Review of Literature
Catheter associated urinary tract infections

In hospital acquired urinary tract infections (UTIs), catheterization of the urinary tract is the most important factor which predisposes the host to these infections and is associated with significant morbidity, high cost and nearly three fold increase in mortality (Nickel et al, 1985). Catheter acquired UTI accounts for as much as 35% of all nosocomial infections (Warren et al., 1987).

Garibaldi et al., (1974) reported that despite the use of aseptic insertion techniques and closed drainage system, bacteriuria develop in 10-27% of catheterized patients within 5 days. Incidence of bacteriuria rises from 0.5-1% for single ‘in and out’ catheterization to 10-30% for catheters in place for upto 4 days and upto 95% for catheters in place for 30 days and more.

Nickel et al., (1985) developed a closed sterile drainage system in catheterized rabbit animal model. Pseudomonas aeruginosa strain isolated from a patient with catheter associated UTI was used for causing infection. Evaluation of intraluminal versus extraluminal route of catheter acquired UTI was done. Their study indicated that in short term catheterization, contamination of the drainage tube resulted in bacteriuria. Extraluminal route assumed more importance in long-term catheterization for the development of bacteriuria if a sterile closed drainage system was maintained.

Parsons, (1986) defined the mechanisms through which catheters contribute to the pathogenesis of UTIs. The most obvious is the disruption of the normal valvular function of urethra. Catheter serves as a direct conduit for pathogens. Microorganisms may be carried from the external meatus to the bladder when the catheter is introduced. Microorganisms may spread along the meatal area or they may pass in a retrograde fashion through the lumen. Catheters may also traumatize urethral and bladder mucosa, disrupting the normal
mucopolysaccharide coating of the epithelium, damaging its cellular structure and rendering it more susceptible to the attachment of bacteria and allowing entry of bacteria through surface erosions.

Dickinson and Bisno, (1989) outlined clinical and microbiological aspects of extravascular infections associated with implanted medical devices. The pathogens usually encountered in catheter associated UTIs were found to be gram-negative bacilli. These infecting organisms were either from the bowel flora or nosocomially acquired highly antibiotic resistant bacteria. It was observed that indwelling catheter enhances colonization of the urethra with infecting bacteria which is then pushed or dragged into the bladder from where they ascend and cause infection of the kidneys leading to acute or chronic pyelonephritis. The lumen and external surface of catheter act as conduits for bacterial entry into the bladder. It was concluded that catheter offers a niche on its luminal and external surfaces for bacteria to develop a microenvironment within its lumen.

Richter et al, (1991) assessed the relation between preoperative infected urine and postprostatectomy wound infection in patients with and without indwelling bladder catheters. In patients with indwelling catheters prior to operation, wound infection was 22.4% when urine was infected and 8.3% when it was not. In patients without catheters, infected urine was associated with 40% of wound infections, as compared with 8.9% of wound infections in patients with sterile urine. Wound infection has been demonstrated to be a postprostatectomy complication directly related to the presence of urinary infection at surgery; therefore, elective prostatectomy should be deferred until urine becomes sterile.

catheter was 11.0%. It was observed that duration of catheterization is the most important risk factor which predisposes patients to UTIs. It was recommended to limit to the minimum the time of catheterization in hospitalized patients.

Richards et al., (1999) described the epidemiology of nosocomial infections in medical intensive care units (ICUs) in the United States. Nosocomial infections were analyzed by infection site and pathogen distribution. Urinary tract infections were most frequent (31%), followed by pneumonia (27%) and primary bloodstream infections (19%). It was concluded that presence of foreign devices like urinary catheters increase the risk of developing nosocomial infections in hospital admitted patients.

Bouza et al., (2001) assessed the incidence of nosocomially acquired urinary tract infections (NAUTI) in Europe. Catheter-associated UTIs (CAUTIs) were present in 187 patients (62.8%). The five most commonly isolated micro-organisms were E. coli, Enterococcus sp., Candida sp., Klebsiella sp. and P. aeruginosa. A closed drainage system was used in only 78.5% of catheterised patients. Opening of the closed drainage system was the most frequent major error in catheter management (16.8%). It was suggested that improvement in the area of bladder catheterisation, catheter care and medical management may help in preventing NAUTI.

Urli et al., (2002) reported infections acquired in intensive care units. The most common infections were pneumonia, urinary tract infections, central venous catheter infections CVC-related sepsis and bacteraemic sepsis. The most frequent pathogens were Staphylococcus aureus, P. aeruginosa, other Gram-negative aerobes and Candida spp. Antimicrobial resistance was substantial, with 68% methicillin-
aureus, P. aeruginosa, other Gram-negative aerobes and Candida spp. Antimicrobial resistance was substantial, with 68% methicillin-resistance in S. aureus and 76% of P. aeruginosa displaying antibiotic resistance.

Vapnek et al., (2003) compared the incidence of hematuria, pyuria and clinical urinary tract infection in patients using a hydrophilic coated catheter. Use of the hydrophilic coated catheter by patients on intermittent self-catheterization is associated with less hematuria and a significant decrease in the incidence of urinary tract infections.

Verhaz et al., (2003) identified the microorganisms associated with CAUTIs and studied their antibiotic susceptibility pattern. The results showed that in 89% of cases infections were caused by gram-negative bacteria, in 7% by gram-positive bacteria and in 4% by Candida. The most common bacteria was E. coli (33.6%) followed by P. aeruginosa (14.1%), Proteus mirabilis (13.3%), and Enterobacter (10.5%). Majority of bacteria showed extremely high resistance (72-100%) to ampicillin, gentamycin and cotrimoxazole, and in some cases a significant resistance to ciproflaxacine, nalidixic acid, ceftriaxone and ceftazidime was also observed. It was concluded that poor hygienic and epidemiological conditions as well as irrational use of antibiotics contribute to uncontrolled development of urinary tract infections in catheterized patients.

Taneja et al., (2004) carried out study to identify organisms isolated from urine of patients suffering from UTIs and to test their antibiotic susceptibility. P. aeruginosa was the commonest (76) followed by B. pickettii (10), P. putida (6), P. fluorescence (2), P. stutzeri (20) P. vesicularis (2), S. putrefaciens (2) and Stenotrophomonas maltophilia (2). Seventy six per cent of P. aeruginosa produced beta-lactamases as compared to 45% of other pseudomonads. Maximum
uropathy followed by post operative period and surgery on urinary tract were the commonest risk factors leading to development of UTIs.

Al-Asmary et al., (2004) assessed the rates of nosocomial infections and UTIs in hospital admitted patients as well as risk factors associated with these infections. It was observed that UTIs were the most common nosocomial infections accounting for about 31.7% of overall nosocomial infections. Duration of hospital stay, unit of admission, history of diabetes mellitus or debilitating diseases and duration and number of urinary catheters were independently associated with increased risk of nosocomial UTIs (NUITs). It was concluded that since UTIs comprise about one third of nosocomial infections, therefore, there is a need to carefully control factors leading to development of these infections.

Zotti et al., (2004) studied the prevalence of hospital acquired infections (HAI). UTIs were found to be most common HAI (52.7%). A significant correlation was found with major risk factors related to medical procedures (urinary catheter, mechanical ventilation, surgical drainage, intravascular catheters). Indwelling urinary catheter and mechanical ventilation were the risk factors more significantly associated with HAI. Patients with HAI were found to be older and had a greater mean length of stay in hospital.

Verhaz et al., (2005) conducted a retrospective study on causative agents of catheter associated UTI (CAUTI). The seven most commonly isolated microorganisms were E. coil (31.0%), Pseudomonas aeruginosa (13.8%), Proteus mirabilis (12.9%), Klebsiella (12.3%), Enterococcus spp. (5.2%), Pseudomonas spp. (4.3%) and Serratia spp. (4.0%). The majority of pathogens were highly resistant to ampicillin (64-100%), gentamycin (63-100%), and trimethoprim-sulfamethoxazole (68-100%), while some bacterias, like Pseudomonas
sulfamethoxazole (68-100%), while some bacteria, like *Pseudomonas aeruginosa* and Serratia spp. showed high rates of ciprofloxacin resistance, 42.8% and 72.7% respectively.

Bagshaw and Laupland, (2006) reviewed the occurrence, microbiology, risk factors for acquisition, and outcomes associated with intensive care unit-acquired urinary tract infections. Placement of indwelling catheter into urinary tract lead to development of intensive care unit-acquired urinary tract infections in almost all the patients. Other factors associated with the development of these infections included increased duration of urinary catheterization, female sex, intensive care unit length of stay, and preceding systemic antimicrobial therapy. *E. coli* was the most common pathogen associated with these infections followed by *P. aeruginosa*, *Enterococci* and *Candida albicans*. However no mortality was observed in patients suffering from intensive care unit-acquired UTIs. It was concluded that UTI is a common complication of critical illness that is associated with increased patient morbidity but not mortality. It was also stressed that further studies are warranted to define the epidemiology and management of these infections.

To control the incidence of catheter associated UTIs, different approaches have been employed by workers. Pugach *et al.*, (1999) developed an antibiotic, ciprofloxacin liposome containing hydrogel for external coating of silicone Foley catheters and evaluated its efficacy in a rabbit model. 30% decrease in the bacteriuria rate for catheters coated with hydrogel containing ciprofloxacin were noted compared to untreated catheters. Ahern *et al.*, (2000) studied the effect of hydrogel/silver coatings on adhesion of bacteria to catheter surface *in vitro* using radiolabel-cell procedure and scanning electron microscopy. Significant decrease in adhesion of both Gram-positive- and Gram-negative bacteria commonly associated with nosocomial urinary tract infections to the hydrogel/silver catheters was observed.
*P. aeruginosa* showing superior adhesion capacity to silicone catheter was also significantly decreased to hydrogel/silver catheter. Karchmer *et al.*, (2000) assessed the efficacy of a silver-alloy, hydrogel coated latex urinary catheter for the prevention of NUTIs. Cho *et al.*, (2001) described an indwelling urethral catheter coated with gentamicin sulphate on the inner and outer surface of the catheter, and evaluated the efficacy and safety of this catheter in preventing CAUTIs in rabbits. The gentamicin-releasing catheter reduced the incidence of bacteriuria after both 3 and 5 days of catheterization as compared to controls. Scanning electron microscopy showed deterioration of the bacterial biofilm on the surface of the gentamicin-releasing catheters. Lai and Fontecchio, (2002) assessed the efficacy of silver-hydrogel urinary catheters in preventing CAUTIs. Rupp *et al.*, (2004) assessed the efficacy of silver coated catheters in preventing catheter associated UTIs (CAUTIs) and to test for the emergence of silver-resistance in urinary microbial isolates. Significant decrease in rate of CAUTIs was observed following introduction of silver coated catheters as compared to when non-coated catheters were used. No silver-resistant microbes were recovered in the susceptibility tests. It was concluded that use of silver coated catheters may help to control CAUTIs. The efficacy of nitrofurazone-coated urinary catheter in inhibitory activity of catheter-associated urinary tract infection (CAUTI) was evaluated by Lee *et al.*, (2004). It was observed that age and period of insertion significantly affect the incidence rate of CAUTI. Cindolo *et al.*, (2004) compared hydrophilic catheter to the standard polyvinyl chloride catheter with regard to bacteriological safety and overall comfort in patients undergoing intravesical immuno- or chemotherapy for bladder cancer. Significant decrease in rate of CAUTI was observed when hydrophilic catheter was used as compared to when standard catheter was used. It was concluded that use of these catheters would provide a
significant clinical advantage while substantially reducing health care costs.

Trautner et al., (2005) investigated whether a colicin-expressing strain of *E. coli* could prevent urinary catheter colonization by a colicin-susceptible, uropathogenic strain of *E. coli* *in vitro*. These workers inoculated segments of urinary catheter with colicin-producing *E. coli* K-12 and then exposed to either colicin-susceptible *E. coli* (a uropathogenic clinical isolate) or colicin-resistant *E. coli* (derived from the susceptible clinical isolate). Catheters were then incubated overnight, rinsed and sonicated. The presence of colicin-producing *E. coli* K-12 on the catheter surface completely prevented catheter colonization by colicin-susceptible *E. coli* but not by resistant *E. coli*. The colicin-susceptible strain but not the colicin-resistant strain disappeared from broth cultures in the presence of colicin-producing *E. coli* K-12. It was concluded that colicin has the potential to prevent catheter colonization by colicin sensitive strains indicating thereby that bacteriocin production by a non-pathogenic organism may have clinical applicability as a means to prevent catheter-associated UTI.

**Alginate and biofilms**

Production of alginate by *P. aeruginosa* has been considered as the most important virulence trait, which enable this pathogen to form biofilms leading to recurrent and chronic infections by evading host defense mechanisms.

Boyd and Chakrabarty, (1994) investigated the role of *P. aeruginosa* alginate lyase in the process of cell sloughing. Increased expression of the alginate lyase in mucoid strain of *P. aeruginosa* led to alginate degradation and increased cell detachment. Similar effects were seen both when the alginate lyase was induced at the initial stage of cell inoculation and when it was induced at a later stage of
growth. Results indicated that high-molecular-weight alginate polymers are required to efficiently retain the bacteria within the biofilms. When expressed from a regulated promoter, the alginate lyase was observed to induce enhanced sloughing of cells because of degradation of the alginate. These workers concluded that alginate lyase play a role in the development of bacterial biofilms.

Effect of muc A alterations on conversion of *P. aeruginosa* to mucoidy phenotype was investigated by Boucher *et al.*, (1997). In addition, these workers also studied the clearance of *P. aeruginosa* strains isolated from cystic fibrosis patients in aerosol infection model established in mice. These workers observed that cystic fibrosis isolates have alterations in muc A leading to overproduction of alginate. Mucoid *P. aeruginosa* strains were able to persist in the experimental animals for longer durations as compared to non-mucoid strains. These workers concluded that alginate production is an important virulence trait of *P. aeruginosa* which enables this pathogen to evade host innate defense mechanisms.

Pasquier *et al.*, (1997) evaluated the effect of chemical composition of alginate and other neutral polysaccharides on phagocytosis and generation of reactive oxygen intermediates by *P. aeruginosa*. These workers pretreated macrophages with characterized EPS and assessed *P. aeruginosa* phagocytosis and reactive oxygen intermediate (ROI) production using chemiluminescence. It was observed that alginate and neutral polysaccharides impaired phagocytosis of *P. aeruginosa*. Macrophages primed with alginate were able to produce more ROI as compared to non-primed macrophages. On the contrary, neutral polysaccharides lead to significant decrease in ROI production by scavenging effect assessed by xanthine-xanthine oxidase system. It was concluded that chemical composition of alginate and neutral polysaccharides is an important factor, which
ultimately influence the behavior of strains towards phagocytosis and oxidative burst.

Virulence potential of mucoid and non-mucoid strains of *P. aeruginosa* isolated from cystic fibrosis patients was investigated by Yu *et al.*, (1998) in mouse model of respiratory tract infection. It was observed that non-mucoid strains were cleared from the lung tissues much more efficiently as compared to mucoid strains. However, the microscopic pathology findings and proinflammatory cytokine levels were similar in mice infected with nonmucoid and mucoid *P. aeruginosa* strains throughout the infection.

Mathee *et al.*, (1999) investigated the effect of release of free oxygen radicals such as hydrogen peroxide by PMNs on *P. aeruginosa*. It was observed that exposure of biofilms of *P. aeruginosa* to hydrogen peroxide led to formation of mucoid variants having mutations in mucA, which encodes an anti-sigma factor leading to the deregulation of an alternative sigma factor (sigma22, AlgT or AlgU) required for the expression of alginate biosynthetic operon. Mucoid variants produced excessive levels of alginate, produced lower amounts of inducible-beta-lactamase, secreted little or no LasA protease, showed only 44% elastase activity and showed an unaltered LPS profile but exhibited no detectable differences in growth rate as compared to parent strain. These workers concluded that PMNs and their oxygen radicals cause phenotypic and genotypic changes in biofilms of *P. aeruginosa* leading to enhanced alginate production and protecting bacteria from host’s innate immune defenses.

Hentzer *et al.*, (2001) studied the effect of alginate overproduction on biofilm structure and architecture of *P. aeruginosa*. Biofilms formed by an alginate-overproducing strain exhibited a highly structured architecture and were significantly more resistant to the antibiotic tobramycin than biofilm formed by an isogenic nonmucoid
strain. These workers concluded that conversion to mucoid phenotype in \textit{P. aeruginosa} is associated with alteration in biofilm architecture leading to increased antibiotic resistance.

Role of algR implicated in production of alginate and twitching motility in pathogenesis of \textit{P. aeruginosa} was investigated by Lizewski \textit{et al.}, (2002). Standard parent strain and its isogenic mutant deficient in production of algR were compared in terms of sensitivity to reactive oxygen intermediates, killing by phagocytes, systemic virulence, and the ability to cause infection in murine lung model. These workers observed that mutant strain was more sensitive to hypochlorite but was resistant to hydrogen peroxide. There was no significant difference in phagocytosis of parent strain and its isogenic mutant assessed in macrophage cell lines. However, mutant strain was significantly less lethal in comparison to parent strain in an acute septicemia infection mouse model. In addition mutant strain was cleared from lungs of experimental animals much more efficiently as compared to parent strain. These workers concluded that algR plays an important role in virulence of \textit{P. aeruginosa}.

