of the capsular polysaccharide K-antigen such as chemical composition, molecular weight, mode of expression and genetic determination (Table 2).

The capsules of most extraintestinal pathogenic *E. coli* strains are thin, patchy, acidic, thermostable and highly anionic and belongs to group II polysaccharides (Orskov, 1978) (Fig. 1 b,c). These polysaccharides tend to aggregate spontaneously because of
phosphatidic acid group at the reducing end of the molecule. Some important group II polysaccharides include K1, K2, K5, K6, K12, K13, K14, K15, K20, K23, K51, K52 and K54. Table-3 describes the structure of few K-antigen most closely associated with urinary tract infections (Jann and Jann, 1990).

Table 2: Classification of capsular polysaccharide antigens of *E. coli*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Property</th>
<th>Capsular polysaccharide group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>1</td>
<td>Molecular weight</td>
<td>&gt;100 Kd</td>
</tr>
<tr>
<td>2</td>
<td>Acidic component</td>
<td>Glucuronic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Galacturonic acid, pyruvate</td>
</tr>
<tr>
<td>3</td>
<td>Expressed below 20°C</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Coexpression with antigens</td>
<td>08, 09, 020</td>
</tr>
<tr>
<td>5</td>
<td>Lipid at reducing end</td>
<td>Core lipid A</td>
</tr>
<tr>
<td>6</td>
<td>Removal of lipid at pH 5-6 or 100°C</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Chromosomal determinant at (close to)</td>
<td>rfb(his), rfc(trp)</td>
</tr>
<tr>
<td>8</td>
<td>CMP-KDO synthetase activity</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Intergeneric relationship with</td>
<td>Klebsiella</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1b : Diagram of outer layer of *E. coli* (Jann and Jann, 1985)

Fig. 1c : Electron micrograph of *E. coli* with a thin capsule (Jann and Jann, 1985)
### Table 3: Structure of Few Important Uropathogenic K-Antigens

<table>
<thead>
<tr>
<th>S. No.</th>
<th>K-Antigen</th>
<th>Repeating Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>KlAc⁺</td>
<td>8 → Neu NAc → 7/9 a OAc</td>
<td>McGuire and Binkley (1964)</td>
</tr>
<tr>
<td>2.</td>
<td>KlAc⁻</td>
<td>8 → Neu NAc → a</td>
<td>Orskov et al (1979)</td>
</tr>
<tr>
<td>4.</td>
<td>K12</td>
<td>3 → Rha → Rha → KDO → α α ↓7(8) OAc</td>
<td>Schmidt and Jann (1983)</td>
</tr>
<tr>
<td>5.</td>
<td>K13</td>
<td>3 → Rib → KDO → 4 S E OAc</td>
<td>Vann and Jann (1979)</td>
</tr>
</tbody>
</table>

**Genetics:**

Group II capsular polysaccharide are encoded by a cluster of genes located near the Ser A locus on the *E. coli* chromosome (Orskov, 1978).
Echarti et al (1983) cloned the genes coding for production of K1 polysaccharide capsule of *E. coli*. The minimum of 9 kilobases of DNA-split into at least two gene blocks is involved in synthesis and assembly of the capsule. One gene block is responsible for biosynthesis of polysaccharide and other is responsible for extracellular appearance of capsular polysaccharide.

Genetic analysis by Boulnois et al (1987) indicated that more than one locus was required for K-antigen biosynthesis. One locus for determination of antigen specificity (Synthesis and polymerization of subunits) another responsible for post polymerization and modification of the polysaccharide, and third is responsible for the translocation across the outer membrane to the cell surface.

To explore the relationship between the different capsule biosynthesis pathways, genes for production of K5, K7, K12, K92 antigens were cloned and characterized and these genes have common organization and closely related (Robert et al 1986, 1988).

To identify and characterize the inner-membrane transport system during synthesis of capsule, the molecular analysis of *E. coli* k5 Kps locus has been done by Smith et al (1990). The complete nucleotide sequence was determined of a region of *E. coli* k5 antigen gene cluster (Kps M and Kps T) postulated to encode function for the translocation of capsular polysaccharide across the inner membrane.

Frinke et al (1991) studied the biosynthesis of *E. coli* K5 polysaccharide *in vitro* and determined the serological specificity of *in vitro* product by monoclonal antibodies. The polymerization occurred at the inner face of the cytoplasmic membrane without the participation of lipid linked oligosaccharides. The k5 polysaccharide obtained from the
membrane after an *in vitro* incubation had 2-keto-3-deoxyoctulosonic acid as the reducing sugar.

