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REVIEW

Urinary tract infections caused by *Pseudomonas aeruginosa*: A minireview

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Summary Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. Infections of the urinary tract are the second most common type of infection in the body. Catheterization of the urinary tract is the most common factor, which predisposes the host to these infections. Catheter-associated UTI (CAUTI) is responsible for 40% of nosocomial infections, making it the most common cause of nosocomial infection. CAUTI accounts for more than 1 million cases in hospitals and nursing homes annually and often involve uropathogens other than *Escherichia coli*. While the epidemiology and pathogenic mechanisms of uropathogenic *Escherichia coli* have been extensively studied, little is known about the pathogenesis of UTIs caused by other organisms like *Pseudomonas aeruginosa*. Scanty available information regarding pathogenesis of UTIs caused by *P. aeruginosa* is an important bottleneck in developing effective preventive approaches. The aim of this review is to summarize some of the advances made in the field of *P. aeruginosa* induced UTIs and draws attention of the workers that more basic research at the level of pathogenesis is needed so that novel strategies can be designed.

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Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span [1,2]. UTIs account for more than 8 million visits to physician's offices, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually [3,4]. UTIs are the second most common infection of any organ system and the most common urological disease in the United States, with a total annual cost of more than $3.5 billion [5]. These infections are more common in females than in men. Incidence in women in the age of 20—40 years ranges from 25 to 30% whereas in older women above 60 years of age it ranges from 4 to 43% [6—8]. UTIs can be classified as uncomplicated or complicated [9,10]. The recognized predisposing factors in complicated UTIs are anatomic defects, vesicouretic reflux (VUR), obstruction, surgery, metabolic diseases like diabetes mellitus and generalized immunosuppression especially in patients of organ transplant [11—16]. Catheterization of urinary tract is one of the most common factor which predisposes the host to complicated UTIs [17—20]. Instillation of catheter may lead to damage of mucosal layer, which disrupts the natural barrier and allows bacterial colonization [21]. Organisms can gain entry via extraluminal route [22] by moving across the outer lumen of catheter or by intraluminal route by directly entering the interior of catheter [23].

The organisms most commonly responsible for catheter-associated UTIs are *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis* [6,24—26]. In case of *E. coli*, the epidemiological, experimental and clinical studies have established the role of multiple virulence factors of *E. coli* like adhesins operative through type-I fimbriae and P fimbriae, O serotypes, K1 capsule, serum resistance, hemolysins, cytotoxic nectrotizing factor (CNF) and siderophores (enterochelin and aerobactin) in relation to uncomplicated and complicated UTIs [2,27]. However, there is paucity of literature in relation to pathogenesis of UTIs caused by *P. aeruginosa*. Despite advances in antimicrobial therapy, the mortality and morbidity associated with *P. aeruginosa* induced UTIs remain significantly high. This unfavorable outcome is due to our inability to develop therapeutic strategies to prevent the disease which in turn is due to incomplete understanding about the pathogenesis of the disease. The aim of this review is to highlight some of the most important advances in understanding the pathogenesis of *P. aeruginosa* induced UTIs.

Virulence factors of uropathogenic *P. aeruginosa*

P. aeruginosa is the third most common pathogen associated with hospital-acquired catheter-associated UTIs [6]. Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell-associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores [28—31]. These factors have been shown to play an important role in pathogenesis of *P. aeruginosa* induced infections like respiratory tract infections, burn wound infections and keratitis [32—36]. However, limited reports are available regarding role of these virulence traits in urinary tract infections. Woods et al. [36] showed high production of elastase and protease in strains isolated from urinary tract infections in comparison to isolates from other infections like burn wounds infection, skin wound infection and acute pneumonia. Quantitative analysis of elastase, phospholipase C, toxin A, and exoenzyme S was assessed in *P. aeruginosa* strains isolated from wound infections, respiratory tract infections and urinary tract infections by Hamood et al. [37] 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