Song \textit{et al.}, (2003) studied role of alginate in mouse model of acute lung infection using standard parent strain PAO1 and its isogenic mutant strains, one overproducing alginate and other deficient in production of alginate. It was observed that at 24 hour postinfection, lung bacterial load as well as pathology was significantly higher in mice infected with alginate deficient as compared to other strains. However significantly lower lung and spleen bacterial loads were observed in mice infected with \textit{P. aeruginosa} strain PAO1 and alginate deficient strain as compared to alginate overproducing strain between 24 and 48 h post-infection time period.

Yagci \textit{et al.}, (2003) carried out study to type \textit{P. aeruginosa} strains isolated from cystic fibrosis patients and to assess production
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of virulence factors by these isolates. These workers employed arbitrarily primed PCR to genotypically compare the strains. Evaluation of virulence factor results revealed that 95.8% of the strains were alginate, 71.7% elastase and 52.1% alkaline protease producers. AP-PCR analysis revealed 35 genotypes and indicated almost a complete discrepancy among the strains. Among aminoglycosides, amikacin was the most effective one and a high level resistance to beta lactams was observed. These workers concluded that alginate is the most important virulence factor in the chronic colonization of cystic fibrosis patients with *P. aeruginosa*.

Ciragil and Soyletir, (2004) evaluated the relationship between the elaboration of virulence factors by *P. aeruginosa* and infected body sites. These workers investigated the production of alginate, elastase and alkaline protease production in *P. aeruginosa* strains isolated from lower respiratory tract samples of cystic fibrosis as well as strains isolated from lower respiratory tract, urine and blood samples of non-cystic fibrosis patients. It was observed that all isolates produced same levels of alginate except uroisolates which produced lower amounts of alginate. There was no statistically significant difference in elastase levels among different groups of isolates. Alkaline protease levels, although not statistically significant, was greater in urine isolates than blood and respiratory isolates. However, it was significantly lower in cystic fibrosis isolates compared to other isolates. It was concluded that alginate, elastase and alkaline protease levels do not differ significantly when different body sites are considered. However, it was observed that alkaline protease was the least produced virulence factor in terms of different body sites. Alginate was found to be the most commonly produced virulence factor in respiratory tract isolates, regardless of presence of cystic fibrosis.
Genetic diversification in biofilms of *P. aeruginosa* was evaluated by Boles *et al.*, (2004). It was observed that biofilm formation by *P. aeruginosa* was associated with extensive genetic diversification affecting multiple traits including the behavior of the bacteria in biofilms. The induced genetic changes were found to produce by a recA-dependent mechanism. Some biofilm-derived variants exhibited an increased ability to disseminate, whereas others manifested accelerated biofilm formation. The presence of these functionally diverse bacteria increased the ability of biofilms to tolerate environmental stresses. These findings suggested that self-generated diversity help biofilms to persist in host milieu despite the presence of adverse environmental conditions.

Lee *et al.*, (2005) assessed non-mucoid *P. aeruginosa* isolates collected during different periods of chronic infection from CF patients with respect to phenotypic changes and *in vitro* biofilm formation. These workers observed that physiological alterations were associated with loss of motility and with decreased production of virulence factors like pyocyanin and proteases. In addition decreased production of quorum-sensing molecules was also observed as 45% of the isolates were unable to produce 3-O-C (12)-homoserine lactone quorum-sensing molecules. Variability in biofilm formation was observed in *P. aeruginosa* isolates recovered from a particular patient at different time periods of chronic infection. Decrease in adherence of biofilm cells of *P. aeruginosa* was observed with progression of the chronic lung infection. It was concluded that adherence may not play an essential role in survival of *P. aeruginosa* in chronic lung infections.

In order to determine whether *P. aeruginosa* form biofilms on mucosal surfaces, Dohar *et al.*, (2005) established chronic suppurative otitis media model in monkeys. One ear of monkeys was perforated and inoculated with biofilm forming strain of *P. aeruginosa* whereas
other ear served as control. At the end of the study period, both ears were irrigated to remove planktonic bacteria, and the middle ear mucosa was removed and examined ultrastructurally using scanning electron microscopy (SEM). It was observed that \textit{P. aeruginosa} had the ability to form biofilms on mucosal membranes.

Ferguson and Stolz, (2005) demonstrated biofilm formation in patients of chronic rhinosinusitis (CRS) using transmission electron microscopy (TEM). These workers observed bacteria enmeshed in glycocalyx in two patients of CRS. These biofilm bacteria were found to be \textit{P. aeruginosa}. Glycocalyx present in these isolates was unique and was not present in \textit{P. aeruginosa} grown in culture medium. Antibiotic treatment, steroids and nasal lavages were found to have no effect in resolving patient’s symptoms infected with biofilm forming \textit{P. aeruginosa}. It was concluded that \textit{P. aeruginosa} formed biofilms on mucosal surfaces \textit{in vivo} leading to antibiotic resistance in this pathogen.

Garcia-Medina \textit{et al.}, (2005) studied the interaction of \textit{P. aeruginosa} with airway epithelial cells \textit{in vitro} using electron microscopy and confocal microscopy. Pod like clusters of intracellular bacteria (\textit{P. aeruginosa}) were demonstrable in airway epithelial cells. Addition of antibiotics capable of penetrating epithelial cells and having the ability to eradicate planktonic cells of \textit{P. aeruginosa} had no effect on growth and survival of these intracellular bacteria. These findings suggested that \textit{P. aeruginosa} has the ability to persist in the milieu of airway epithelial cells by converting to biofilm mode leading to chronic respiratory tract infections.

Kirisits \textit{et al.}, (2005) characterized colony morphology variants from mature \textit{P. aeruginosa} biofilms. These variants were found to autoaggregate in liquid culture, exhibited increased adherence to solid surfaces as well as showed increased hydrophobicity and reduced
motility compared to the wild-type parent strain. In addition, these variants formed biofilms with significant three-dimensional structure and more biomass than the wild-type parent strain. Transcriptional profiles of these variants revealed increased expression of the psl and pel loci, implicated in the adherence of \textit{P. aeruginosa} to solid surfaces. Mutation in the psl locus was observed to have no effect on the colony morphology but hyperadherence and autoaggregation were lost. Further, colony morphology variants were also found in isolates from cystic fibrosis patients, displaying many of the same characteristics as the laboratory variants, suggesting a link between laboratory and cystic fibrosis biofilms.

Sriramulu et al., (2005) developed artificial sputum medium to study effect of sputum components on biofilm formation by \textit{P. aeruginosa}. It was observed that amino acids, lecithin, DNA, salt and low iron were required for tight microcolony formation in biofilms. Amino acids were also found to be involved in diversification of colony morphology, alterations in LPS structure and hyperexpression of OprF in biofilms of \textit{P. aeruginosa}. It was concluded that amino acids play an important role in pathogenesis of cystic fibrosis by helping in biofilm formation leading to persistence of \textit{P. aeruginosa} in lung tissues.

Role of alginate in resistance of biofilm cells of \textit{P. aeruginosa} to host’s immune system was investigated by Leid et al., (2005). These workers tested alginate positive strain as well as the respective isogenic mutants of \textit{P. aeruginosa} which lacked the ability to produce alginate, for their susceptibility to human leukocytes in the presence and absence of IFN-gamma. It was observed that alginate negative strains were phagocytosed by human leukocytes more efficiently as compared to alginate positive strain particularly in presence of interferon –gamma. It was concluded that alginate play an important role in protecting biofilm cells of \textit{P. aeruginosa} from the human immune system.
Role of innate immune mechanisms in biofilm formation by *P. aeruginosa* was investigated by Walker *et al.*, (2005). It was observed that presence of neutrophils enhanced initial *P. aeruginosa* biofilm development over a period of 72 h through the formation of polymers comprised of actin and DNA. F-actin was found to be a site of attachment for *P. aeruginosa*. Since these components have been reported to be present in sputum of cystic fibrosis patients, indicating that cellular components from necrotic neutrophils can serve as a biological matrix to facilitate biofilm formation by *P. aeruginosa*.

Hoffman *et al.*, (2005) reported mouse model of chronic *P. aeruginosa* lung infection without artificial embedding of bacteria mimicking cystic fibrosis. These workers employed stable mucoid CF sputum isolate of *P. aeruginosa* producing higher amounts of alginate due to a deletion in mucA and possessing functional N-acylhomoserine lactone (AHL)-based quorum-sensing systems. It was observed that this strain of *P. aeruginosa* was able to induce infection in mice as indicated by high number of *P. aeruginosa* in the lung tissues. On the contrary, AHL-producing nonmucoid revertant from the mucoid isolate and nonmucoid isolate deficient in AHL were almost cleared from the lungs of the mice when checked on 7th postinfection day. It was concluded that this model can serve useful purpose for evaluating the interaction between mucoid *P. aeruginosa*, the host and antibacterial therapy.

Sarkisova *et al.*, (2005) studied the effect of calcium on alginate and other virulence factor production by biofilm cells of *P. aeruginosa*. It was observed that addition of calcium resulted in biofilms that were thicker than biofilms formed in absence of calcium. Scanning confocal laser microscopy showed increased spacing between cells for the thick biofilms, and Fourier transform infrared spectroscopy revealed that the material between cells is primarily alginate. An algD transcriptional reporter demonstrated that addition of calcium caused
an eightfold increase in alg gene expression in biofilms of *P. aeruginosa*. Calcium addition also resulted in increased amounts of three extracellular proteases (AprA, LasB, and PrpL). Spectrochemical analyses showed that the calcium addition caused a three- to fivefold increase in pyocyanin production. Calcium addition affected the structure and extracellular matrix composition of mucoid *P. aeruginosa* biofilms, through increased expression and stability of bacterial extracellular products. These workers concluded that calcium-induced extracellular matrix of mucoid *P. aeruginosa* consist primarily of alginate and also harbor extracellular proteases and perhaps pyocyanin that may further disrupt cellular calcium levels.

Jain and Ohman, (2005) examined the phenotype of a DeltaalgL mutation in the highly mucoid CF isolate of *P. aeruginosa*. Upon induction of alginate production with isopropyl-beta-D-thiogalactopyranoside, the DeltaalgL mutant cells were lysed within a few hours. Electron micrographs of the DeltaalgL mutant showed that alginate polymers accumulated in the periplasm, which ultimately burst the bacterial cell wall. These workers concluded that AlgL play a role in degrading free alginate polymers in the periplasm and also help in transporting these polymers outside the periplasm leading to formation of alginate in *P. aeruginosa*.

**Siderophores**

Iron is the most important element which decides the ultimate outcome of an infection. Iron-limiting conditions have been reported to prevalent in the milieu of urinary tract, (Shand *et al.*, 1985), therefore the ability of microorganisms to sequester iron from the host becomes a significant factor in determining their growth, metabolic process and pathogenicity (Bullen *et al.*, 2005). *P. aeruginosa* has been reported to produce 2 siderophores, pyochelin and pyoverdin, which help this
pathogen to obtain iron from host’s iron binding proteins like lactoferrin and transferrin.

Cox, (1982) evaluated the effect of pyochelin on the virulence of virulent and avirulent strains of *P. aeruginosa*. When growth and clearance of both virulent and avirulent strains in mice was analysed by these workers, it was revealed that pyochelin increased the growth and lethality of virulent bacteria but only increased the survival of the avirulent bacteria. It was observed that this effect was due to increased bacterial growth in presence of pyochelin. These workers concluded that pyochelin help in increasing virulence of virulent strains of *P. aeruginosa* by stimulating growth as well as by allowing the expression of additional virulence properties.

Effect of siderophores on growth of *P. aeruginosa* in transferrin and human serum was investigated by Ankenbauer et al, (1985) by employing mutant strains of *P. aeruginosa* deficient in production of pyochelin and pyoverdin. No difference in growth of pyoverdin positive but pyochelin negative strain of *P. aeruginosa* was observed as compared to parent strain in presence of transferrin and human serum. On the contrary growth was severely retarded in pyochelin positive but pyoverdin negative strain similar to that observed in mutant strain deficient in production of both the siderophores. These workers concluded that pyoverdin play a significant role in growth of *P. aeruginosa*.

Role of *P. aeruginosa* pyochelin has been proposed in inflammation. It has been reported that siderophore generation in vivo at sites of *P. aeruginosa* infection where O$_2$ and H$_2$O$_2$ derived from phagocytic cells like neutrophils are also present. Coffman et al, (1990) observed that iron bound to *P. aeruginosa* pyochelin could catalyze -OH formation via Haber Weiss reaction. The generation of
-OH could contribute to extensive tissue damage observed in *P. aeruginosa* infections.

Gensberg *et al.*, (1992) carried out study to evaluate role of siderophores in iron acquisition employing parent strain and its isogenic double mutant deficient in production of pyochelin and pyoverdin. It was observed that addition of pyoverdin from the parent strain PAO1 or from a clinical strain induced expression of an 85 kDa iron regulated outer membrane protein (IROMP) and increased the rate of 55Fe-pyoverdin transport. On the other hand, addition of purified pyochelin induced expression of a 75 kDa IROMP accompanied with increased 55Fe-pyochelin uptake without affecting 55Fe-pyoverdin transport. Addition of pyoverdin from the parent strain or a chromatographically distinct pyoverdin caused increased reactivity with an anti-85 kDa monoclonal antibody in Western blotting, indicating that the same receptor is being induced. These workers concluded that *P. aeruginosa* respond to different siderophores differently and induce different cognate receptors.

Visca *et al.*, (1992) assessed production of virulence determinants in *P. aeruginosa* strains isolated from patients suffering from urinary tract infections. It was observed that uropathogenic strains of *P. aeruginosa* produced at least one type of siderophore i.e. pyochelin and/or pyoverdin. However not all the uropathogenic strains produced both siderophores.

Role of pyoverdin in virulence of *P. aeruginosa* was studied by Meyer *et al.*, (1996) *in vitro* and *in vivo*. Parent strain of *P. aeruginosa* and its isogenic pyoverdin-deficient mutants were grown in bicarbonate-containing succinate medium containing apotransferrin. It was observed that mutant strains were unable to grow in this medium. However, growth was restored following supplementation of medium with pyoverdin. Further when parent strain of *P. aeruginosa*
was grown at higher temperatures inhibiting pyoverdin production, it behaved like mutant strains with growth inhibited in presence of apotransferrin and restored following supplementation with purified pyoverdin. Pyoverdin deficient mutant strains of \textit{P. aeruginosa} were found to be avirulent in mouse model of burn wound infection as compared to parent strain. On the other hand, when purified pyoverdin was injected following infection with mutant strains, virulence of these mutant strains was restored. It was concluded that pyoverdin play an important role in virulence of \textit{P. aeruginosa} by helping this pathogen to sequester iron from host's iron binding proteins.

Takase \textit{et al.}, (2000) investigated the role of pyochelin and pyoverdin in virulence of \textit{P. aeruginosa}. These workers constructed single isogenic mutant strains of standard parent strain PAO1 deficient in production of pyochelin or pyoverdin as well as double mutant deficient in production of both pyochelin and pyoverdin. Standard parent strain as well as its isogenic mutant strains were injected into the calf muscles of immunosuppressed mice. It was observed that there was no difference in virulence of parent strain and single mutant strains \textit{in vivo} assessed in terms of calf muscle infection and mortality of experimental animals. On the contrary, double mutant failed to show any lethal virulence although it was able to infect muscles of mice. When these \textit{P. aeruginosa} strains were inoculated intranasally into immunosuppressed mice, it was observed that pyoverdin deficient single mutant and double mutant strain showed decreased virulence indicated by poor growth in lungs and decreased mortality of experimental animals as compared to pyochelin deficient single mutant strain and parent strain of \textit{P. aeruginosa}. In addition when \textit{in vitro} growth of double mutant was checked under free-iron-restricted condition with apotransferrin, significant reduction in growth of this strain was observed. However when medium was
supplemented with hemoglobin no reduction in growth was observable. These workers concluded that siderophores play most important role in pathogenesis of infections caused by *P. aeruginosa* however pyoverdin comparatively plays more important role in bacterial growth and dissemination than pyochelin.

Britigan *et al.*, (2000) reported that human neutrophils, macrophages and myeloid cell lines can acquire iron from two *P. aeruginosa* siderophores. It was suggested that acquisition and sequestration of iron bound to bacterial siderophores by host phagocytes efficiently could provide a secondary mechanism to limit iron availability to microbes.

Effect of siderophore signaling on production of virulence factors was evaluated by Lamont *et al.*, (2002). It was observed that pyoverdin regulate the production of three virulence factors namely exotoxin A, endoprotease and pyoverdin itself. Regulation was found to occur through transmembrane signaling system that include an outer membrane receptor for ferripyoverdin and sigma factor. It was concluded that siderophore signaling may impart survival advantage to *P. aeruginosa* in host tissues specially biofilm cells.

**Protease and Elastase**

Kharazmi *et al.*, (1984) carried out study to examine the effect of alkaline protease and elastase purified from *P. aeruginosa* on human neutrophil function in terms of neutrophil chemotaxis, oxygen consumption, glucose oxidation, superoxide production, and nitroblue tetrazolium reduction. It was observed that both the exoenzymes significantly inhibited chemotaxis in a dose dependent manner. Alkaline protease but not elastase inhibited opsonized zymosan-stimulated neutrophil oxygen consumption. However both the enzymes had no effect on glucose oxidation and nitroblue tetrazolium-reducing activity of stimulated neutrophils. Elastase but not protease
also inhibited superoxide production. It was concluded that *P. aeruginosa* exoproducts interfere with host defense mechanisms and help in protecting this pathogen against phagocytic defense of host leading to persistence and chronicity of infections.