Gottesman and Stout (1991) found that K12 polysaccharide synthesis is regulated by a complex network of regulatory proteins which are encoded by regulatory genes RcsA, RcsB and RcsC. For maximum expression of capsule, two positive regulators RcsA and RcsB genes are necessary. They further proposed a model for the capsule synthesis in which RCSA interacts with RCSB to stimulate transcription of the CPS genes.

**Immunogenicity of Capsular Polysaccharide**

Capsular polysaccharides from uropathogenic *E. coli* are poor immunogen in animals and humans.

Kaijser and Olling (1973) analyzed antibody response to infecting *E. coli* strain in rabbits with hematogenous pyelonephritis. K13 antibody response was very moderate and in several animals, no K13 antibodies were detected at all. This might be due to the fact that antigenic stimulation was not sufficiently intensive or could be due to the induction of tolerance to K-antigen.

Kaijser and Ahlstedt (1977) raised antibodies to K-antigen in rabbit by repeated immunization with whole, formalin killed and later live bacteria. Serum samples studies contained varying level of antibody to different K-antigens. The amount of K1 antibody were lower than those of anti K13 antibody in the immunized animals.

Only 12% of the humans with pyelonephritis due to K1 strain and one third of the rabbits immunized with K1 strain produce measurable antibody response (Hanson *et al.*, 1977).

Kaijser (1981) studied the *E. coli* K-antibody response in rabbits
which were immunized with a mixture of five *E. coli* strains containing K-antigen of strains of uropathogenic importance. For each of the K-antigen, no statistically significant difference in K antibody formation was shown if one K-antigen containing strain or five different strains were given to the animals. However, some *E. coli* K-antigens induced generally higher K antibody titre than others. K1 produced fairly low titre or no antibody was detected against K1.

Poor immunogenicity of capsular polysaccharide is because of molecular mimicry with the host immune system. capsular polysaccharide *E. coli* K5 found to have the same structure as that of non sulphated precursor polysaccharide in heparin biosynthesis, and K1 is similar to the important moiety found on the surface of mammalian cell i.e. sialic acid (Vann *et al* 1981).

Kaijser (1983) noted that peroral immunization of the healthy adults with live *E. coli* 04:K12 elicited an antibody response of IgG and IgM to K12 antigen in serum. In addition, secretory antibody of IgA were also shown in the saliva.

The immunogenicity of poor immunogenic *E. coli* K-antigens has been shown to increase to certain extent by conjugating it with carrier proteins. The coupling of K1 and K13 with bovine serum albumin elicited IgG and IgM anti K antibody in majority of the animals. These higher antibody titres to K-antigen had the capacity to partially protect the animals against ascending pyelonephritis (Kaijser *et al*, 1983 a,b).

**Anticomplementary and antiphagocytic activity**

Presence of capsular polysaccharide enable the bacteria to overcome host defence and protect its cell against the bactericidal action of complement and phagocytosis (Fig. 1d).
Horwitz and Silverstein (1980) determined the influence of *E. coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. It was reported that for phagocytosis and killing of encapsulated *E. coli*, both complement and antibody were required. In the absence of antibody, *E. coli* K1 capsule blocked complement fixation to the bacterial surface probably by masking surface components like lipopoly-saccharide antigen.

Aguero and Cabello (1983) studied the relevance of capsular K1 antigen in the pathogenicity of *E. coli*. Their observation was that presence of K1 antigen protected the *E. coli* from bactericidal activity of serum and phagocytosis by mouse peritoneal macrophages. However, K1 *E. coli* variants were efficiently phagocytosed in presence of normal mouse serum due to complement deposition by activation of alternate pathway of complement, which otherwise is inhibited by presence of K1 capsule.
The importance of K-antigen as virulence factor in nephropathogenicity was emphasized by Verweig-Van Vught et al (1983). The loss of K-antigen from K+ strains caused decreased serum resistance and K- strains induced better chemiluminescence in PMN leucocytes. In addition, *E. coli* variants without capsule had 3-16 fold increased LD50 in animals.

Presence of K1 capsule on *E. coli* impart serum resistance to majority of the strains by shielding these cells from alternate pathways of complement activation. The resistance can be broken with addition of anti K1 or anti LPS antibodies in the serum (Pluschke and Achtman, 1984).