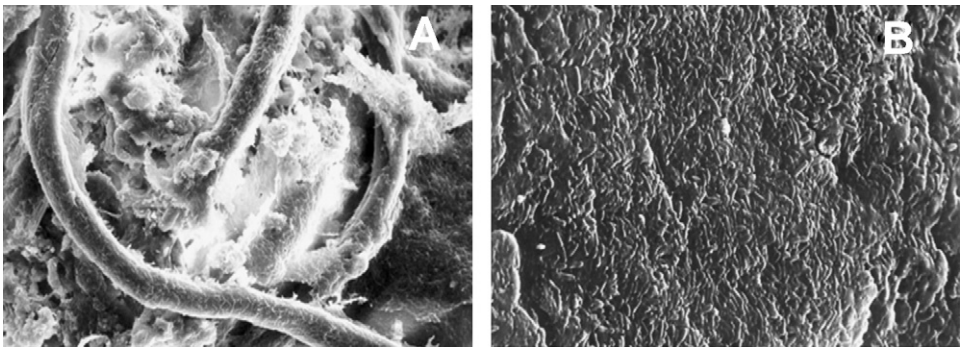

Figure 1 Photograph showing complete encrustation of urinary catheter by biofilms of *P. aeruginosa* (A) and a higher magnification showing rod shaped bacteria on the surface of catheter (B).

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. Ciragil and Soyletir [38] investigated 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*. Visca et al. [39] 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. We reported that uroisolates of *P. aeruginosa* produce high levels of alginate, siderophores, exoenzymes and hemolysin [40]. However uroisolates possessing high hemolytic property showed significantly high renal bacterial counts and marked tissue damage compared to low producers indicating direct association between hemolysin production and renal colonization. It was suggested that besides considering levels of all extracellular enzymes, high levels of hemolysin production *in vitro* could be used as surrogate information for assessing pyelonephritic potential of *P. aeruginosa*. Further studies employing mutant strains of *P. aeruginosa* defective in hemolysin production are required to elucidate the precise contribution of this virulence trait to the incidence of UTIs.

Biofilm formation by *P. aeruginosa*

In addition to elaboration of virulence factors, *P. aeruginosa* has a tendency to form biofilms on the surface of urinary catheters. Growth of *P. aeruginosa* begins in the form of microcolonies, which later coalesce together to form biofilms (Fig. 1) [41—43]. Alginate, which is an acetylated polymer of beta-D-mannouronic acid and alpha-L-guluronic acids, is the most important component of *P. aeruginosa* biofilms. However, some other exopolysaccharides like psl and pel have also been shown to play an important role in biofilm forming ability of non-alginate producing strains of *P. aeruginosa* [44,45]. Psl is a mannose-rich and galactose-rich polysaccharide, however the precise Psl structure has not been elucidated [46—50]. This is an area requiring future research. As with Psl, the Pel structure is unknown and further biochemical analyses of Pel polysaccharide is necessary [51]. Biofilms are resistant to antimicrobial agents as well as to host defense mechanisms and hence are difficult to eradicate. Biofilms contribute towards pathogenicity of *P. aeruginosa* as these often lead to persistent and recurrent infections [52—54].