Kapur and Srinivas, (1986) carried out study to evaluate relationship between pyocin typing and production of protease and elastase by *P. aeruginosa*. These workers qualitatively determined levels of protease and elastase in culture supernatants of *P. aeruginosa*. It was observed that 45.4% of the clinical isolates were both protease and elastase producers (P + E +); 40.9% were only protease producers (P + E -) and 13.6% were non producers (P - E -). Majority of protease and elastase producers was found to belong to aeruginocine code 7777. It was concluded that this aeruginocine type is most virulent and responsible for majority of *P. aeruginosa* induced infections probably due to higher production of protease and elastase.

Elsheikh *et al.*, (1987) carried out study to assess the role of elastase in mink model of respiratory tract infection employing elastase producing parent strain and its isogenic mutant strains deficient in production of elastase. Significant decrease in mortality was observed following intratracheal infection with mutant strains as compared to parent strain. Microscopic examination of lung tissue revealed more pronounced tissue damage as well as haemorrhage following infection with parent strain. On the contrary, very mild tissue damage was observed when mutant strains were used to induce infection in mink. In addition these workers also compared virulence potential of *P. aeruginosa* strains isolated from natural infection which differed in elastase production in mink. It was observed that strains producing higher levels of elastase were significantly more virulent as indicated by higher rate of mortality and severe lung damage in comparison to strains elaborating lower levels of elastase. These workers concluded that elastase is an important virulence trait of *P.*
aeruginosa which help this pathogen in tissue colonization and infection.

Horvat and Parmely, (1988) carried out study to investigate immunomodulatory activity of alkaline protease. These workers determined the effect of P. aeruginosa alkaline protease on IFN-gamma production by antigen-stimulated human T-cell clones. It was observed that culture filtrates obtained from P. aeruginosa elaborating protease were able to inhibit IFN-gamma production by T-cells. On the other hand, bacterial filtrates prepared from mutant strains of P. aeruginosa deficient in production of protease were unable to inhibit IFN-gamma production by T-cells. The inhibitory activity was abolished following treatment with antiserum to alkaline protease. Further purified protease and inactive filtrate from protease-deficient mutant strain reconstituted by the addition of alkaline protease was also able to inhibit IFN-gamma production by T-cells. Alkaline protease treated recombinant IFN-gamma showed altered migration on Western blots (immunoblots) of polyacrylamide gels. Treatment of IFN-gamma with alkaline protease also diminished its ability to enhance expression of Fc receptors on cells of the U-937 histiocytic cell line. It was concluded that alkaline protease has potential to alter immunomodulatory activities of IFN-gamma which may help in persistence of this pathogen in host tissues.

Trancassini et al., (1989) carried out study to investigate role of protease and elastase on adherence of P. aeruginosa to cell lines (WEHI cell line). These workers screened P. aeruginosa strains for alkaline protease production using casein as substrate while elastase activity was investigated with the elastin-congo red as substrate. It was observed that strains elaborating high amounts of protease and elastase were significantly more adherent in comparison to strains producing low amounts of these exoenzymes. These workers concluded that in addition to tissue damage, protease and elastase
also help in adherence of *P. aeruginosa* to host cells which is the most crucial step in initiation of infection.

To investigate inactivation of cytokines by alkaline protease and elastase of *P. aeruginosa* a study was carried out by Parmely *et al.*, (1990). These workers observed that human recombinant gamma interferon (rIFN-gamma) and human recombinant tumor necrosis factor alpha were inactivated by both proteases but human recombinant interleukin-1 alpha and recombinant interleukin-1 beta were resistant to the effects of both proteases Murine rIFN-gamma was relatively resistant to alkaline protease but was inactivated by elastase. It was concluded that proteolytic cleavage of cytokines by *P. aeruginosa* is dependent on production of both alkaline protease and elastase.

Kharazmi, (1991) reviewed mechanisms involved in evasion of host defense by *P. aeruginosa*. One of the mechanism was attributed to the production of alkaline protease and elastase which inhibited the function of the cells of the immune system (phagocytes, NK cells, T cells), inactivate several cytokines (IL-1, IL-2, IFN-r, TNF), cleave immunoglobulins and inactivate complement components. Interference with host innate immune mechanisms is likely to favour persistence of *P. aeruginosa* in host tissues. It was concluded that exoenzymes play an important in virulence of *P. aeruginosa* by helping this pathogen to evade host defense mechanisms.

To compare different procedures for quantitative determination of elastase production in culture supernatant of *P. aeruginosa* isolated from cystic fibrosis patients, two immunological assays (ELISA and radioimmunoassay) and two enzymatic assays using elastin or tetraalanine as substrate were employed by Saulnier *et al.*, (1992). Standard curve was plotted using purified elastase and results were expressed in mg of elastase per litre of supernatant. It was observed
that values of elastase production for the same strain varied significantly for different procedures. ELISA values were three to five times higher whereas radioimmunoassay (RIA) values were three to five times lower than other assays. Enzymatic assays with elastin gave higher values than those using tetraalanine. These workers observed increase in elastase activity following incubation of elastin with lasA indicating that presence of lasA in culture supernatant interfere with elastase determination. But this increase was not observed when tetraalanine was used as substrate. It was concluded that enzymatic assays using synthetic substrates like tetraalanine are best procedures for elastase determination which give true values of elastolytic activity in culture supernatants of *P. aeruginosa*.

Correlation between exoenzyme production and pulmonary exacerbations of cystic fibrosis was determined by Grimwood et al., (1993). These workers isolated *P. aeruginosa* sputum isolates from seriously ill, hospitalized patients with cystic fibrosis and from cystic fibrosis clinic patients who were in relatively better health. It was observed that exoenzyme levels (protease and elastase) were maximum during admission which decreased significantly during hospitalization. However, deterioration in cystic fibrosis lung disease lead to increase in *P. aeruginosa* exoenzyme production, especially by nonmucoid strains. Treatment of patients with antibiotics (cephalosporins) lead to significant decrease in exoenzyme production. These workers concluded that exoenzymes play an important role in pathogenesis of cystic fibrosis and antibiotics may improve pulmonary function in patients with cystic fibrosis by decreasing *P. aeruginosa* exoenzyme expression.

Kernacki et al., (1995) carried out study to detect protease and elastase production in vivo in mouse model of corneal infection and to check ability of purified protease to mount nonocular antibody response in presence or absence of adjuvant. After infection
supernatants from homogenized corneas were collected and analysed for proteolytic activity by zymography and immunoreactivity by immunoblotting. It was observed that corneas infected with *P. aeruginosa* demonstrated presence of protease but not elastase production. Peak production of protease was observed during peak bacterial load in corneal tissue and protease was not detectable when corneas were sterile. In addition a nonocular antibody response was observed in mice following immunization with microgram quantities of purified protease in presence of adjuvant. It was concluded that alkaline protease play significant role in *P. aeruginosa* induced corneal infections.

Quantitative analysis of elastase, phospholipase C, toxin A, and exoenzyme S was done in *P. aeruginosa* strains isolated from wound infections, respiratory tract infections and urinary tract infections Hamood *et al.*, (1996). It was observed that most of the isolates produced all the four virulence traits. However depending on infection site, the isolates produced varied levels of these virulence determinants. High levels of elastase and phospholipase C were produced by most isolates obtained from trachea, urinary tract, and wounds. Significantly higher levels of toxin A was produced by wound isolates, while significantly higher level of exoenzyme S was produced by wound and urinary tract isolates. It was observed that persistent infection isolates from different sites produce significantly higher levels of exoenzyme S. These workers concluded that elastase, phospholipase C, toxin A, and exoenzyme S are important virulence traits which help *P. aeruginosa* to cause a variety of persistent infections.

*Azghani et al.*, (2002) carried out study to evaluate effect of *P. aeruginosa* elastase on induction of mitogen-activated protein (MAP) kinase activity and IL-8 production in alveolar epithelial cells using western blot analysis and ELISA kit. It was observed that *P.
aeruginosa elastase induced phosphorylation of extracellular signal-regulated (ERK1/2) proteins of the MAPK pathway in epithelial cells. Significant enhancement in production of IL-8 was also observed following stimulation of alveolar epithelial cells with elastase which was abolished of the ERK activation inhibitor. These workers concluded that P. aeruginosa elastase activates MAP kinase pathway leading to enhanced production of IL-8 which can enhance pulmonary inflammation.

Schmidtchen et al., (2003) carried out study to assess role of elastase in chronic ulcers. These workers observed that elastase producing P. aeruginosa strains induced degradation of complement components, various antiproteinases, kininogens, fibroblasts proteins and proteoglycans in vitro. In addition these strains significantly degraded human wound fluid as well as human skin proteins ex vivo. Further elastase containing P. aeruginosa medium and purified elastase inhibited fibroblast cell growth. It was concluded that elastase play a significant role in pathophysiology of chronic ulcers.

Caballero et al., (2004) investigated distribution of Protease IV gene, its production and types in different strains of P. aeruginosa. These workers determined role of protease IV in corneal infections by employing isogenic mutant strains. Protease IV gene was located using PCR, and pulse field gel electrophoresis. Quantitative determination of protease production was assessed using western blot analysis, colorimetric assay and zymography. These workers observed that protease IV gene was present in all strains of P. aeruginosa but not in non-aeruginosa strains. All the strains of P. aeruginosa produced protease however levels varied from strain to strain. Although mutant strain of P. aeruginosa deficient in production of protease IV was able to produce protease but it was less virulent as compared to parent strain assessed in terms of corneal pathology and corneal bacterial load. It was concluded that protease IV gene is present in all strains of
*P. aeruginosa* and played a significant role in pathogenesis of corneal infections.

Ciragil and Soyletir, (2004) carried out study to investigate relationship between production of virulence traits and site of infection. These workers isolated *P. aeruginosa* strains from cystic fibrosis patients as well as from lungs, urine and blood of non cystic fibrosis patients. It was observed that urinary isolates produced least amount of alginate and maximum amount of alkaline protease as compared to other isolates. Significantly lower levels of alkaline protease were observed in cystic fibrosis isolates as compared to other isolates. No significant difference in elastase levels was observed among different strains of *P. aeruginosa*. However these workers observed no correlation between elaboration of virulence factors and site of infection. It was concluded that virulence factors play an important role in pathogenesis of infections caused by *P. aeruginosa*.

Marquart *et al.*, (2005) investigated effect of calcium and magnesium on production of Protease IV by *P. aeruginosa*. These workers determined protease IV activity in culture supernatants using caseinase assay and colorimetric test. It was observed that addition of calcium or magnesium to culture medium significantly enhanced protease IV production. To assess the outcome of this *in vitro* observation *in vivo*, these workers established corneal model of *P. aeruginosa* in rabbits. It was observed that infection of rabbit corneas did not change magnesium concentration but significantly increased calcium concentration in corneas. It was concluded that increase in calcium concentration in cornea during infection with *P. aeruginosa* could favour persistence of this pathogen mediated through increase in protease IV production.
Hemolysins and Phospholipase C

Iron acquisition in vivo can be mediated by production of hemolysins also known as rhamnolipids by the pathogen. Hemolysins are a group of cytotoxic polypeptides which cause lyses of RBCs. Besides erythrocytes, hemolysins are also toxic for a wide range of additional cells including PMNs, monocytes and fibroblasts in vitro. Hemolysins are thought to induce membrane damage by acting as a pore forming cytolysin. The transmembrane pore created by hemolysin allows the diffusion of hydrophilic molecules up to molecular weight of 1000 Daltons. Liu, (1966) demonstrated production of second type of hemolysin, PLC, by P. aeruginosa. These workers also reported that injection of PLC preparation of P. aeruginosa into skin of animals produced a central abscess surrounded by an area of redness and induration within 24 hours.

Histopathological studies carried out in inflamed mouse foot pads indicated that purified PLC caused marked signs of inflammation within 15 minutes postinjection, as indicated by cellular infiltration and post edema (Coutinho et al., 1988). The foot pad studies carried out in mice have suggested that PLC is a potent inflammatory agent and may have an important role in P. aeruginosa infections.

Effect of rhamnolipids purified from culture supernatants of Pseudomonas aeruginosa on human peripheral blood neutrophil and monocyte function was evaluated by Kharami et al., (1989). Preincubation of monocytes with rhamnolipid enhanced the oxidative burst response of these cells to phorbol myristate acetate (PMA) and to opsonized zymosan. This priming effect was observed both in a superoxide assay and in a chemiluminescence assay. However, rhamnolipid did not prime the neutrophil oxidative burst response. It was concluded that the priming of monocytes by rhamnolipid for enhanced generation of oxygen radicals may play a role in the
pathogenesis of tissue damage in the lungs of cystic fibrosis patients with P. aeruginosa infections.

Bergman et al., (1989) analysed the ability of P. aeruginosa strains isolated from burn wound infections to induce inflammatory-mediator release from rat mast cells or human granulocytes. These workers characterized bacterial strains with respect to their cell-associated hemolysin activity as well as their secreted hemolysin and phospholipase C activities. It was observed that P. aeruginosa expressing heat-labile hemolysin and phospholipase C induced histamine release from rat mast cells and leukotriene formation from human granulocytes, while bacterial strains expressing heat-stable hemolysin were potent releasers of histamine but did not lead to leukotriene formation. It was suggested that both hemolysins of P. aeruginosa contribute to its pathogenicity by inducing and modulating inflammatory-mediator release from various cells.

Meyers and Berk, (1990) assessed the inflammatory potential of P. aeruginosa PLC. Marked inflammatory response was observed following injection of PLC into peritoneal cavity of mice. Inflammation was characterized by the accumulation of inflammatory cells and plasma protein as well as the release of arachidonic acid metabolites. Heat-inactivated PLC failed to show any of these effects, indicating that enzyme activity is necessary for PLC-induced inflammation. When human granulocytes were stimulated with PLC in vitro, generation of leukotrienes and prostaglandins was observed. Stimulation of mouse peritoneal cells with PLC lead to generation of thromboxane in addition to generation of leukotrienes and prostaglandins. Both human granulocytes and mouse peritoneal cells stimulated with PLC generated significantly increased levels of arachidonic acid metabolites as compared with cells incubated with heat-inactivated PLC. It was concluded that PLC is a potent inflammatory agent playing an important role in P. aeruginosa infections.
PLC has been reported to exert an important pathological effect in *P. aeruginosa* induced lower respiratory tract infections (Vasil *et al.*, 1991). PLC caused enzymatic degradation of phosphatidylcholine component of lung surfactant leading to atelectasis. Further derivatives of phosphatidylcholine induced production of additional PLC thus reinforcing enzyme effect.

Puzova *et al.*, (1994) analysed 33 uropathogenic strains of *P. aeruginosa* for hemolytic activity in both bacterial broth culture filtrates and isolate lyzates, resistance to bactericidal activity of human serum, plasmid DNA profile and resistance to six antibiotics namely tetracycline, kanamycin, chloramphenicol, septrin, ampicillin, streptomycin, gentamicin. It was observed that 24 of the 33 (73%) bacterial filtrates showed lysis of rabbit erythrocytes, whereas 3 showed hemolysis after guinea-pig erythrocyte treatment. 12 of 33 isolate lysates showed in parallel lysis of both types of erythrocytes used. Serum resistance was found in 17 (52%) isolates, intermediate resistance in 15 (45%) isolates and only one isolate showed serum sensitivity. 94% isolates showed resistance to tetracycline, 79% to kanamycin, 76% to chloramphenicol, 73% to septrin, 64% to ampicillin, 45% to streptomycin and 18% to gentamicin. Plasmid DNA was present in all the strains of *P. aeruginosa* except in one strain.

Effect of *P. aeruginosa* PLC and lipase was analysed on release of inflammatory mediators by human platelets, neutrophilic and basophilic granulocytes, and monocytes by König *et al.*, (1996). It was observed that addition of purified PLC from *P. aeruginosa* but not purified lipase, induced 12-hydroxyeicosatetraenoic acid (HETE) generation from human platelets, the generation of leukotriene B4 (LTB4) and oxygen metabolites, enzyme release from human neutrophils, and histamine release from basophils but decreased the release of interleukin-8 (IL-8) from human monocytes in a dose-dependent manner. On the other hand, the addition of purified lipase
enhanced PLC-induced 12-HETE and LTB4 generation, had no effect on enzyme, histamine, or IL-8 release, but decreased the PLC-induced chemiluminescent response. When well-defined calcium ionophore A23187 and phorbol-12-myristate-13-acetate (PMA) were used as stimuli, *P. aeruginosa* lipase enhanced calcium ionophore-induced LTB4 generation and beta-glucuronidase release but reduced calcium ionophore-induced and PMA-induced chemiluminescence. Analysis of lipase-inactivated culture supernatant revealed reduced generation of HETE and LTB4. These workers concluded that simultaneous secretion of lipase and PLC by *P. aeruginosa* residing in an infected host may result in severe pathological effects which cannot be explained by the sole action of the individual virulence factor on inflammatory effector cells.

Effect of PLC, both hemolytic (PLC-H) and non-hemolytic (PLC-N), on neutrophil accumulation following infection with *P. aeruginosa* mediated through release of IL-8 was assessed by Konig et al., (1997). These workers employed purified PLC-H as well as culture supernates of *P. aeruginosa* strain capable of producing both PLC-H and PLC-N, and mutant strains deficient in the production of one or other phospholipase, or deficient in production of both the siderophores. It was observed that lower concentrations of PLC-H and PLC-N enhanced IL-8 production and IL-8-specific mRNA expression whereas higher concentrations of PLC-H decreased IL-8 production and IL-8-specific mRNA expression. These results suggested that PLC play an important role in neutrophil recruitment in *P. aeruginosa* infections by upregulating release of IL-8.