One way of providing virulence by capsular polysaccharide is stearic hindrance. C3b is deposited on the cell wall underneath the polysaccharide capsule such that capsule may act as a mechanical barrier to recognition of C3b by phagocytic cells (Brown et al, 1983).

The anticomplementary effect of capsular polysaccharide may occur, since it provides a favourable binding site for regulatory factor B1H to C3b. The deposition of H-C3b on the bacterial cell surface prevents the formation of C3 convertase which results into breakdown of amplification loop and blocking of complement cascade (Harber et al, 1986).

Allen et al (1987) showed the direct evidence of K1 capsule interference against host defence system by constructing mutant without K1 antigen from parent strain. K1' mutants were more sensitive to serum, easily phagocytosed by PMN and produced higher chemiluminescence response than K+ parent strain. Electron microscopic studies revealed that most intraphagocytosed *E. coli* K+ (wild type) were
able to remain intact up to 1h after endocytosed in PMN, but not the K strains.

There is a threshold level of K1 needed to encounter serum lysis effect. Vermeulen et al. (1988) found that amount of bacterial cell wall associated capsular polysaccharide K1 required for serum resistance was present during the log phase of growth. It was reported that loss of capsule at low pH may be an additional mechanism by which host defend against invasive infection by K1 encapsulated *E. coli*.

Another evidence of antiphagocytic activity of K1 antigen came from the results of Sokolowska et al. (1989) who showed that addition of K1 capsular polysaccharide to opsonin before mixing with PMN, decreased the rate of phagocytosis.

Kim et al. (1990) evaluated murine hybridoma antibody directed against the K1 capsule for their ability to enhance bactericidal activity of serum against *E. coli* strains. The antibodies to K1 efficiently enhanced PMN mediated killing of K1 encapsulated *E. coli*. The opsonization with anti K antibody were 10-fold better than the antibody to O side chain of the LPS.

Herpay et al. (1991) examined the surface hydrophobicity of 136 *E. coli* strains by salt aggregation test and deduced that possession of antigen K1 provides the bacterial surface a hydrophilic character and provides covering to its relative surface hydrophobicity.

**Association with pyelonephritis and other virulent factors**

Not only, certain K types are more associated with the urinary tract infections, but K1, K2 and K13 are present in greater amount among *E. coli* from cases of pyelonephritis than among strains from cystitis or from stools of healthy children (Kaijser, 1973). Kaijser et al. (1977)
reported that the most frequently associated K-antigens with the acute pyelonephritis are K1, K2, K3, K12 and K13 which account for 70% of isolates from patients with acute pyelonephritis in children.

Hughes *et al* (1983) found an association of hemolysin production and type of K-antigen on the surface of the *E. coli*. It was reported that strains from symptomatic and asymptomatic patients were mostly K5⁺ Hyl⁺ or K1⁺ Hyl⁺, which showed a strong association of K5 capsule with hemolysin secretion.

Urinary tract infections are quite common in the women during pregnancy. Stenqvist *et al* (1987) reported that serotypes K1, K5 and K20 were most frequently (62.5%) found in the urinary isolates from pregnant women with acute pyelonephritis as compared to isolates from asymptomatic bacteriuria cases (32%).

Sandberg *et al* (1988) studied the relationship between bacterial characteristics and severity of UTI in adults. Nine serotypes comprised 65% of the strains in uncomplicated pyelonephritis and 63% of isolates possessed either K1 or K5. Out of these, most frequently present was K1, which was 37% in pyelonephritis and 17% in cystitis isolates.

Domingue *et al* (1988) studied the importance of K-antigen in experimental infections in mice. They observed that when K- antigen was present in large amounts, it favoured increased lethality, bladder and renal colonization and renal pathology.

The K-antigen 2, 1, 5 and 12 were the capsular types found in the isolates from boys with urinary tract infections accounting for 18%, 13%, 13% and 11% of strains respectively. Significant difference was not shown between strains from lower UTI or pyelonephritis in boys in comparison with strains from pyelonephritis in girls. 80% strains from
boys were encapsulated, while all the strains from girls with pyelonephritis were encapsulated (Westerlund et al, 1988).

Falkenhagen (1991) analysed the 253 strains of E. coli which had been isolated from urinary tract infections. 86% strains were encapsulated and belonged to eight common O-serotypes associated with UTI. K1 and K5 capsules could be found in 32 and 33% of isolates.

2.3.1.6 Serum Resistance

Ability of Escherichia coli and other gram negative bacteria to resist bactericidal action of serum has been recognized as virulence factor as early as in 1968 (Feingold et al, 1968).

Guze et al (1973) carried out experiments to compare the nephropathogenicity of serum resistant and serum sensitive strains of E. coli. They found that serum resistant strains were usually more nephropathogenic in comparison to serum sensitive strains in variety of models of UTI. But these resistant strains were not associated with increased lethality.

Lindberg et al (1975) observed that prevalence of serum resistant among ABU patient isolates was even lower than among faecal strains, suggesting that either serum sensitive strains are selected from the faecal flora for symptomatic colonization of the urinary tract or faecal strains entering the bladder may adopt to new environment by becoming more serum sensitive.

Brooks et al (1980) reported that isolates from patients with pyelonephritis and cystitis are more commonly serum resistant than are those from patients with ABU or faecal isolates. Their observations suggest that serum resistant is important in the pathogenesis of symptomatic UTI, regardless of the severity.
Montengro \textit{et al} (1985) reported that bacterial resistance to killing by serum results from the individual or combined effects of capsular polysaccharide, O-polysaccharide side chains and surface proteins. Further Goldman \textit{et al} (1984) conclude that O- antigen polysaccharide side chains protect against complement lysis not by blocking complement activation but by causing complement to be activated at a location distant from sensitive membrane.
PART - II : IMMUNITY AND IMMUNOPROPHYLAXIS

2.4 Immunity in pyelonephritis

Urinary tract infections caused by E. coli are associated with a local and systemic immune response which has been fairly well characterized over past 30 years (Habson et al., 1977). The main interest in the immunological reactions occurring in the cases of pyelonephritis has focussed on events like the possible role of acquired immunity directed against bacterial antigens. This immunity could be protective by eliminating bacteria from the renal tissue or infection may stimulate autoimmune reaction against the renal tissue and thus could produce tissue damage even after eradication of infection.

2.4.1 Humoral immune response

After acute Pyelonephritis, about 90% of the patients develop diagnostic changes in the antibody titre against infecting bacteria, whereas, this occurs in 50% of the cases with cystitis (Vosti et al., 1965). IgG is the predominant immunoglobulin synthesized during pyelonephritis in addition to IgM and IgA antibodies.

Role of bacterial antigens in the antibody response was investigated by Hanson et al. (1977). They reported that in patients with acute pyelonephritis, there was consistently an increase in the level of O-antibodies in contrast to those with cystitis. Such antibodies were mainly of IgM class, but in patients with recurrent infection, IgG antibodies to LPS were also seen. However, antibodies to O and K antigen were secreted in urine of the patients with acute pyelonephritis. They also noticed that patients with acute pyelonephritis had increased titre of IgG to Tamm Horsefall protein (THP), but the possible significance of these antibodies was unknown.
Maltspy and Baltzer et al. (1981) investigated antibody response to lipid A. They found that it was significantly elevated in children with cystitis, pyelonephritis or asymptomatic bacteriuria. Rene and Silverblatt (1982) found that pyelonephritic patients developed an increase in specific antipilus antibodies of the different classes like IgG, IgM and IgA. These antipilus antibodies were cross reactive to some extent. However these antibody were absent or present in very less concentration in the urine of patients with acute pyelonephritis (Salit et al., 1988).

Bacterial antigens have been shown to be present in renal scars following infection in form of amorphous material and may contribute to continued appearance of local antibody producing cells in the kidneys (Thomson and Olesen, 1977).

Experimentally induced *E. coli* pyelonephritis has given information on the kinetics of both locally and systemically produced antibodies. Despite the presence of local and systemic antibody, the infection is not eradicated which suggests that the components of immune response are ineffective. This inability may be explained on the basis of the observation that K-antigen which is virulent factor of bacteria, rarely induces serum or local antibody response in pyelonephritis (Smith and Kaijser, 1976).

### 2.4.2 Cell mediated immune response

The role of cell mediated immune reactions directed against bacterial antigen in pyelonephritis is difficult to interpret and not well characterized.

Miller and Creaghe (1975) laid stress on the role of cell mediated immune response in pyelonephritis. They found that experimental *E. coli* renal infection affected the distribution of lymphocytes and have marked
effect on the functional capacity of the splenic lymphocytes in early infection.