Okazaki et al., (1997) assessed O-serotyping as well as production of virulence factors like hemolysin, pyocyanin, pyoverdin, elastase and caseinase in 18 multiple-antibiotic resistant (MARPA) strains and in 13 strains of antibiotic sensitive *P. aeruginosa* (ARPA) isolated from clinical specimens. It was observed that majority of
isolates MARPA strains belonged to serotype C whereas ARPA strains belonged to serotype G, C and E. MARPA strains showed no production of virulence factors whereas strain to strain variation in production of virulence factors was observed in ARPA strains. Hemolysin and pyoverdin was observed in all the 13 strains (100%), pyocyanin in 8 strains (61.5%), elastase and caseinase in 9 strains (69.2%) of ASPAs strains of *P. aeruginosa*. It was concluded that emergence of MARPA strains is associated with loss of elaboration of virulence factors as compared to ARPA strains which produced a variety of virulence factors.

Lanotte *et al.*, (2003) investigated the correlation between the production by *Pseudomonas aeruginosa* isolates of four exoenzymes (protease, elastase, neuraminidase, and phospholipase C (PLC)) and the clinical state of cystic fibrosis (CF) patients. It was observed that elastase and neuraminidase activities were higher in strains isolated from patients with excellent or good clinical status whereas PLC levels were higher in isolates from patients with poor or weak clinical status. It was concluded that PLC may help in persistence of *P. aeruginosa* by impairing lung function.

A novel extracellular phopholipase C was identified by Barker *et al.*, (2004) in *P. aeruginosa*. It was observed that whereas all presently known PLCs of *P. aeruginosa* (PlcH, PlcN and PlcB) hydrolyse phosphatidylcholine (PC), but only PlcB was active on phosphatidylethanolamine (PE). This novel PLC was also found to be involved in chemotaxis of phopholipids.

Effect of Touildine blue (TBO) on secretion of virulence factors by *P. aeruginosa* was investigated by Sharma *et al.*, (2005). Viability of MCF-7 cells incubated with culture supernatants of bacteria photosensitized with TBO (experimental) was found to be higher than that for MCF-7 cells incubated with culture supernatants of bacterial
cells treated either with TBO in dark (control II) or not receiving any treatment (neither TBO nor light (control I)). Furthermore, whereas MCF-7 cells incubated with supernatants of bacteria, control I and II, showed significant morphological alterations, no such changes were observed in MCF-7 cells incubated with supernatant of experimental cells. The activities of phospholipase C and proteases of *P. aeruginosa* were also found to decrease on photosensitization. These workers suggested that potency of virulence factors is reduced in cells surviving photodynamic treatment with TBO and this may have implication in treatment of *P. aeruginosa* infections.

**Exotoxins**

*P. aeruginosa* has been reported to produce a variety of exotoxins like exotoxin A, exotoxin S, exo U and exoT which have been implicated in the ability of this pathogen to cause a wide spectrum of infections.

Role of *P. aeruginosa* exoenzymes in cystic fibrosis lung infection in the presence and absence of specific serum antibodies was studied by Doring et al., (1985). Exoenzymes were not detectable in sputum samples of patients. Positive serum antibody titers to bacterial exoenzymes were found in the majority of patients. The results of this study suggested that exoenzymes do not directly contribute to lung damage after induction of immune response to bacterial antigens.

Miyazaki et al., (1995) studied the effects of exotoxin A on PMNs *in vitro* and in mouse model. Exotoxin A inhibited *in vitro* phagocytosis and killing of *P. aeruginosa* by human and murine PMNs. *In vivo*, parent strain of *P. aeruginosa* was found to be more virulent than its isogenic mutant strain deficient in production of exotoxin A. Bacterial load in blood and internal organs were significantly higher in mice infected with parent strain as compared to mutant strain. Monoclonal
antibody against exotoxin A prevented the death of the mice if it was given simultaneously with or 2 h before, infection with parent strain of \textit{P. aeruginosa}. It was concluded that exotoxin A is toxic \textit{in vivo} and promoted the growth of \textit{P. aeruginosa in vivo}.

Schultz \textit{et al.}, (2001) studied the effect of exotoxin A in pathogenesis of respiratory tract infections caused by \textit{P. aeruginosa} in mouse model of acute pneumonia. Lung bacterial load was significantly higher in mice infected with parent as compared to mutant strain deficient in production of exotoxin A. However, influx of neutrophils was similar in broncho-alveolar lavage fluids (BALF) during pneumonia in mice infected with parent and mutant strain. Lung levels of cytokines (tumor necrosis factor-alpha, interleukin-6) and chemokines (macrophage inflammatory protein-2, KC) were higher in mice inoculated with parent strain whereas BALF concentrations of NO were similar in mice treated with parent and mutant strains. These data suggested that exotoxin A play an important role during pneumonia caused by \textit{P. aeruginosa} by impairing host defense.

The contribution of exotoxin A (ETA) in \textit{P. aeruginosa} induced keratitis was evaluated by Pillar and Hobden, (2002) \textit{in vitro} and \textit{in vivo}. It was observed that mutant strain adhered to scarified corneal epithelium in \textit{in vitro} organ culture model and initiated ocular disease similar to that in wild-type strains. However, mutant strains were cleared from the eye much more rapidly than patent strain. It was concluded that although ETA had no effect on the ability of \textit{P. aeruginosa} to adhere to corneal wounds or to initiate \textit{P. aeruginosa} keratitis, it was crucial for the organism to persist in the eye and ultimately cause disease.

Fogle \textit{et al.}, (2002) assessed the efficacy of exotoxin A anti-IgG antibodies (ETA-Ab) to neutralize the \textit{in vivo} effects of ETA. ETA
induced apoptosis in hepatocytes and induced production of specific cytokines. ETA-Ab was observed to neutralize these effects. In addition these workers also evaluated the role of exotoxin A in vivo in mouse model of burn wound infection employing parent strain and its isogenic mutant deficient in production of exotoxin A. The lethality, local spread, and systemic spread were significantly reduced following infection with mutant strain compared to parent strain. It was concluded that ETA contribute to the spread of P. aeruginosa during burn wound infection.

Lee et al. (2003) evaluated the contribution of exotoxin A regulated proteins toward corneal infections in vivo. Mutation of either exoU or exoT alone was found to have no significant effect on virulence, whereas simultaneous mutation of both exoT and exoU or of exsA resulted in a significantly reduced capacity to cause corneal disease in case of cytotoxic strain. Complementation of the double exoUexoT mutant with exoU alone restored bacterial colonization levels and disease severity to wild-type levels. Complementation with exoT alone increased colonization and increased virulence to almost the same levels as wild-type or exoU-complemented infections. However, mutations in exo A or exo T or exo U was found to have no effect on the virulence of invasive strain. It was concluded that exotoxin A contributed to corneal virulence of only cytotoxic P. aeruginosa strain, with contributions made by both exoU and exoT to bacterial survival and disease severity.

Rapid quantitation of exoU and exo T in clinical isolates of P. aeruginosa was done by employing ELISA (Li et al., 2005). Findings were compared with results of standard immunoblotting and correlated with virulence of P. aeruginosa isolates towards cultured epithelial cells. It was suggested that this simple technique will facilitate in screening large number of isolates for elaboration of Exo U and Exo T. This may help in predicting the course of clinical disease in
a patient and should be taken into account in determining optimal treatment strategies for infected patients.

Rao et al., (2005) tested the hypothesis that children with tracheostomies are colonized by *P. aeruginosa* that express these virulence factors and have antibodies directed against these virulence factors. Serum from majority of the patients was found to be positive for presence of antibodies to ETA and components of the type III system by Western blot analysis. Sera from patients who were antibody-positive for ETA were also seropositive for either ExoS or ExoU. These data identified a seropositive reaction to *P. aeruginosa* cytotoxins in some patients with tracheostomies indicating infection by cytotoxic strains of *P. aeruginosa*.

**Quorum sensing systems**

A variety of gram negative and gram positive have been reported to monitor their cell density as well as expression of virulence factors through chemical signals. These signals known as quorum sensing signals are mainly operative through autoinducers generally acylhomoserine lactones (AHLs). In *P. aeruginosa* two types of quorum-sensing systems, *las* (Gambello and Iglewski, 1991) and *rhl* (Ochsner and Reiser, 1995) have been reported which consist of two signal-generating synthetases (*LasI* *RhlI*) and two cognate transcriptional regulators (*LasR* *RhlR*). The major products of *LasI* and *RhlI* are N-(3-oxododecanoyl)-homoserine lactone (OdDHL or 3OC₁₂-HSL) (Pearson et al., 1994) and N-butanoylhomoserine lactone (BHL or C₄-HSL) (Pearson et al., 1995; Winson et al., 1995), respectively. The *lasIR* encoded quorum-sensing system has been shown to modulate expression of *lasI* itself (Seed et al., 1995), *lasB* (elastase) (Passador et al., 1993; Pearson et al., 1997), alkaline protease (Gambello et al., 1993), secretion pathway (Chapon-Herve et al., 1997) and *rhlR* (Latifi et al., 1996; Pesci et al., 1997). The *rhlIR*-encoded quorum sensor
modulates expression of *rhll* itself (Latifi *et al.*, 1996), *rhlAB* (rhamnolipid biosynthesis) (Ochsner and Reiser, 1995; Pearson *et al.*, 1997), *lasB* (Brint and Ohman, 1995; Pearson *et al.*, 1995, 1997) and *rpoS* (Latifi *et al.*, 1996). Both these quorum-sensing systems have also been shown to regulate twitching motility (Glessner *et al.*, 1999) and are involved in the differentiation of planktonic cells to biofilm mode (Davies *et al.*, 1998). These signals have been reported to interfere with the host immune system, where it specifically down-regulate the production of the cytokines IL-12 and TNF-α which support the bactericidal Th-1 milieu and protect the host (Telford *et al.*, 1998).

Telford *et al.*, (1998) carried out study to investigate immomodulatory potential of AHLs, N-(3-Oxodecanoyl)-L-Homoserine Lactone (OdDHL) and N-(3-Oxohexanoyl)-L-Homoserine Lactone (OHHL) in murine and human leukocyte immunoassays *in vitro*. These workers observed that OdDHL but not OHHL inhibited lymphocyte proliferation and tumor necrosis factor-α production by lipopolysaccharide-stimulated macrophages. Decrease in production of IL-12, a Th-1 supportive cytokine, in presence of OdDHL was also observed. Lower concentrations (<7x10⁻⁵ M) of OdDHL was found to increase IgG1 production whereas higher concentrations (>7x10⁻⁵ M) inhibited antibody production. OdDHL also promoted IgE production by IL-4 stimulated human peripheral blood mononuclear cells indicating that this molecule is active against human lymphocyte populations. These workers concluded that AHL molecules play an important role in virulence of *P. aeruginosa* by downregulating the production of cytokines influencing Th-1 and Th-2 balance and promoting colonization of this pathogen in host tissues.

Role of quorum sensing signals in pathophysiology of burn wound infections caused by *P. aeruginosa* was investigated by
Rumbaugh et al., (1999). These workers tested and compared the virulence potential of parent strain possessing functional las and rhl systems and its isogenic mutants deficient in production of quorum sensing signals (lasR mutant, lasI mutant and rhlI mutant) in burn wound infection model established in mice in terms of lethality, dissemination of *P. aeruginosa* within body and burned skin. These workers observed significant decrease in bacterial load in liver, spleen and burned skin in comparison to parent strain. Complementation of mutant strains with plasmids carrying lasI or rhlI genes lead to restoration of *in vivo* virulence of these strains. These workers concluded that quorum sensing systems play an important role in the horizontal spread of *P. aeruginosa* within burned skin as well as within liver and spleen and contribute to virulence of *P. aeruginosa* in burn wound infections.

Winzer et al., (2000) carried out study to investigate influence of quorum sensing signals on production of lectins PA-IL and PA-IIL by *P. aeruginosa*. For both PA-IL and PA-IIL, mutation of lasR delayed but did not abolish lectin production. Loss of both the lectins and their restoration following the introduction of plasmid borne rhl locus indicated that rhl system was responsible for controlling lectin production. These workers concluded that quorum-sensing systems are required for expression of lectins by *P. aeruginosa* which play crucial role in adherence of this pathogen to host cells.

Effects of quorum sensing signal molecules, N-butanoyl-L-homoserinelactone (C4-HSL) and N-(3-oxodecanoyl)-L-homoserinelactone (3-oxo-C12-HSL), on resistance of planktonic cells of *P. aeruginosa* to hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) was studied by employing parent strain and its isogenic mutants deficient in production of AHLs (Huang and Shih, 2000). In double mutant of *P. aeruginosa* deficient in production of lasI and rhlI, the viable cell concentration decreased with time and was reduced by about 4 log
cycle after 2 h of 7.5 mM H₂O₂ treatment in comparison to only a 2 log cycle reduction observed with parent strain. However when this double mutant was cultured with 20% PAO1 spent medium, it showed similar hydrogen peroxide resistance to that seen in parent strain. However, adding synthetic homoserine lactones alone did not increase resistance of mutant strain to H₂O₂ as seen in the experiments adding PAO1 spent medium. Results of these workers indicated that the difference in cell resistance against H₂O₂ between parent and mutant strain was related to the existence of gene products of the lasI and rhlI systems.

Pearson et al., (2000) carried out study to identify role of quorum sensing signals in respiratory tract infections. These workers tested the virulence potential of quorum sensing producer strain and its isogenic mutants in model of acute pneumonia. They observed that quorum sensing producer strain possessing functional las and rhl systems was comparatively more virulent as compared to lasI mutant and lasI rhlI double mutant assessed in terms of lung bacterial load. Histopathology of lung tissue also revealed more severe confluent pneumonia in mice infected with quorum sensing producer parent strain as compared to mild focal pneumonia observed in experimental animals infected with mutant strains. These workers concluded that quorum-sensing systems are essential in pathogenesis of respiratory tract infections caused by *P. aeruginosa*.

Radiometric techniques were employed to detect quorum-sensing signals in sputum of patients infected with cystic fibrosis patients (Singh et al., 2000). It was observed that sputum samples from patients colonized with *P. aeruginosa* produced significantly higher amounts of AHLs as compared to broth grown cultures of standard *P. aeruginosa* strain in planktonic mode. However when standard *P. aeruginosa* strain was grown in biofilm mode, levels of AHL production was comparable to that produced in sputum samples
indicating that cystic fibrosis lungs were infected with biofilms of *P. aeruginosa*. It was concluded that production of AHLs is strongly influenced by environmental conditions.

Mcknight *et al.*, (2000) reported a second type of intercellular signal involved in lasB induction. These workers identified this signal as 2-heptyl-3-hydroxy-4-quinolone and designated it as the Pseudomonas quinolone signal (PQS). PQS was observed to be part of the quorum-sensing hierarchy since its production and bioactivity depended on the las and rhl quorum-sensing systems, respectively. In order to define the role of PQS in the *P. aeruginosa* quorum-sensing cascade, lacZ gene fusions were used to determine the effect of PQS on the transcription of the quorum-sensing system genes lasR, lasI, rhlR, and rhlI. It was observed that in *P. aeruginosa*, PQS caused a major induction of rhlI'-lacZ and had lesser effects on the transcription of lasR'-lacZ and rhlR'-lacZ. Transcription of both rhlI'-lacZ and lasB'-lacZ was cooperatively effected by C(4)-HSL and PQS. Further results indicated that PQS was produced maximally during late stationary phase of growth. It was concluded that PQS act as a link between the las and rhl quorum-sensing systems but this signal is not involved in sensing cell density.

Calfee *et al.*, (2001) carried out study to identify precursor of PQS and to assess efficacy of precursor analogs against elaboration of PQS and exoenzyme production by *P. aeruginosa*. Using radioactivity, these workers identified anthranilate as precursor of PQS and methyl anthranilate as analog of anthranilate. PQS production monitored in presence of methyl anthranilate revealed decreased production of PQS. In addition, decrease in elastase production by *P. aeruginosa* was observed when cells were grown in presence of increasing concentrations of methyl anthranilate. These workers concluded that quorum sensing syttems can serve as potential candidates to develop effective preventive approach against *P. aeruginosa* infections.
Production of AHLs in ocular gram negative bacteria was investigated by Zhu et al., (2001). These workers isolated strains of Acinetobacter, Aeromonas hydrophila, Escherichia coli, Haemophilus influenzae, Klebsiella oxytoca, Serratia liquefaciens, Serratia marcescens, Stenotrophomonas maltophilia and Pseudomonas aeruginosa from contact lens adverse response patients as well as from asymptomatic subjects. The biosensor strains Chromobacterium violaceum mutant CV026 and Agrobacterium tumefaciens A136 were used for detection of AHL signal molecules. However, AHLs were detectable only in Serratia marcescens. It was concluded that AHL signals were produced during ocular infections. It was also stressed that different types of AHL molecules may be associated with other bacteria, which were not detectable, by this bioassay and hence profiles of AHLs varied with type and site of infection.

Smith et al., (2002) carried out study to investigate the role of AHLs in vivo in acute model of acute pneumonia. It was observed that mutant strains of P. aeruginosa deficient in production of lasI or lasR were significantly less virulent as compared to parent strain possessing functional quorum sensing systems assessed in terms of lung bacterial load. Further these workers assessed the effects of AHLs (30-C12-HSL) injected into the skin of experimental animals. It was reported that 30-C12-HSL stimulated production of variety of cytokines like interleukin-1alpha (IL-1alpha) and IL-6 and the chemokines macrophage inflammatory protein 2 (MIP-2), monocyte chemotactic protein 1, MIP-1beta, inducible protein 10, and T-cell activation gene 3. Further it also activated T cell response promoting Th1 environment and induced the production of cyclooxygenase 2 (Cox-2) enzyme. Cox-2 enzyme is important for the conversion of arachidonic acid to prostaglandins and has been implicated in edema, inflammatory infiltrate, fever, and pain. It was concluded that quorum sensing signals play an important role in pathogenesis of respiratory
tract infections by promoting bacterial growth and inducing inflammation causing tissue damage leading to bacterial colonization.

Hentzer et al., (2002) carried out study to develop novel molecular tools which allow in situ detection of AHL-mediated quorum sensing and its inhibition in *P. aeruginosa* biofilms. These workers developed green-fluorescent protein (Gfp) based reporter technology to detect single-cell level quorum sensing in laboratory based *P. aeruginosa* biofilms. The technique was found to be highly sensitive capable of detecting odDHL at concentrations as low as 20nM.

Pathophysiological impact of quorum sensing systems on course of acute pneumonia was investigated by comparing the virulence of parent strain possessing functional *las* and *rhl* systems with that of its *lasR* deleted mutant employing rat model of acute pneumonia (Lesprit et al., 2003). Significantly higher lung bacterial load was observed in experimental animals following infection with parent strain in comparison to mice infected with mutant strains. Pulmonary lesions assessed by Haemotoxylin-eosin staining revealed severe necrosis of bronchioalveolar structures, vascular congestion and neutrophil infiltration following infection with parent strain. In contrast, lung injury in rats infected with mutant strains was characterized by absence of congestion and macrophage infiltration. It was concluded that quorum sensing systems play an important role in pathogenesis of *P. aeruginosa* induced acute pulmonary infections. It was indicated by reduced ability of mutant to induce mortality, lower lung bacterial load and less severe pulmonary lesions observed with mutant strain.

Huang et al., (2003) carried out study to investigate utilization of AHLs as growth substrates by environmental and clinical strains of *P. aeruginosa*. Both clinical and environmental strains were found to be capable of degrading long chain AHLs but not short chain AHLs. However growth of clinical strains commenced only after long lag
phases. To elucidate the mechanism of AHL utilization, these workers carried out liquid-chromatography-atmospheric pressure chemical ionization-mass spectrometry. It was observed that *P. aeruginosa* utilize AHLs via an AHL cyclase and a homoserine-generating homoserine lactonase.

Williams *et al.*, (2004) investigated the effect of autoinducers on host gene expression. It was observed that *P. aeruginosa* autoinducers N-3-oxododecanoyl-homoserine lactone and N-butanoylhomoserine lactone can enter eukaryotic cells and activate artificial chimeric transcription factors based on their cognate transcriptional activators, LasR and RhlR respectively. It was speculated that the effects of autoinducers on the transcription of genes in host cells may result from specific interactions of these bacterial proteins with host proteins.

Schaber *et al.*, (2004) carried out study to determine if deficiency within the QS system compromised the ability of *P. aeruginosa* to establish infections in humans. These workers isolated 200 strains of *P. aeruginosa* from patients with urinary tract, lower respiratory tract and wound infections. 5 strains were found to lack LasB and LasA activities and produced either no or very low levels of the autoinducers N-(3-oxododecanoyl) homoserine lactone and N-butyryl homoserine lactone. PCR analysis revealed that three isolates contained all four QS genes (lasI, lasR, rhlI and rhlR) while two isolates lacked both the lasR and rhlR genes. The isolates produced variable levels of exotoxin A and, with one exception, were deficient in pyocyanin production. One isolate produced the type III secretion system (TTSS) effector proteins ExoS and ExoT, two isolates produced ExoT only and two isolates produced no TTSS proteins. The isolates produced weak to moderate biofilms on abiotic surfaces. One QS-deficient clinical isolate (CI-1) lacked all tested virulence factors and produced a weak biofilm. These workers concluded that naturally
occurring QS-deficient strains of *P. aeruginosa* do occur and are capable of causing infections; and, that besides the known virulence factors, additional factors may contribute to the ability of certain strains to establish an infection.

Importance of *Pseudomonas aeruginosa* quorum-sensing systems in the pathogenesis of corneal infection was investigated by Zhu *et al.*, (2004). Clinical isolates of *P. aeruginosa* were isolated from keratitis patients and screened for possession of quorum-sensing genes lasI, lasR, rhlI, rhlR, by polymerase chain reaction and Southern blot hybridization. Elastinolytic activity, controlled by the *las* system, was assayed using elastin Congo red and rhamnolipid production controlled by the *rhl* system was assessed using agar plates containing methylene blue/cetyltrimethyl ammonium bromide. In addition virulence potential of these strains was examined *in vivo* in a scarified inbred BALB/c mouse model. It was observed that quorum deficient strains elaborated less elastase as well as produced less rhamolipids and these strains were less virulent in comparison to quorum sensing producer strains assessed in terms of eye colonization in mouse keratitis model. These workers concluded that quorum sensing systems play an important role in development of keratitis.

Wu *et al.*, (2004) carried out study to investigate effects of quorum-sensing on immunoglobulin G responses in a rat model of chronic lung infection induced with *P. aeruginosa*. These workers observed serum antibodies against *P. aeruginosa*. Significantly weaker responses of serum IgG and IgG1 and a lower ratio of IgG1/IgG2a were observed in rats infected with quorum-signal-deficient strain as compared to parent strain. Lungs were found to be sterile in case of rats infected with quorum deficient strain as compared to experimental animals infected with parent strain. These workers concluded that quorum-sensing signals play a crucial role in
persistence of infections caused by *P. aeruginosa* by modifying immune responses.

Effect of quorum sensing signals on production of cytokines in relation to burn wound infections by employing murine model of thermal injury was studied by Rumbaugh *et al.*, (2004). Mice were infected with quorum sensing producer parent strain and its isogenic mutant strain defective in production of both las and rhl system. Using multi-probe RNase protection assay, these workers detected mRNA for TNF-alpha, IL-6, TGF-beta, and G-CSF within the burned skin of mice infected with parent strain. On the contrary, expression of these cytokines was not observable in experimental animals infected with mutant strain. It was concluded that quorum sensing signals play an important role in induction of cytokine response in burn wound infections.

Gould *et al.*, (2004) elucidated the crystal structure of the acyl-homoserine lactone (AHL) synthase LasI that produce 3-oxo-C12-AHL from the substrates 3-oxo-C12-acyl-carrier protein (acyl-ACP) and S-adenosyl-L-methionine. The LasI six-stranded beta sheet platform, buttressed by three alpha helice, forms a V-shaped substrate-binding cleft that lead to a tunnel passing through the enzyme that can accommodate the acyl-chain of acyl-ACP. This tunnel place no apparent restriction on acyl-chain length, in contrast to a restrictive hydrophobic pocket seen in the AHL-synthase Esal. Interactions of essential conserved N-terminal residues, Arg23, Phe27 and Trp33, suggest that the N-terminus form an enclosed substrate-binding pocket for S-adenosyl-L-methionine. Analysis of AHL-synthase surface residues identified a binding site for acyl-ACP, a role that was supported by *in vivo* reporter assay analysis of the mutated residues, including Arg154 and Lys150. These workers concluded that availability of structure of AHL will help in developing effective preventive approach to control *P. aeruginosa* induced infections.
Furanones were injected intravenously in mice infected with *P. aeruginosa*. In a study of Wu *et al.*, (2004) mice infected with *P. aeruginosa* lung infection were treated with different doses of furanones to evaluate the therapeutic effects of furanones on the chronic lung infection. Results showed that furanone compounds interfered with quorum sensing systems and suppressed bacterial quorum-sensing in lungs indicated by decrease in expression of green fluorescent protein, increased lung bacterial clearance and reduced severity of lung pathology. Further treatment with furanone compounds significantly prolonged the survival time of the mice in a lethal *P. aeruginosa* lung infection. These workers concluded that furanone compounds could be used as therapeutic agents for treatment of *P. aeruginosa* induced infections.

George *et al.*, (2005) evaluated the effects of C12- and C4-AHLS of *P. aeruginosa* and a synthetic furanone signaling antagonist on growth, primary adhesion to hydrogel contact lenses, and elastase B production of *P. aeruginosa* strains, including QS mutants, and isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*. These workers added AHLs and furanone to cultures of bacteria at various growth stages. Primary adhesion of *P. aeruginosa* to lenses soaked in AHLs and furanone was examined by adenosine triphosphate analysis. Elastase B activity of cultures exposed to AHLs and furanone was determined by an elastin congo red assay. It was observed that AHLs had no significant effects on maximal cell densities or primary adhesion. However, adhesion of a lasl-rhl- QS mutant to lenses was significantly lower than its wild-type strain, regardless of added AHL or furanone. Exogenous AHLs had negligible effects on elastase production of wild-type *P. aeruginosa* but restored elastase production in QS-deficient mutants. The furanone was biocidal for *Staphylococci* but enhanced growth of the lasl-rhl- QS mutant. Furanone decreased elastase production of wild-type strains
but increased production in QS mutant. It was concluded that exogenous AHLs and the furanone were of minor importance in initial adhesion of *P. aeruginosa* to lenses. The data further indicated strain diversity in *P. aeruginosa* and complexity of AHL systems.

Chambers *et al.*, (2005) carried out study to identify and quantitate AHLs in mucopurulent respiratory secretions from cystic fibrosis patients. These workers subjected mucopurulent secretions to reverse-phase fast pressure liquid chromatography and analyzed the presence of AHLs using a tral-luxCDABE-based reporter that respond to AHLs with acyl chains ranging between 4 and 12 carbons. Using this assay system, these workers detected and identified a broad range of AHLs being present at low concentrations in limited sample volumes. N-(3-oxo-dodecanoyl)-l-homoserine lactone, N-(3-oxo-decanoyl)-l-homoserine lactone and N-octanoyl-l-homoserine lactone (OHL) were the AHLs most frequently identified. OHL and N-decanoyl-l-homoserine lactone were detected in nanomolar concentrations compared to picomolar amounts of the 3-oxo-derivatives of the AHLs identified.

Kaufmann *et al.*, (2005) discovered a new nonenzymatically formed product from N-(3-oxododecanoyl)-L-homoserine lactone. These workers observed that both the N-acylhomoserine and its novel tetramic acid degradation product, 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl) pyrrolidine-2,4-dione, are potent antibacterial agents. Bactericidal activity was observed against all tested Gram-positive bacterial strains, whereas no toxicity was seen against Gram-negative bacteria. They propose that *Pseudomonas aeruginosa* utilizes this tetramic acid as an interference strategy to preclude encroachment by competing bacteria. Additionally, they have discovered that this tetramic acid bind iron with comparable affinity to known bacterial siderophores, possibly providing an unrecognized mechanism for iron solubilization.
Environmental Stress

*P. aeruginosa* has been reported to continuously sense and respond to various environmental stimuli such as osmotic stress, pH and iron content through its cell to cell signaling systems (Bullen *et al.*, 1978; Bjorn *et al.*, 1979; Erickson *et al.*, 2000). In addition, this pathogen also responds to starvation and dessication. Iron is the most important factor which has been reported to play a crucial role in biofilm formation and virulence of *P. aeruginosa*. The total iron concentration in biological fluids is >20 μM which is bound to iron binding proteins like lactoferrin and transferrin. However the free iron available is less than 10^(-6) μM, which is also the amount required by bacteria for maximum growth. Therefore availability of iron may have influence on ultimate outcome of an infection. Effect of iron in pathogenesis of *P. aeruginosa* induced mouse corneal infections was investigated by Woods *et al.*, (1982). Parental strain PAO1 when cultured in high-iron medium (5 micrograms of Fe per ml) was less virulent than when it was cultured in low-iron medium (0.05 microgram of Fe per ml). The iron concentration of the growth medium had no effect on the virulence of *P. aeruginosa* mutant which was resistant to the iron regulation of toxin A yields (PAO-toxFeR-18). A severely defective iron transport mutant, PAO-toxFeR-10, was avirulent regardless of the iron concentration of the growth medium. These studies indicate that both iron acquisition and iron regulation of toxin production are important factors in the determination of *P. aeruginosa* virulence which is strain dependent.

Boyce *et al.*, (1984) investigated the effects of cations on the stability in culture of mucoid strains of *P. aeruginosa* isolated from patients with cystic fibrosis by studying their effect on the selection of nonmucoid derivatives which arise by spontaneous mutation in cultures of mucoid organisms. Calcium and magnesium ions were found to have no effect on growth or stability of the mucoid cultures.
On the contrary, iron had profound effect on the selection of nonmucoid mutants in unshaken cultures of mucoid organisms. In medium containing 0.01 mM iron, nonmucoid mutants rapidly accumulated to a greater than 100-fold-higher frequency than the mucoid forms. It was suggested that iron play an important role in selection of nonmucoid cells from a population of mucoid *P. aeruginosa*.

Sokol and Woods, (1984) examined the effect of iron on virulence of *P. aeruginosa* in a chronic pulmonary infection model in rats. Rats were infected by giving transtracheal inocula of agar beads in which 10^4 cfu of *P. aeruginosa* strain PAO and the mutants of strain PAO, Fe5 and Fe18 were embedded. When strain PAO was grown in low-iron medium before infection, it caused severe parenchymal changes including a dense mononuclear cell infiltration in the alveolar spaces, as well as intra- and peribronchial inflammation. When strain PAO was grown in high-iron medium, the pathological changes in lungs were restricted to intra- and peribronchial inflammation. Strain Fe5, in which the effect of iron on yields of elastase is deregulated, produced similar pathological changes regardless of whether it was grown in low- or high-iron media. All rats infected with strain Fe18, in which the effect of iron on yields of toxin A is deregulated, died within 48 h after infection. These results brought out that iron concentration of the culture medium can influence the pathogenesis of chronic respiratory tract infections caused by *P. aeruginosa* in a chronic respiratory infection.

Role of ferric uptake regulator (Fur) in growth and iron uptake ability of *P. aeruginosa* was assessed by Hasset et al., (1996). Mutants, expressing altered Fur proteins, were found to be defective in both ferricpyochelin- and ferripyoverdin-mediated iron uptake. These mutants required a significant lag phase prior to log-phase aerobic growth. Significant increase in growth of these mutants was observed
following addition of iron in culture medium. On the other hand, iron deprivation by the addition of the iron chelator 2,2'-dipyridyl to wild-type bacteria produced an increase in manganese superoxide dismutase (Mn-SOD) activity and a decrease in total catalase activity. It was concluded that mutations in *P. aeruginosa* fur locus affect aerobic growth and SOD and catalase activities in *P. aeruginosa*.

Frederick *et al.*, (2001) studied factors affecting catalase expression in planktonic and biofilm cells of *P. aeruginosa*. Biofilm cells of *P. aeruginosa* were found to require more iron whereas planktonic cultures required no addition of iron. However, iron-stimulated catalase activity in biofilms was significantly less in planktonic cells. These workers also studied effect of oxygen on catalase activity. Nitrate-respiring planktonic cultures produced approximately twice as much catalase activity as aerobic cultures grown in the presence of nitrate. This nitrate stimulation effect was also demonstratable in biofilm cells. It was concluded that iron availability, but not oxygen availability, is a major factor affecting catalase expression in biofilms.

Tsuburai *et al.*, (2004) examined whether the transfer of exogenous heme oxygenase (HO)-1 cDNA, a rate-limiting enzyme in the oxidative degradation of heme to biliverdin, carbon monoxide (CO), and iron, in the lung would provide therapeutic effect in a murine model of lung inflammation induced by *P. aeruginosa*. HO-1 overexpression attenuated neutrophil influx and decreased numbers of apoptotic bronchial epithelial cells. In addition, the overexpression of Bcl-2, a known antiapoptotic factor, was observed and thought to be the mechanism that inhibited bronchial epithelial cellular apoptosis. It was suggested that HO-1 overexpression may be useful for treating *P. aeruginosa*-associated lung inflammation by attenuating neutrophil influx and apoptotic cell death.
Effect of iron limitation on outer membrane composition of six strains of *P. aeruginosa* using conventional and microchip electrophoresis was studied by Kustos *et al.*, (2005). It was observed that iron limitation caused change in outer membrane protein (OMP) profile of *P. aeruginosa*. Appearance of a 92 kDa protein in all six *P. aeruginosa* strains and a 94 kDa protein in one strain could be demonstrated. Besides that up and downregulation of certain proteins was also detectable.

Musk *et al.*, (2005) studied the effect of iron compounds on mature biofilms of *P. aeruginosa*. Ferric ammonium citrate inhibited biofilm formation in a dose-dependent manner. Other iron salts also functioned similarly. In addition to biofilm inhibition in static culture, it was observed that pregrown biofilms could be disrupted and cleared by switching to iron-rich media in flow-chamber experiments. Furthermore, *P. aeruginosa* isolated from sputum of 20 cystic fibrosis (CF) patients showed a similar response to elevated iron levels. These workers found that high levels of iron repress the expression of genes whose products are essential for scavenging iron and that expression of these genes is critical for virulence and biofilm formation.