In a study of Miller et. al. (1976) it has been shown that T lymphocytes were the predominant lymphocytes in the inflammatory infiltrate, but did not respond to PHA in vitro. They further manipulated the immune capacity of rats using immuno suppressive drugs. Pyelonephritic animals treated with cycloheximide were unable to produce antibacterial antibodies. Despite this, organisms were eliminated from the infected kidneys more readily than untreated animals with normal humoral response.

In another study of Miller et al (1978) T lymphocyte mitogens, Con A and PHA have been used to determine the association between cell mediated immunity and renal infection. A marked suppression of "T" lymphocyte response to Con A in vitro has been found as early as 2 h after induction of renal infection. Immune responsiveness showed maximum suppression after 48-72 h of challenge, when 90% of immune reactivity was consistently lost despite an active B-cell response and antibody production. It was, therefore, concluded that the thrust of rapid bacterial proliferation during the critical 48-72 h period after incubation coincided with the ablation of cell mediated immune mechanism.

Further Miller et al. (1983) showed that reduced immune capacity of T lymphocytes in pyelonephritis is mediated through an antigen activated suppressor cells. It was proposed that the generation of a similar cell population in the pyelonephritic animals could account for the depressed cell mediated immune response. Experiments suggesting this hypothesis have clearly shown that the immune function of non responsive splenic lymphocytes from pyelonephritic animals could be
restored by removing a suppressor cell population generated during renal infection. This was confirmed by *in vitro* experiments in which suppressor cells from pyelonephritic animals were able to suppress 96% of mitogenic responsiveness of normal splenic lymphocytes.

Kirillov *et al.* (1988) quantitatively characterized the subpopulations of lymphocytes in blood of acute pyelonephritic patient, while Kurnick *et al.* (1988) showed that T lymphocytes play an important role in the kidney in response to invading bacteria. These T lymphocytes, especially with helper/inducer phenotypes accumulate in the lesions of acute pyelonephritis in rats. They are mostly activated cells and cells with specific reactivity to infecting bacterial stain. Further it was shown that P-pili had no or little importance in modulating the local immune response in the infected kidney.

Glauser *et al.* (1978) reported that PMNL infiltrating the kidney in the renal infection may play an important role in damaging the renal parenchyma tissue, producing renal scarring during acute pyelonephritis.

Sullivan *et al.* (1977) reported that polymorphonuclear leukocytes infiltrating the kidney in renal infection may play some role in damaging renal parenchyma tissue in acute pyelonephritis. There was suppression of infiltration of these cells in kidney of rats treated with snake venom.

Pryor *et al.* (1980) showed that type-I fimbriae of *E. coli* stimulated the respiratory burst activity or release of granular enzymes during pyelonephritis. However, bacteria resisted ingestion by phagocytic cells which produced large quantities of potentially toxic reactive oxygen species.
Shimamura (1981) observed that structural elements of renal tissue were well preserved in leukocyte depleted rats, while tubular basement membrane and tubular epithelia were extensively destroyed where massive infiltration of leukocytes had occurred. This shows the role of PMNLs in renal tissue damage following pyelonephritis.

It was suggested that interaction between bacteria and phagocytes during inflammatory reaction is crucial to the outcome of infection. Reactive oxygen species were proposed to disturb the normal balance between released proteinases and tissue antiproteinases or directly cause tissue injury (Carb and Janoff, 1979).

Harber et al. (1986) observed stimulation of PMNL chemiluminescence and release of granular proteins in vitro provoked by type 1 fimbriae of E. coli which were not phagocytosed but induce renal scarring. These observations offered a biochemical explanation for the pathogenesis of tissue damage and scar formation associated with chronic pyelonephritis.

Kaack et al. (1986) reported that granulocytes aggregation observed during acute pyelonephritis might lead to vascular occlusion and mediate tissue damage due to resultant renal ischemia. Complement activation mediating inflammatory response in kidneys within 48 h was observed.

Shah et al. (1987) have reported that neutrophils alter the renal function by some unknown mechanisms involving direct or indirect reactions. Direct injury is caused to glomerular basement membrane or DNA and other constituents.
Ivanyi et al. (1988) showed the appearance of leukocytes in the lumen of tubules during acute human pyelonephritis leading to disruption of cortical collecting tubules. There was a focal inflammatory disruption of uriniferous ducts forming morphological basis of intratubular accumulation of leukocytes.