Osmolarity is another important factor which has been reported to effect growth and virulence of *P. aeruginosa*. Growth of pathogens in hypertonic urine in order to establish and cause UTI form an important criteria. Kunin *et al.*, (1992) assessed the effect of glycine betaine on osmotolerant ability of *E. coli* isolated from blood, urine, or stool and 12 representative enteric strains. Glycine betaine is a powerful osmoprotectant molecule present in the inner medulla of the kidney and is excreted into urine. These workers observed no association between salt tolerance and site of isolation. Salt-sensitive enteric strain that responded poorly to glycine betaine and mutant strains lacking the ability to synthesize or transport glycine betaine were not able to grow well in hypertonic urine. It was suggested that
accumulation of glycine betaine appear to be a mechanism by which
*E. coli* adapt to external osmotic forces and grow in hypertonic urine.

Mourino *et al.*, (1994) assessed the effects of osmolarity on
haemolysin production by standard and uropathogenic strains of *E. coli*. It was observed that low osmolarity increased haemolysin
production in all the strains of *E. coli*.

Effect of glycine betaine, an osmoprotectant, on growth and
cytoplasmic contents of *E. coli* was assessed by Smirnova and
Oktyabrsky, (1995). Glycine betaine stimulated the growth rate of *E. coli* in minimal medium with normal osmolarity at alkaline pH (pH
8.2). Betaine also caused a reduction in the intracellular pools of K+
and low molecular weight thiols in *E. coli* growing both in medium
with high osmolarity and at alkaline pH. These effects of betaine were
absent at pH 7.0. In cells growing in high osmolarity medium, 10 mM
sodium acetate or 10 microM N-ethylmaleimide reduced expression of
the osmosensitive gene *proU* to the same extent as treatment with
betaine; however, under these conditions, sodium acetate and N-
ethylmaleimide did not stimulate the growth of *E. coli*. It was proposed
that low molecular weight thiols and intracellular pH may participate
in the response of *E. coli* to betaine.

Boucher *et al.*, (1997) studied the role of gene *mucC* in the
regulation of stress sensitivity and alginate production. Insertional
inactivation of mucC in *P. aeruginosa* strain PAO1 did not cause any
effect on alginate synthesis. However, it affected growth of *P. aeruginosa*
under conditions of combined elevated temperature and
increased ionic strength or osmolarity. Inactivation of *mucC* in *mucA*
or *mucB* mutant backgrounds resulted in a mucoid phenotype when
the cells were grown under combined stress conditions of elevated
temperature and osmolarity. Each of the stress factors tested
separately did not cause comparable effects. The combined stress
factors were not sufficient to cause phenotypically appreciable enhancement of alginate production in $mucA$ or $mucB$ mutants unless $mucC$ was also inactivated. It was suggested that multiple stress signals play a role in the regulation of $algU$-dependent functions.

The sigma factor RpoS (sigmaS) has been described as a general stress response regulator that control the expression of genes which confer increased resistance to various stresses in some gram-negative bacteria. Suh et al., (1999) elucidated the role of RpoS in $P. aeruginosa$ physiology and pathogenesis by employing parent strain and its isogenic RpoS mutants. It was observed that mutant strain was more sensitive to carbon starvation, was hypersensitive to heat shock at 50 degrees C, increased osmolarity, and prolonged exposure to high concentrations of $H_2O_2$. The rpoS mutant produced less exotoxin A, elastase and Las A protease than the parent strain. However, levels of phospholipase C and casein-degrading proteases were unaffected by a mutation in $rpoS$. On the contrary, increased production of pyocyanin and pyoverdine was observed in mutant strain as compared to parent strain. Mutant strain was able to cause more lung damage than parent strain in rat model of chronic lung infection. It was concluded that RpoS imparts unique properties to $P. aeruginosa$ including ability to cope up with environmental stresses prevalent in host milieu.

Schwan et al., (2002) determined the effects of pH, osmolarity, and human urine on the transcription of several fim genes, as well as the overall expression of type 1 pili in uropathogenic $E. coli$. Growth in acidic medium slightly reduced expression of all the fim promoters ($fimA$, $fimB$, and $fimE$). Increased osmolarity (400 mM NaCl) in neutral-pH medium repressed $fimA$ and $fimB$ transcription which was further repressed when osmolarity was increased to 800 mM NaCl. This effect was more pronounced in high-osmolarity acidic media. Enzyme immunoassays with anti-type 1 pilus antibody and hemagglutination assays showed that fewer type 1 pili were detected.
with cells in a low-pH high-osmolarity environment. It was suggested that the environment prevalent in host milieu may regulate expression of type 1 pili in *E. coli*.

Bazire *et al.*, (2005) assessed the effect of osmotic stress on production of quorum sensing signals and rhamnolipid by *P. aeruginosa*. It was observed that hyperosmotic shock applied during the exponential growth phase interfered with production of quorum sensing signals and prevented rhamnolipid production. These defects were found to result from lower expression of genes involved in quorum sensing production and rhamnolipid syntheses. The osmoprotectant glycine betaine partially restored the production of quorum sensing signal molecules and rhamnolipid production. The results of this study indicated that osmolarity of the medium has a profound influence on production of quorum sensing signals by *P. aeruginosa*.

Internal and external pH is kept under fairly tight regulation by the cell. Changes in pH result in adjustment of activity and synthesis of proteins associated with many different processes, including proton translocation, amino acid degradation, adaptation to acidic or basic conditions and virulence. Booth, (1985) showed that the regulation of cytoplasmic pH control the permeability of the cell membrane to protons. This control is exerted by controlling the activity of the ion transport systems involved in proton entry. It has also been confirmed that there is no constant intracellular pH value for all organisms.

Most bacteria have been reported to possess membrane bound pumps which remove protons from the cytoplasm to generate a transmembrane electrochemical gradient of protons called the protonmotive force. Biological membranes exhibit a low proton permeability of the lipid bilayer and the very specific control of ion movement through the protein complexes in the membrane. Since
long it has been suggested that pH of the growth medium could affect the metabolism of bacteria. When bacteria are growing at an alkaline pH, they produce acidic fermentation products and when they grow at an acidic pH, neutral products are formed. Foster, (1991) provided additional examples of genes induced or repressed during various pH treatments based on protein gel analysis and gene/operon fusions. Most of these genes have not been implicated in adaptation or habituation. It was reported that expression of several membrane proteins is affected by acid treatment and adjusting level of these proteins is required to compensate for changes in activities brought about by changes in pH.

Gargan et al., (1993) assessed the effect of pH and osmolality on phagocytosis and killing of E. coli by PMNs in vitro. Maximum phagocytosis was seen in urine as well as in Hanks balanced salt solution at both 485 and 200 mosM between pH 6 and 8. Phagocytosis was abolished at pH 5. Killing of the strains by PMN was optimal between pH 6.5 and 7.5 in urine at 485 mosM. It was observed that the bactericidal activity of PMN in urine was more sensitive to alterations in pH than phagocytic function. It was suggested that raising the pH of urine and reducing the osmolality may increase the ability of natural defense mechanisms to eliminate infecting organisms.

The effect of the properties of urine and its components on the chemiluminescence (CL) response of PMNs was evaluated by Kubo et al., (1998) by employing artificial urine medium. CL response of PMNs was found to be significantly suppressed at a pH of 6 or 5, but not suppressed at a pH of 7 or 8. It was observed that function of PMNs in urine was reduced mainly by urine pH, concentration of urea, sodium, potassium, and creatinine. It was suggested that controlling these parameters in urine may restore functions of PMNs to clear bacteria in patients with urinary tract infections.
Carlsson et al., (2001) assessed the effects of pH, nitrite and ascorbic acid on growth of *P. aeruginosa*. Growth of *P. aeruginosa* was markedly reduced by the addition of nitrite to acidified urine. This inhibition was enhanced by ascorbic acid. Bacteriostatic effect of acidified nitrite was related to the release of NO and other toxic reactive nitrogen intermediates. These results may help to explain the well-known beneficial effects of urinary acidification with vitamin C in treatment and prevention of UTIs.

**Tamm-Horsfall protein (THP)**

Tamm-Horsfall protein is the most abundant urinary glycoprotein in mammals. It is a glycosyl-phosphatidyl-inositol (GPI)-anchored membrane protein that mainly reside at the luminal face of cells of the thick ascending limb of Henle’s loop (TAL) and early convoluted tubules of nephron. Tamm Horsfall protein contain exclusively N-linked glycans, mainly of polyanterary type largely sialylated and fucosylated, but also have high mannose glycans.

Tamm and Horsfall, (1952) isolated and identified a urinary glycoprotein by salt precipitation method from urine and found it to be responsible for urinary inhibition of Myxovirus-induced hemagglutination.

In 1964, Bayer used electron microscopy to confirm the binding of influenza virus to urinary mucoprotein. Urinary THP was visualized as a network of filaments composed of smaller fibrils with a diameter of 4 to 24 nm, but the length could not be detected because of their tendency to form end-to-end aggregates.

Parrkinen *et al.*, (1988) investigated whether human urine contained inhibitors of adhesins of *E.coli*. Normal human urine was found to inhibit hemagglutination by S and type-1 fimbriae but not P fimbriae. The major inhibitor of S fimbriae in normal urine was

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identified as THP and interaction with S fimbriae was probably mediated by its sialyloligosaccharide.

Uropathogenic adhesion was measured using a range of polymer materials with differing surface tension properties using phosphate buffered saline (controls), Tamm Horsfall protein (THP), and human urine by image analysis by Hawthorn and Reid, (1990). Results showed that THP did interfere with adhesion by binding directly to these surfaces as well as to *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and, to a lesser extent, *Proteus mirabilis*. Incubation of the uropathogens in THP and urine resulted in altered adhesion profiles to polymer surfaces, with no single trend apparent.

Reinhart *et al.*, (1990) studied the binding of THP to uropathogenic *E.coli*. It was found out that THP binding to uropathogenic *E.coli* bearing type-1 fimbriae was proportional to THP concentration and size of the bacterial inoculum. These workers showed that fimbrial receptor site for THP has lectin like properties and THP bind to fimbriae via its mannose side chain.

Hawthorn *et al.*, (1991) isolated epithelial cells from a healthy human kidney and found that they secreted THP in tissue culture. They noted adherence of uropathogenic bacteria to THP coated tubular cells. Extraneous THP bound to type-1 piliated *E.coli* but not to P-piliated strains and caused reduction in the adhesion of type-1 fimbriated *E.coli* to renal cells although it was not able to competitively exclude adhesion. On the contrary, adherence of THP coated *Proteus mirabilis* and *P. aeruginosa* to renal epithelial cells was found to be enhanced *in vitro*. These workers stressed that THP may not necessarily act as host defense component rather it may promote adhesion of uropathogens to epithelial cells promoting bacterial colonization.
Role of increasing concentration of calcium in the reduction of anti-adherence activity of Tamm-Horsfall protein was investigated by Sobota and Apicella, (1991). THP, at concentrations occurring in normal urine, was demonstrated to show anti-adherence activity for type-1 fimbriated *Escherichia coli*. Concentrations of calcium higher than those normally occurring in the urine significantly decreased the anti-adherence activity of THP. It was suggested that individuals with above normal concentrations of calcium in the urine might be at increased risk for urinary tract infections since the protective effect of THP was compromised.

Olczak *et al.*, (1999) carried out study to investigate alteration in composition of THP under different pathological conditions. These workers isolated THP from patients suffering from urinary tract infection, glomerulonephritis or interstitial nephritis and Bartter's syndrome (BS). Monosaccharide profile, N-glycan profile, THP reactivity with specific lectins and some other proteins was analyzed. It was observed that there were reduced levels of N-acetylgalactosamine in THP isolated from all the three disease conditions as indicated by decreased reactivity to *Phaseolus vulgaris* lectin. N-acetylglucosamine levels were reduced in THP isolated from urinary tract infection and glomerulonephritis or interstitial nephritis. In THP isolated from UTI patients there were reduced amounts of galactose and alpha 2, 6-linked sialic acid assessed by decreased reactivity to *Datura stramonium* lectin and *Sambucus nigra* lectin. In patients with BS there was a shift from tetrasialylated glycans towards less-sialylated chains. THP isolated from all the patients exhibited increased adherence to IgG1. These workers concluded there is alteration in composition of THP under different pathological and physiological conditions which is accompanied with variation in binding of THP to different uropathogens.
Akiyama et al., (2000) proposed that THP is a cytoprotective agent that protect the urothelium from cationic species. To test this hypothesis, these workers isolated THP from normal and interstitial cystitis urine to see if it could protect cultured cells from damage induced by the polyamine and protamine sulfate (PS). Toxicity of PS was significantly reduced by incubation with THP but not reduced by incubation with another protein, albumin. THP from patients having interstitial cystitis was less protective as compared to equal quantity of THP from normal urine. It was concluded that THP had an important role in bladder mucosal defense mechanisms, protecting the bladder surface from injury.

In vivo phagocytic activity of mouse peritoneal macrophages after treatment of bacteria with THP was examined by Bastos et al., (2001). At low THP concentrations (12.5 μg/ml and 50 μg/ml) no significant difference was observed in the phagocytosis of E.coli. However at high THP concentration (500 μg/ml and 1250 μg/ml) a reduction of bacterial phagocytosis by mouse peritoneal macrophages was observed.

Rooijen et al., (2001) carried out study to examine the patterns of the complex- and oligomannose-type glycans of uromodulin (Tamm-Horsfall glycoprotein) in the course of pregnancy. Uromodulin was isolated from urine of three pregnant women. Each batch of uromodulin was enzymatically N-deglycosylated and the released N-glycans were isolated, quantified and profiled by high-pH anion-exchange chromatography. It was observed that in the course of pregnancy no significant changes were detected in the negative charge distribution stemming from sialic acid and sulfate residues on the complex-type carbohydrate chains of uromodulin.

Pak et al., (2001) showed that THP was the principal urinary protein binding specifically to type-1 fimbriated E.coli. This binding
was highly specific and saturable and could be inhibited by D-mannose and abolished by endoglycosidase H treatment of THP, suggesting that binding was mediated by high mannose moieties of THP. It was species-conserved, occurring in both human and mouse THPs. Binding to THP was much greater with an \textit{E.coli} strain bearing a phenotypic variant of type-1 fimbrial Fim H adhesin, characteristic of those prevalent in UTI isolates hence concluding that THP contained conserved high mannose moieties capable of specific interaction with type-1 fimbriae. This major urinary glycoprotein was found to be a key urinary anti-adherence factor, which prevented type-1 fimbriated \textit{E.coli} from binding to the urothelial receptors.

Comparison of THP from human kidney with urinary THP using western blotting showed that molecular weight of kidney THP was greater than that of urinary THP (Cavallone et al., 2001). No difference in electrophoretic mobility of urinary and kidney THP was observed. This observation strongly suggested that a proteolytic cleavage at the juxtamembrane ectodomain of kidney THP was responsible for the urinary excretion.

Cavallone et al., (2002) compared two methods for purification of Tamm Horsfall glycoprotein, the method of Tamm and Horsfall (T and H method) and DEF method. Purity of THP preparations was evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and Western blotting developed with anti immunoglobulin G (IgG) antibodies and antichorionic gonadotropin antibodies. All THPs isolated by T and H method from proteinuric patients were contaminated by IgG and one of the five preparations from pregnant women contaminated by chorionic gonadotropin hormone. A smaller or no contamination was found in THPs isolated by diatomaceous earth filtration (DEF) method in which filtration of urine through a diatomaceous earth filter was carried out. They concluded that although albumin is the most
abundant protein in the anomalous urine, it never appear in THP preparations. The consistent contamination with IgG of THP prepared by salt precipitation-method might be related to the formation of a stable complex between the two proteins.

Rhodes, (2002) carried out study to characterize the interaction between THP and complement 1q to determine the robustness of this reaction under different environmental conditions. The influence of pH coupled with ionic strength was evaluated with an ELISA that demonstrated immobilized THP bound complement 1q strongly with a KD in the nM/L range from pH 9 to pH 5.5. Increasing the ionic strength from 10 mM/L sodium chloride (NaCl) to 154 mmol/L NaCl decreased the affinity of THP for complement 1q slightly (2-7-fold) at pH 9 to pH 6.5. These studies indicated that, compared to at pH 8.2, THP bound complement 1q at pH 5.8 with an almost two-fold higher affinity due to a faster association rate. The capacity of THP for complement 1q decreased significantly at pH 5.8, suggesting that a site for complement 1q binding to THP may be lost at acidic pH. Biosensor studies suggested that THP bound complement 1q at a site other than the region of its collagenous tail where C1r2 and C1s2 bind. Further it was demonstrated by western blot analysis that THP bound preferentially to the C chain of complement 1q.

A study was carried out study to investigate interaction of THP with Bordetella pertussis toxin. Mennozi et al., (2002) observed that THP bind to virulent strains of *Bordetella pertussis* and prevent its adherence to renal and pulmonary epithelial cells. However THP failed to bind mutant strains of *Bordetella pertussis* lacking functional pertussis toxin. These workers demonstrated that glycosylation (carbohydrate) moiety of THP is essential for binding of this molecule to THP. These workers concluded that THP not only possess anti-adherence activity but also has the ability to trap toxins produced by pathogens colonizing mucosal surfaces.
Carvalho et al., (2002) determined the behavior of Tamm Horsfall and uromodulin in calcium oxalate crystallization in vitro. They studied a group of 10 male stone formers who had formed at least one kidney stone composed of calcium oxalate. Ten normal men were used as controls. It was suggested that THP play a differential role, THP may act on nucleation and inhibit crystal aggregation, while uromodulin may promote aggregation of calcium oxalate crystals.

To determine the effects of THP on the protection of MCDK cells from oxalate induced free radical injury a study was carried by Hsieh et al., (2003). It was observed that as a free radical scavenger, THP reduced the amount of free radicals and provided significant protection at a critical concentration of 500 nM. The deglycosylated THP decreased the protection of the MDCK cells from oxalate-induced injury. It also increased adhesion of the calcium oxalate monohydrate crystals to the MDCK cells. It was concluded that effect of THP on the protection of oxalate induced radical injury may be partly due to its intact glycosylation and its adhesion to the cell membrane.

Cavallone et al., (2004) carried out study to characterize structural and functional differences between human THP (hTHP) and pig THP (pTHP). pTHP was found to contain a much higher proportion (47%) of Man5GlcNAc2 than hTHP (8%). In addition significant enhanced adherence of E. coli to pTHP was observed as compared to hTHP. Shorter mannose chains present in pTHP bound to Fim H receptor of E. coli with greater affinity than long mannose chains present in hTHP. hTHP was found to be more susceptible to leukocyte elastase hydrolysis than pTHP indicating that pTHP is much less prone to urinary degradation than hTHP. These workers concluded that variation in mannose chains lead to differential binding of THP to type 1-fimbriated E. coli leading to species specific variation in binding of THP to E. coli.
Role of THP in *E. coli* induced UTIs was elucidated by Bates *et al.*, (2004). These workers generated THP gene knockout mice (THP-/-) and compared ability of different *E. coli* strains possessing type I or P fimbriae to cause infection in this mice as well as in wild type mice (THP+/+). It was observed that THP-/- mice inoculated with type 1 fimbriated *E. coli* had a longer duration of bacteriuria, and more intense colonization of the urinary bladder indicated by significant increase in bacterial load in urine, bladder and renal tissue in comparison with THP+/+ mice. However no difference in renal bacterial load was observed in mice infected with P fimbriated strain of *E. coli*. These workers concluded that THP play an important role in host defense mechanisms by entrapping type I fimbriated *E. coli* and flushing them out with flow of urine.

Mo *et al.*, (2004) examined the *in vivo* role of Tamm-Horsfall protein (THP), in innate urinary defense. They genetically ablated the mouse THP gene and found that THP deficiency predisposes mice to bladder infections by type 1-fimbriated *E. coli*. In contrast, THP deficiency did not enhance the ability of P-fimbriated *E. coli* to colonize the bladder. They suggested that potential THP defects could predisposed the urinary bladder to *E. coli* infections.

Chakraborty *et al.*, (2004) compared the immunogold localization of THP in diabetic and control kidney tissue specimens with or without kidney damage. The majority of diabetic samples were found to have slightly lower degree of THP, while patients with known renal dysfunction had lowest THP. Their results indicated that decreased gold labeling is associated with known renal damage including damage to the thick ascending limb of the loop of Henle and the early distal convoluted tubule, irrespective of presence or absence of diabetes.
Shihabi et al., (2004) described high-performance liquid chromatography (HPLC) method for measurement of THP. Urine was mixed with 30% NaCl and left at 37°C for 30 min and then centrifuged. The precipitate was vortex-mixed and dissolved in a triethanolamine buffer. 20 μl aliquot was injected on a Macrosphere GPC column that was eluted with phosphate buffer and the effluent was detected by a fluorometer set at 280 nm for excitation and 325 nm for emission. It was found to elute the first peak.

Correlation between urinary excretion rate of Tamm-Horsfall protein (THP) is dependent on salt intake in healthy, genetically hypertension-prone individuals. This was investigated by Torffvit et al., (2004). Study subjects were put on a low-salt diet (10 mM of sodium and 70 mM of potassium per day) for 1 week. During the second week, sodium chloride capsules (230 mM/day) were added to the diet to achieve a high-salt intake of 240 mM/day. Urine samples (24-hour and overnight collections) were collected at the end of the high- and low-salt diet weeks. A low salt intake induced a decrease in the urinary excretion rate of THP during the night (11.7 μg/min) compared with baseline (19.5 μg/min; p < 0.05) and high salt intake increased the urinary excretion rate (23.1 μg/min; p < 0.01). These workers concluded that urinary excretion of THP is dependent on sodium intake. Patients with a high salt sensitivity responded to the high salt intake with an even greater increase in the urinary excretion rate of THP.

Wimmer et al., (2004) investigated the effects of THP in its soluble form on distinct polymorphonuclear leukocyte (PMNL) functions. PMNL were isolated from the venous blood of healthy adult donors and incubated at low THP concentrations (70-350 ng/ml), resembling plasma concentrations, and at high THP concentrations (1.75-8.75 μg/ml), resembling urinary concentrations. These results showed that high (urinary) THP concentrations inhibited PMNL
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apoptosis and chemotaxis and stimulated PMNL phagocytosis, while low (plasma) THP concentrations increased PMNL chemotaxis. They concluded that THP influences several PMNL functions, suggesting a crucial immunomodulatory role for this glycoprotein in host defence mechanisms of the kidney and genitourinary tract.

Tamm-Horsfall protein was reported as multilayered defense molecule against urinary tract infection by Saemann et al., (2005). The evolutionary conserved Tamm-Horsfall protein (THP) was found to be capable of both mediating direct antimicrobial activity and alerting immune cells. The study indicated that beside antimicrobial activity, THP also exerted potent immunoregulatory activity. Furthermore, genetic ablation of THP gene led to severe infection and lethal pyelonephritis in an experimental model of UTI. Recent data demonstrated that THP linked the innate immune response with specific THP-directed cell-mediated immunity. Role of THP as a specialized defense molecule was discussed and it was proposed that THP integrated model of protective mechanisms against UTI. It acted by two principle nonmutually exclusive mechanisms involving the capture of potentially dangerous microbes and induction of robust protective immune responses against uropathogenic bacteria.

Dou et al., (2005) carried out study to investigate the consequences of deletion of cyclooxygenase-2 (COX-2) gene in mice. Using cDNA microarrays analysis these workers demonstrated that in COX-2 -/- mice the genes encoding pre-pro-epidermal growth factor (pre-pro-EGF) and Tamm-Horsfall protein (THP)/uromodulin were aberrantly expressed in the kidneys. It was observed that in renal tissue of COX-2 -/- mice, levels of mRNA coding for THP were severely reduced assessed by Western blot and immunohistological methods followed by corresponding decrease in levels of THP excreted in urine. To assess the outcome of decreased expression of THP in COX-2 -/- mice, these workers established ascending urinary tract infection in
mice with *E. coli*. It was observed that bladders of COX-2 -/- mice were highly susceptible to colonization by *E. coli* as compared to wild type mice. These workers concluded that THP is an important component of host defense and deficiency of this molecule predisposes the host to UTIs.

**Reactive nitrogen intermediates (RNI), Reactive oxygen species (ROS) and Myeloperoxidase (MPO)**

As early as 1959, Sbarra and Karnovsky reported that phagocytosis of microorganisms by granulocytes is accompanied by a dramatic increase in oxygen consumption which is not cytochrome linked. They also found that there is an increase in oxidation of glucose through the hexose monophosphate shunt following phagocytosis.

Tsujimura *et al.*, (1980) assessed the possible cause of the excessive increase in the number of polymorphonuclear leukocytes in the urine of patients suffering from urinary tract infections in regard to the chemotactic factors for the leukocyte. These workers separated urinary protein fractions of 2 controls and 10 patients with urinary tract infections by gel filtration on a Sephadex G-50 column, and tested chemotactic activity *in vitro* by using Boyden's apparatus and *in vivo* by injecting the protein fractions into guinea pig skins. Chemotactic activity was found in the urinary protein fractions of 6 patients. The fractions of these 6 patients showed typical infiltration of polymorphonuclear leukocytes in the local tissue of guinea pigs. A high correlation was found between the activity and the leukocyte increase, although there was no relation between the type of infecting bacteria and chemotactic activity. The data suggested that the marked increase in the number of urinary leukocytes is attributable to the chemotactic factors in the urine.
Shah et al., (1983) studied the relationship between NADPH induced chemiluminescence (CL) (a measure of respiratory burst) and lipid peroxidation (a measure of tissue injury) in kidney microsomes and found that peak value of CL positively correlated the lipid peroxidation suggesting the role of products of respiratory burst in tissue injury. They further examined the role of reactive oxygen radicals in these processes by utilizing several scavengers of ROS like catalase. They found that in presence of catalase (250μg/ml) there was 14% decrease in CL and 11% decrease in lipid peroxidation.

Effect of various oxygen free radical scavengers in preventing tissue injury caused by *Escherichia coli* in pyelonephritic mice was studied by Kaur et al., (1988). These workers found a significant decrease in the levels of malondialdehyde formation in the mice treated with free radical scavengers like superoxide dismutase, dimethylsulfoxide and allopurinol even in the presence of infection. Renal brush border membrane enzymes activity was found to be decreased which was used as markers of renal tissue damage in these groups.

Meylan et al., (1989) studied the role of PMN oxidative metabolism in generating tissue injury during acute pyelonephritis in rats. For this they treated the rats with dapsone, a compound that had no antibacterial effect but is known to prevent PMN oxidant damage due to the extracellular release of the myeloperoxidase system by activated PMN during acute suppurative pyelonephritis. Dapsone inhibited myeloperoxidase-mediated reactions. Dapsone-treated animals showed 65% reduction in renal scar formation as compared to controls when sacrificed after 2 months of acute pyelonephritis.

Cu et al., (1990) carried out study to investigate the effect of iron limitation on generation of ROS by PMNs. Decrease in generation of ROS was observed by PMNs isolated from patients suffering from
UTIs. It was suggested that oral iron supplementation could effectively improve the impaired PMN ROS generation.

The interaction of human neutrophils with *P. aeruginosa* biofilms was examined by Jensen *et al.*, (1990) using a chemiluminescence assay. The biofilms induced an oxidative burst response by polymorphonuclear leukocytes which was slow and only 25% of the response was induced by planktonic bacteria. It was suggested that reduced response to *P. aeruginosa* biofilms could play a role in the persistence of bacteria in chronic infections.

The ability of urinary PMNs to generate active oxygen species was assessed by Kataoka and Fujita, (1991). The ability of urinary PMNs to generate active oxygen species (AO) was found to be significantly inhibited as compared to that of PMNs in blood, and this suppression was observed to be time-dependent. PMNs were affected significantly by osmolarity of reaction medium. PMNs exposed to hyposmotic medium generated active oxygen, whereas PMNs exposed to hyperosmotic medium did not generate active oxygen. These results suggested that despite of the regulation of the ability of urinary PMNs by urine osmolarity, the urinary PMNs remain to be primed and maintain bactericidal activity.

The effect of pyocyanine, a pigment produced by *Pseudomonas aeruginosa*, was investigated by Shellito *et al.*, (1992) on production of reactive nitrogen intermediates by macrophages. They found that addition of pyocyanine to cultures of murine alveolar macrophages inhibited the capacity of these cells to produce reactive nitrogen intermediates (measured as nitrite) in a dose-dependent manner without altering cell viability, cytokine-induced Ia expression, or production of tumor necrosis factor.

Smith *et al.*, (1994) measured urinary nitrite levels by the Griess method and assayed urinary nitric oxide (NO) synthase activity. NO
synthase activity was significantly higher in soluble and particulate fractions of urine obtained from patients suffering from UTIs. It was concluded that human infected urine contained an isoform of NO synthase which is an endogenous source of urine nitrite.

Role of ROS in pathogenesis of pyelonephritis in BALB/c mice was demonstrated by Gupta et al., (1996). A clear correlation between extent of ROS generation and subsequent lipid peroxidation and DNA damage in kidneys was observed during the course of infection, from 2 to 14 days. Administration of antioxidants, superoxide dismutase, catalase and dimethylsulfoxide significantly reversed the histopathological changes, reduced the extent of lipid peroxidation in renal brush border membrane, and also reversed the altered enzyme activities to near normal situation. These results suggested that interaction of ROS with various cellular organelles in kidneys has a significant deleterious effect, and this could be the underlying mechanism for renal dysfunction in pyelonephritis.

Mathy-Hartert et al., (1996) studied the effect of myeloperoxidase uptake on cytocidal activity against *Pseudomonas aeruginosa*. It was concluded that loading macrophages with exogenous MPO could enhance their microbicidal activity, thereby suggesting a potential useful therapeutic application.

Impairment of Cl-/H₂O₂/MPO-mediated bacterial killing of neutrophil by cyanate formed from urea was thought to be one of the predisposing factor leading to increased susceptibility of uraemic patients to UTI. This was shown in the study of Qian et al., (1997). It was observed that cyanate inhibits both peroxidative and halogenating activities of MPO and also inhibits the enzyme within intact human neutrophils. Inhibition was observed to be H₂O₂-dependent and irreversible.
Wheeler et al., (1997) demonstrated increase in nitric oxide synthase activity in neutrophil-enriched fractions from urine of patients with UTIs compared to that in neutrophil-enriched fractions from noninfected controls. Partially purified inducible nitric oxide synthase was membrane associated, calcium independent, and inhibited by arginine analogues. Elevated inducible nitric oxide synthase activity and elevated nitric oxide synthase protein measured in patients with urinary tract infections and treated with antibiotics does not decrease until 6-10 days of antibiotic treatment. The extended elevation of neutrophil inducible nitric oxide synthase during urinary tract infections may have both antimicrobial and proinflammatory functions.

Dowling et al., (1998) studied the effect of nitric oxide synthase (NOS) inhibitor asymmetric dimethyl ariginine (ADMA) and the inactive enantiomer N\(^\text{6}\)-methyl D- ariginine (D-NMMA) on *P. aeruginosa* infection of the respiratory mucosa in nasal turbinate organ cultures. Results of their study suggested that NO may be a mediator of epithelial damage caused by *P. aeruginosa*.

Levels of free oxygen radicals, blood urea, serum creatinine, serum protein and uric acid were estimated in acute renal failure by Dubey et al., (2000). Raised levels of enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) and lipid peroxide (LPO) as estimated in blood showed that these enzymes could be used as markers of renal injury.

Oh-oka et al., (2001) examined the involvement of reactive oxygen species (ROS) and myeloperoxidase (MPO) in bacterial killing by human polymorphonuclear leukocytes (PMN) by means of a chemiluminescence assay. Using one standard strain and three uroisolates of *Pseudomonas aeruginosa*, they examined whether the production of ROS and changes in MPO activity altered the number of...
bacteria in contact with PMN. They concluded that MPO-dependent processes were strongly favored by human PMN for the oxidative killing of bacteria.

The cellular localization and time course of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) expression in the mouse bladder and kidney tissues after intravesical inoculation with uropathogenic *E. coli* was investigated by Poljakovic *et al.*, (2001). Significant enhancement in nitrite levels in urine was observed at 6 and 12 hours postbacterial instillation. Bladders from mice instilled with *E. coli* showed a large number of iNOS and COX-2 expressing inflammatory cells. The inflammatory cell activation peaked at 6 and 12 hours postinstillation and vanished by 72 hours. iNOS expression was detected in some urothelial cells after 24 and 72 hours, but COX-2 expression was not detected. In the kidney, infection activated an iNOS and COX-2 response, as shown by immunoreactivity in inflammatory cells at all time points. A strong epithelial iNOS response was observed in the renal pelvis at 12, 24, and 72 hours postinstillation, but COX-2 was not detected. Enhanced tissue expression of iNOS and COX-2 after bacterial instillation was also demonstrable by RT-PCR. It was suggested that epithelial iNOS response is not caused by direct bacterial activation, but more likely is by mediators involved in the inflammatory response.

Vaziri *et al.*, (2002) tested the hypothesis that chronic renal failure (CRF) result in enhanced ROS-mediated NO inactivation and protein nitration which can be ameliorated with antioxidant therapy. CRF resulted in marked elevations of blood pressure, plasma MDA, and tissue nitrotyrosine abundance, but did not change plasma L-arginine level. This was coupled with depressed vascular tissue NO production and reduced immunodetectable NOS proteins in the vascular, renal, and cardiac tissues. Antioxidant therapy ameliorated the CRF-induced hypertension, improved vascular tissue NO
production, lowered tissue nitrotyrosine burden, and reversed downregulations of NOS isoforms. In contrast, antioxidant therapy had no effects in the controls. CRF was associated with oxidative stress which promote NO inactivation by ROS leading to functional NO deficiency, hypertension, and widespread accumulation of protein nitration products. It was suggested that amelioration of oxidative stress by high-dose vitamin E enhance NO availability, improve hypertension, lower protein nitration products, and increase NOS expression and vascular NO production in CRF animals.

Characterization of the interaction of human neutrophils with biofilms of \textit{P. aeruginosa} with regard to morphology, oxygen consumption, phagocytosis, and degranulation was carried out by Jesaitis et al., (2003). Scanning electron and confocal laser microscopy indicated that the neutrophils retained a round, unpolarized, unstimulated morphology when exposed to \textit{P. aeruginosa} biofilms. However, transmission electron microscopy demonstrated that neutrophils, although rounded on their dorsal side, were phagocytically active with moderate membrane rearrangement on their bacteria-adjacent surfaces. The settling of neutrophils on biofilms caused an increase in oxygen consumption apparently resulting from a combination of a bacterial respiration and escape response and the neutrophil respiratory burst but with little increase in the soluble concentration of H$_2$O$_2$. Results of this study indicated that host’s defense become compromised as biofilm bacteria escape while neutrophils remain immobilized with a diminished oxidative potential.