Meylan et al. (1989) have shown the relationship between neutrophils mediated oxidative injury during acute experimental pyelonephritis and chronic renal scarring by demonstrating that renal damage results from excessive infiltration and activation of PMNL in renal parenchyma rather than from direct damage by bacterial virulence factors.

Gupta et al. (1992) studied the mechanism of tissue injury at the cellular level by following the chemiluminescence response of various phagocytic cells in the *E. coli* induced experimental pyelonephritis. There was marked increase in the capacity of phagocytic cells to produce reactive oxygen species with increasing days postinfection, whereas the peak chemiluminescence response time was observed to be decreased with the progress of the disease.

2.5 Immunoprophylaxis against pyelonephritis

2.5.1 Fimbrial vaccines

Silverblatt et al. (1982) evaluated the role of type 1 pili in providing protection against ascending pyelonephritis and further suggested the mechanism of protection. It was reported that rats immunized with type 1 pili of *E. coli* and animals infected with *E. coli* developed anti pili antibodies in their serum. Active and passive immunization of rats with pili protected the animals from ascending pyelonephritis. In response to study the mechanism, they reported that anti pili antibodies did not
mediate complement dependent bacteriolysis, opsonophagocytosis or promote more rapid intravascular clearance of infected *E. coli*. However, this anti pili immunity effectively inhibited the bacterial adherence to epithelial cells.

Robert *et al.* (1984) reported that Gal-Gal pilus vaccine prepared from a clinical isolate prevented histopathological changes in a non obstructive monkey model following ureteral infection of a P-fimbriated strain. In vaccinates animals bacteria covered with antibodies were free within the renal tubules, with no evidence of tubular cell damage, whereas in control animals bacteria showed adherence to the tubules with death of tubular cells.

Some studies provide evidence that bacterial colonization can be blocked or interrupted by antibodies against either the adhesin or the complementary host cell receptor of pathogenic bacteria. Abraham *et al.* (1985) reported that hybridoma antibodies directed against quaternary structural epitopes of type 1 fimbrial adhesin of *E. coli* or against D-mannose receptor prevented the attachment of mannose sensitive *E. coli* to various eukaryotic cells. Further passive intra-peritoneal administration of the fimbrial specific or D-mannose specific antibodies protected mice against retrograde colonization with mannose sensitive *E. coli*. However, it was shown that monoclonal antibodies directed against fimbrial subunits rather than quaternary structural epitopes or against N-acetyl galactosamine D-mannose residues lacked protective activity.

In a study carried out by O’Hanlay *et al.* (1985) the protective efficacy of Gal-Gal pili, mannose sensitive pili and somatic "O" antigen in BALB/c mouse model Gal-Gal pili emulsified in complete freud adjuvant was tested. When injected subcutaneously or intramuscularly protected
the mice after challenge with ID50. Renal colonization by J96 Strain occurred only in 13% of mice (p < 0.05) and renal invasion in 9% mice. Immunization with adjuvant alone or somatic O antigen or mannose sensitive pili did not block renal colonization or invasion by parent strain.

Garg et al. (1987b,c) evaluated the protection afforded by P pili antibody using brush border membrane enzyme as marker. The significant difference in the Vmax of various BBM enzymes of infected and immunized-infected groups at various stages of infection revealed the partial protective role of anti pili antibody against ascending pyelonephritis in rats.

Dima et al. (1989) carried out a study in guinea pigs and found a significant protection against acute pyelonephritis by immunization with purified type 1 fimbriae of E. coli. The immunoprotection was induced by oral vaccination with multiple doses of fimbriated antigen.

Kaack et al. (1988) reported that infants from the monkeys, immunized with purified P-fimbriae during the third trimester of pregnancy were protected against UTI following bladder challenge with the homologous P-fimbriated strain. In the further studies, Kaack et al. (1989) tested the cross protectivity of the two different P-fimbriae vaccines from E. coli strain ER2 and JR1. Vaccinated animals showed elevated antibody titres to P-fimbriae from each of the E. coli strain with true cross reactivity.

Pecha et al. (1989) studied the protective potential of Gal-Gal pili against pyelonephritis in murine model and 49% of the animal were protected from renal colonization after a single dose of Gal-Gal pili. Light microscopic examination of the kidneys demonstrated less histopathological changes among pili immunized mice. O’Hanley et al. (1991)
determined and evaluated the pathogenic significance of Gal-Gal pili and $\alpha$-hemolysin in ascending non obstructive BALB/c mouse pyelonephritis model. A purified Gal-Gal pili vaccine prevented subsequently colonization by a wild type strain exhibiting homologous pili.