Role of iNOS in \textit{Escherichia coli}-induced urinary tract infection (UTI) was evaluated by Poljakovic and Persson, (2003) using iNOS-deficient mice. Increase in urine nitrite levels was observed following infection in iNOS$^{+/−}$ but not in iNOS$^{−/−}$ mice. Results of this study indicated that wild-type and iNOS-deficient mice were equally
susceptible to \textit{E. coli}-induced UTI and the outcome of NO toxicity to \textit{E. coli} may depend on bacterial virulence. Furthermore, myeloperoxidase may contribute to nitrotyrosine formation in the absence of iNOS.

Poljakovic \textit{et al.}, (2005) studied the effect of proinflammatory cytokines on the host iNOS response and the influence of uropathogenic \textit{E. coli} strains with different fimbrial expression. It was observed that \textit{E. coli} induced NO production and iNOS mRNA in kidney epithelial cells but only in presence of nuclear factor-kappaB (NF-kappaB). Proinflammatory cytokines namely IL-6, IL-8 and transforming growth factor-\(\alpha\) did not affect NO production in A498 cells. It was concluded that uropathogenic bacteria are weak inducers of human uroepithelial iNOS, which may be related to insufficient binding of NF-kappaB to iNOS promoter.

Watt \textit{et al.}, (2005) assessed the relationship between levels of neutrophil apoptosis and sputum microbiology in cystic fibrosis (CF) patients. Patients infected with \textit{P. aeruginosa} had a significantly lower percentage of viable neutrophils and significantly higher levels of secondary necrotic granulocytes in the sputum than those with no Gram-negative infection. Number of neutrophils in \textit{P. aeruginosa} infected patients was significantly higher than in the group with no Gram negative infection. These results suggested that cell death and clearance may be altered in CF patients colonized with \textit{P. aeruginosa}.

Walker \textit{et al.}, (2005) reported that neutrophils enhanced biofilm formation by \textit{P. aeruginosa} through formation of polymers composed of actin and DNA. Significant enhancement in biofilm formation was observed following growth of \textit{P. aeruginosa} in presence of purified neutrophil components like actin. The results of this study brought out that pathogens can exploit host innate immune defense for its own survival. When host fail to eradicate the infection, cellular
components from necrotic neutrophils can serve as matrix for biofilm formation by *P. aeruginosa*.

**MSPs and Cytokines**

The invading organism while trying to establish in the urinary tract comes in contact with phagocytes which include macrophages (Stuart and Ezekowitz, 2005; Blasi *et al.*, 2005; Nakanishi, 2006). Macrophages form an important component of the initial line of host’s defense (Aderem and Underhill, 1999; Stevans, 2005; Minakami *et al.*, 2006; Yamamoto and Takaya, 2006). These cells are specialized phagocytes that play an important role in clearance of effete host cells and molecules as well as in immune defense against foreign invasion and infection (Gordon *et al.*, 2002; Janeway and Medzhitov, 2002; Jutras and Desjardins, 2005). Nathan, (1987) reviewed that macrophages of tissues congregate in most acute and chronic inflammatory reactions. They respond to antigenic stimuli and secrete a range of over 100 substances, which vary in their biological activities ranging from induction of cell growth to cell death. Secretory products of macrophages include peptide hormones, complement components, enzymes, bioactive oligopeptides and lipids, reactive oxygen and nitrogen species and other biological substances. The principal constituents of macrophage secretory products (MSPs) are cytokines, which include mainly tumor necrosis factor (TNF)-α, TNF-β, Interleukin (IL)-1, Interferon (IFN)-α, IL-12, IL-16 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Nathan, 1987; Mattana and Singhal, 1995; Mattana *et al.*, 1995; Bosca *et al.*, 2005).

Rugo *et al.*, (1992) evaluated local production of cytokines like IL-1 IL-6, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), TNF-α, TNF-β, IFN-γ, transforming growth factor (TGF beta), and cytokine synthesis inhibitory factor (CSIF)/IL-10 in kidneys, draining lymph nodes, and
spleens in a murine model of pyelonephritis. It was observed that mRNA encoding IL-1, IL-6, G-CSF, GM-CSF, TNF alpha, H400 (a protein homologous to a family of chemotactic factors and identical to MIP-1 beta), and CSIF/IL-10 in the kidney at 12 h and 1, 2, and 3 day postinfection time period. However mRNA for these cytokines was not observable after 5th day postinfection as well as in normal mice. These cytokines were not detectable in other tissues indicating localized production of these cytokines. By immunohistochemical staining these workers demonstrated that IL-6 is produced by mesengial cells of renal tissue in response to infection. It was concluded that in urinary tract there is a time course production of cytokines following infection which may help host to combat infections.

Local immune responses in mice with an impaired chemotaxis of neutrophils in *P. aeruginosa* induced UTIs was studied by Yokoo et al., (1993). Markedly increased susceptibility to bacterial infection was observed when experimental ascending urinary tract infection was induced with *P. aeruginosa* in mice with an impaired chemotaxis of neutrophils as compared to normal mice. On the contrary significant increase in macrophage infiltration was observed in mice with an impaired chemotaxis of neutrophils as compared to control. In addition levels of T and B cells were also enhanced in this mice. It was suggested that when neutrophil infiltration is impaired other immune cells compensate this deficiency in order to control infection.

Production of cytokines by bladder and kidney epithelial cell lines as well as by human peripheral blood monocytes exposed to *E. coli* was studied by Agace et al., (1993). Bladder epithelial cells stained positive for IL-6 and IL-8 with peak production at 2 hour and IL-1 alpha showing peak at 6 hour. Kidney cell line showed production of IL-8 with peak at 2 hour and IL-6 with peak at 6 hour. Peripheral blood monocytes stained for the cytokines IL-1α, IL-1β, IL-8, IL-6, and
TNF-α but not for TNF-β and GM-CSF after stimulation with *E. coli*. This showed that epithelial cells produce cytokines following stimulation with pathogens which may play role in limiting microbial growth.

Ko *et al.*, (1993) investigated role of IL-8 in pyruia in patients suffering from UTIs. These workers observed that almost all the patients had significantly higher levels of IL-8 in their urine as compared to normal individuals in whose urine IL-8 was not detectable indicating local production of this chemokine. Urine IL-8 levels correlated with PMN recruitment into urinary tract. Treatment of patients with anti IL-8 antibody abrogated the influx of neutrophils. These results showed that IL-8 is involved in migration of PMN into urinary tract and development of pyuria.

Role of adherence of *E. coli* to mucosal cytokine generation was evaluated by Svanborg *et al.*, (1994). Adherence of *E. coli* to uroepithelial cells lead to production of cytokines IL-6 and IL-8. IL-6 caused the fever and acute phase response that may accompany systemic urinary tract infections whereas IL-8 lead to neutrophil recruitment. This indicated that bacterial adherence lead to stimulation of epithelial cells leading to secretion of cytokines.

Nickolic-Paterson *et al.*, (1996) demonstrated that cytokines like IL-1, IL-6, GM-CSF, TNF-α, TGF-β and IL-10 play an important role in fibrotic response. These cytokines stimulated leukocyte recruitment at sites of inflammation by inducing synthesis of chemotactic molecules such as monocyte chemoattractant protein-1 (MCP-1). Furthermore, these cytokines induced production of ROS, nitric oxide, eicosanoids and platelet activating factor, which could mediate tissue injury. Thus, they participate in tissue fibrosis by promoting the deposition of extracellular matrix.
Sharma et al., (1996) studied interaction of macrophages with *E. coli* in cell lines *in vitro*. These workers observed increased expression of tumour growth factor-β (TGF-β) in mesengial cells exposed to *E. coli* treated macrophage culture supernatant. It was suggested that macrophage secretory products play an important role in development of chronic glomerular lesions by enhancing synthesis of TGF-β leading to increased synthesis of laminin.

Agace (1996) reported that deliberate colonization of the human urinary tract with *E. coli* induced the local production of IL-8 and levels correlated with urinary neutrophil numbers suggesting a role for IL-8 in neutrophil migration. *In vitro* in urinary tract epithelial cell lines, *E. coli* induced transepithelial neutrophil migration and this process was blocked with anti-IL-8 antibody. These results suggested that interaction of *E. coli* with epithelial cells lead to generation of cytokines which help in neutrophil migration into urinary tract.

Benson *et al.*, (1996) observed that urine IL-6 levels were higher in girls than in boys suffering from urinary tract infections. IL-6 levels increased with age and were positively associated with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum IL-6, and urine leukocyte counts. On the other hand, urine IL-8 response was not influenced by age, but it was influenced by P-fimbriae and was associated with ESR, CRP, urine leukocytes and female sex. These results showed that cytokine responses to urinary tract infection vary with the severity of infection and that cytokine activation is influenced by a variety of host and bacterial variables.

Production of cytokines following induction of pyelonephritis with *E. coli* in mouse model of ascending UTI established following obstruction was investigated by Khalil *et al.*, (1997). Kidney cells were found to express higher levels of IL-1, IL-6 and TNF-alpha mRNA at 12th postinfection hour and persisted till 6th postinfection day. A
marked increase in levels of IL-4, IL-10, TGF-beta and IFN-gamma mRNA was also observed in kidney cells. Un-infected obstructed mice also exhibited production of all these cytokines but levels were significantly lower as compared to infected obstructed experimental animals. Splenocytes showed maximum production of mRNA for IL-1 followed by mRNA for IL-6, TNF-alpha, IL-4, IL-10, IFN-gamma and TGF-beta. These showed that urinary tract produce a variety of inflammatory and immunoregulatory cytokines following infection with uropathogens some of which may be contributing to process of tissue damage and post-infectious renal scarring.

Kernacki et al., (2000) examined role of MIP-2 in neutrophil recruitment into corneal tissue following infection with *P. aeruginosa* and contribution of these inflammatory mediators to tissue pathology. These workers observed that initially from 1st day to 3rd day postinfection time period the number of neutrophils in corneal tissue assessed by myeloperoxidase assay were same in resistant and susceptible mice. However on 5th and 7th postinfection day, number of neutrophils in corneal tissue of experimental animals was more in susceptible mice in comparison to resistant mice. A correlation between MIP-2 production and neutrophil recruitment was observed. Administration of recombinant MIP-2 (rMIP-2) to resistant mice resulted in significant neutrophil influx leading to significantly exacerbated corneal disease. On the contrary, treatment of susceptible mice with neutralizing MIP-2 polyclonal antibody lead to decreased neutrophil influx and decreased corneal tissue damage. These workers concluded that exaggerated innate immune responses during infection contribute to tissue damage and timely down-regulation of host inflammatory response is essential for resolution of infection.

Kinetics of IL-1 production by *P. aeruginosa* in ocular infections was evaluated by Rudner et al., (2000) using susceptible (B6) and resistant (BALB/c) strains of mice. Significantly greater amounts of
both IL-1α and IL-1β was observed in B6 mice as compared to BALB/c mice on 1st and 3rd postinfection day. Decrease in production of IL-1 was observed in BALB/c whereas levels remain elevated in B6 mice on 5th postinfection day. Treatment of B6 mice with polyclonal neutralizing antibody against IL-1beta significantly reduced corneal disease accompanied with reduction in corneal PMN number, bacterial load and MIP-2. It was suggested that optimal production of IL-1 help in clearing infection without damaging cornea whereas higher production of IL-1 caused tissue damage mediated through increased production of MIP-2 and corresponding increased infiltration of PMNs into corneal tissue.

Jantausch et al., (2000) reported production of urinary IL-6 (UIL-6) and urinary IL-8 (UIL-8) in patients suffering from urinary tract infections. Levels of both the cytokines were elevated in patients infected with hemolysin producing strains of E. coli. A correlation between production of these cytokines with urine white blood cells (WBC) was observed. This showed that UIL-6 and UIL-8 may play a role in mounting inflammatory response in patents suffering from urinary tract infections.

Olszyna et al., (2001) carried out study to investigate contribution of CXC chemokine receptor 2 to host defense in an experimental model of urinary tract infection. These workers treated mice with an antibody against the major CXC chemokine receptor in the mouse, CXCR2, before infection with Escherichia coli. It was observed that administration of antibody prevented influx of neutrophils into urine and kidneys. These workers suggested that CXC chemokines play an important in mounting inflammatory response following infection.

Godaly et al., (2001) reported that E. coli stimulate chemokine response in uroepithelial cells and chemokine repertoire depend on
the bacterial virulence factors as well as on the specific signaling pathways that they activate. CXCR1 and IL-8 direct neutrophil migration across the epithelial barrier into the lumen. mIL-8Rh knockout mice showed impaired transepithelial neutrophil migration with tissue accumulation of neutrophils leading to renal scarring. These mice also exhibited impaired clearance of bacteria from renal tissue and developed acute pyelonephritis with bacteremia. These results bring out that cytokines help in neutrophil influx into urinary tract which may help in clearing infections.

Gene expression of cytokines like IL-1, IL-6, MIP-2, TNF-α and KC in ocular infections induced with three different phenotypes (invasive, cytotoxic and contact lens induced acute red eye strains, CLARE) of *P. aeruginosa* was investigated by Xue et al., (2002). Ocular infection with the invasive strain induced the highest levels of IL-1 beta, IL-6, MIP-2 and KC mRNA, followed by the infection with the cytotoxic strain. Ocular infection with the CLARE strain induced the lowest levels of IL-1 beta, IL-6, MIP-2 and KC mRNA. The expression of TNF-alpha mRNA was very low and irregular following *P. aeruginosa* challenge. It was concluded that overexpression of proinflammatory cytokines caused exaggerated immune response leading to tissue damage in corneal infections.

Cole et al., (2003) found that in IL-10 deficient mice there was a significant decrease in bacterial load in corneas at 7 days postchallenge with *P. aeruginosa*. This decrease was accompanied by a reduction in neutrophil numbers in the cornea and changes in cytokine levels compared to those of wild-type mice. A characteristic increase in neovascularization in the cornea was found in IL-10 deficient mice. This increased angiogenesis correlated with an increased expression of KC, whereas the kinetics of macrophage inflammatory peptide 2 expression correlated with neutrophil numbers. The findings of this study suggested important role of IL-10
in *P. aeruginosa* induced keratitis by regulating the expression of proinflammatory cytokines.

Role of macrophages in *P. aeruginosa* corneal infections was studied by McClellan *et al.*, (2003). Macrophages were depleted in both susceptible and resistant strains of mice using clodronate-containing liposomes before corneal infection. Both the strains of mice exhibited more severe corneal disease following macrophage depletion as compared to controls. This was associated with increase in levels of MIP-2, IL-1β, IFN-γ and MIP-1α and corresponding enhanced levels of PMNs at 3rd and 5th postinfection days. On the other hand decrease in production of IL-10 was observed. Significant increase in bacterial load was observed in BALB/c mice at 3rd and 5th postinfection day whereas this increase was observed only on 5th postinfection day in case of B6 mice as compared to control. This showed that macrophages play a key role in deciding ultimate outcome of *P. aeruginosa* induced corneal infections by regulating PMN number, bacterial killing and balancing pro- and anti-inflammatory cytokine levels.

Xue *et al.*, (2003) elucidated the expression of chemokines, their role and regulation in bacterial corneal infection using three types of *P. aeruginosa* strains - invasive, cytotoxic and contact lens induced acute red eye strains. MIP-2 was found to be most predominant chemokine produced following infection with all three types of strains. Prolonged expression of MIP-2 was correlated with neutrophil influx into corneal tissue leading to enhanced inflammation. Administration of anti MIP-2 antibody was able to reduce MIP-2 levels and neutrophil influx however it failed to reduce bacterial load. Neutralization of IL-1beta activity alone using monoclonal antibodies lead to significant reduction in MIP-2 levels indicating that chemokine levels were regulated by IL-1beta. These workers concluded that MIP-2 play a significant role in development of *P. aeruginosa* induced keratitis and
downregulation of this chemokine may help in preventing tissue damage.

Smithson et al., (2005) assessed the surface expression of IL-8 receptors, CXCR1 and CXCR2 and the existence of polymorphisms in the CXCR1 gene in premenopausal women with recurrent UTIs. The levels of CXCR1 and CXCR2 expression on neutrophils were measured and analyzed by flow cytometry by measuring the mean fluorescence intensity (MFI) channel. The promoter and coding regions of the CXCR1 gene were analyzed for the presence of polymorphisms by a sequence-based typing method. No difference in levels of CXCR1 expression was observed whereas CXCR2 levels were lower in patients with recurrent UTIs as compared to in patients without previous UTIs (control). No polymorphisms were detected in CXCR1 gene either in the patients or in the controls. These results suggested that a low level of CXCR2 expression may increase the susceptibilities of premenopausal women to urinary tract infections.

Allen et al., (2005) evaluated effect of P. aeruginosa pyocyanin on neutrophil infiltration in a murine model of acute pneumonia. Peak production of neutrophil was observed at 18th postinfection hour following intratracheal instillation of wild type strain of P. aeruginosa. On the contrary, neutrophil numbers increased up to 48 h in mice infected with pyocyanin-deficient strain of P. aeruginosa. Bacterial load was significantly higher in mice infected with parent strain as compared to in mice infected with mutant strains. In addition, increased neutrophil apoptosis was observed in mice infected with wild-type compared with the phenazine-deficient strain or two other strains that lack pyocyanan production, but produce other phenazines. Levels of chemokines (MIP-2, KC) and cytokines (IL-6, IL-1beta) were significantly reduced in mice following infection with parent strain with phenazine-deficient strain-infected mice at 18 h. These results indicated that P. aeruginosa pyocyanin downregulates inflammatory processes by accelerating neutrophil apoptosis thus promoting persistence of P. aeruginosa in host's tissues.