Synthetic pap A pilin vaccine represent another strategy for immunoprophypaxis. The primary structure and DNA sequence for pap A (structural unit of pili) protein of *E. coli* HU849 strain was determined by automated Edman degradation of overlapping fragments and DNA sequence of structural genes (Baga *et al.* 1984 and O’Hanely *et al.* 1984).

Schmidt *et al.* (1988) studied the various synthetic peptides corresponding to pap A pilin protein. Immunization of BALB/c mouse with the synthetic peptide vaccine corresponding to amino acid residue 5-12 and 65-75 conjugated to carrier protein conferred protection from renal colonization with homologous strain. Antibodies corresponding to these two peptides bound to intact pili indicating that these two regions encode linear antigenic determinant of pap A pilin protein. The protection was also correlated with specific IgG antibodies in the serum.

### 2.5.2 Non fimbrial Vaccines

Kaijser and Olling (1973) studied the antibody response and its protective effect against hematogenous pyelonephritis in rabbits. They showed that antibody to O6 and K13 antigen which were produced by hyperimmunization of different rabbits with various strains of uropathogenic *E. coli* protected against the hematogenous pyelonephritis.

Brooks *et al.* (1974) achieved a good protection against retrograde pyelonephritis due to homologous strains by formalinized or heat killed whole cell vaccines of *E. coli* O111 and *E. coli* O6 Williams. Since there
was no bacteriemia and no urinary antibody, the vaccination appeared to protect by immune reaction operating in the kidney itself. However, vaccine failed to protect against a highly virulent form of E. coli O6 (Riffle).

In another study, Kaijser et al. (1978) reported that intraperitoneal or intravesicular immunization with formalin killed E. coli protected the ascending pyelonephritis in rats by homologous bacteria. In addition, the passive transfer of urine containing O6 and K13 antibodies also protected against the ascending pyelonephritis. Further by absorption experiments, it was shown that K13 antibody was especially important. Balzer et al. (1982) reported that peroral immunization with a live strain of E. coli O6:K13:H1 had the potential of protecting significantly against the pyelonephritis. Sera collected one week after the infection from the immunized group were increased in IgG anti O6 and IgM anti K13 in comparison with non-immunized groups. Protection against pyelonephritis was correlated with anti O6 antibody.

Kaijser et al. (1983a) found that isolated K13 antigen conjugated to bovine serum albumin in contrast to non conjugated K13 was highly immunogenic and induced 55% protection against acute pyelonephritis. The protective effect against experimental pyelonephritis was noted after two injections of conjugate given subcutaneously. Immunization with corresponding amount of isolated K13 antigen or BSA alone induced no detectable K13 antibody or protection against experimental pyelonephritis. In addition, no protection was seen after oral administration of conjugate in rats.

Kruze et al. (1989) carried out the study with Solco Urovac vaccine, which was polyvalent intramuscularly applied vaccine consisting
of bacteria most frequently causing UTI in humans. They found that immunization of mice with Solco Urovac (Solco Basle Ltd., Switzerland) vaccine induced an approximate 10 fold increase of total amount of IgG and 2 fold increase of IgA immunoglobulins in urine which might protect against urinary tract infection.

O'Hanley et al. (1991) tested the efficacy of hemolysin and pili vaccine. They found that hemolysin vaccine did not abrogate subsequent bacterial renal colonization on challenge but it did protect mice which survived challenge from subsequent renal injury. The combination of Gal-Gal pili and hemolysin prevented both renal colonization and renal injury in mouse pyelonephritis model.

Kruze et al. (1992) studied the protective effect of immunization with a polyvalent vaccine (Solco Urovac) in mouse and rat model. The intramuscular immunization increased the resistance of mice to challenge infection with all the homologous strains of bacteria. Protection against heterologous E. coli was also achieved and persisted for about 20 weeks. One third kidneys from vaccinated rats showed abscess as often as in controls.

Roberts et al. (1993) tested the efficacy of O8 oligosaccharide-protein conjugate vaccine against experimental pyelonephritis in monkey model. This vaccination with this conjugate did not significantly alter the duration of bacteriuria or interfer with the infection. But vaccinated animals showed significantly less intratubular infiltration of the neutrophils and reduced degree of renal scarring.