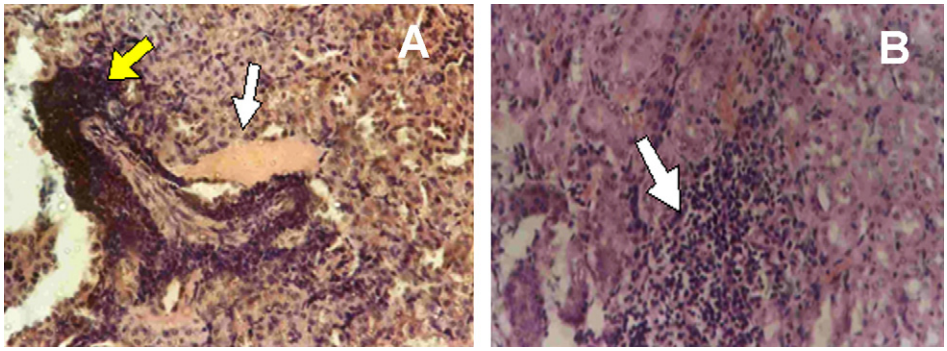

Figure 2 (A) Photograph showing abscess (yellow arrow) and casts (white arrows) along with necrotic changes in renal tissue of mice infected with biofilm cells of *P. aeruginosa*. (B) Photograph showing mild inflammation in renal tissue (white arrow) of mice infected with planktonic cells of *P. aeruginosa*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Once an opportunistic pathogen like *P. aeruginosa* enters the host, its ability to cause infection has been correlated with its tendency to form biofilms [55,56]. *P. aeruginosa* has an innate propensity to stick to the surfaces of catheters and form biofilms leading to higher incidence of UTIs in patients with long-term indwelling bladder catheterization [41,47—59]. In addition, previous microbial urethral colonization could be the cause of most UTIs where introduction of bacteria into the bladder takes place subsequently at the time of catheterization [23,60]. Besides disruption of the normal valvular function of urethra, catheters can also traumatize urethral and bladder mucosa, hence disrupting the normal mucopolysaccharide coating of the epithelium [61]. This damage of cellular structure renders it susceptible to attachment as well as entry of bacteria through surface erosions [62,63]. Therefore, catheter serves as a direct conduit for pathogens which may be carried from the external meatus to the bladder when the catheter is introduced [64]. In addition, internal and external surfaces of catheters have intrinsic irregularities providing convenient sites for organism's implantation as demonstrated by scanning electron microscopy [65]. Following initial adherence, bacteria may exude or attract some products to further solidify attachment [66]. Costerton et al. [67] related the pathogenesis of catheter-associated UTIs to the production of biofilms by the infecting organisms in which bacterial population adhered to catheter surface through pili and/or exopolysaccharides. The organisms in biofilms are able to persist in host's tissues for longer durations and are able to cause continuous damage to the host [52]. *In vivo* biofilm formation was reported by Nickel et al. [68] where colonizing bacterial population was observed embedded in glycocalyx on the external and internal surfaces of Foley's catheter removed from patient. Ganderton et al. [69] examined 50 Foley bladder catheters that had been indwelling for periods ranging from 3 to 83 days in patients for the presence of bacterial biofilms. Scanning electron microscopy revealed biofilm formation on the luminal surfaces of 44 of these catheters. These workers observed very thin to very thick biofilms embedded in a matrix. Stickler et al. [70] compared nature of biofilms formed in urease producing and non-urease producing organisms. It was observed that urease producing organisms, *P. mirabilis*, *Proteus vulgaris* and *Providencia rettgeri* formed crystalline nature of biofilms whereas urease-negative bacteria, *Morganella morganii*, *Klebsiella pneumoniae* and *P. aeruginosa* produced non-crystalline biofilms on urethral catheter. Similar observation of biofilm formation *in vivo* by *P. aeruginosa* on indwelling catheter in mice was made by Kurosaka et al. [71]. In their study, scanning electron microscopy revealed a thick biofilm formation on the surface of polyethylene tubing from day 2 onwards which gradually increased till day 14. Repeatable pattern of cell death and lysis has been documented to occur in biofilms of *P. aeruginosa* during the normal course of development. During the onset of biofilm development and biofilm killing thereafter, a bacteriophage capable of superinfecting and lysing the *P. aeruginosa* parent strain has been detected in the fluid effluent from the biofilm [72,73]. The bacteriophage implicated in biofilm killing was closely related to the filamentous phage Pf1 which existed as a prophage within the genome of *P. aeruginosa*. It has been proposed that prophage-mediated cell death could be an important mechanism of differentiation inside microcolonies that facilitate dispersal of a subpopulation of surviving cells. From our laboratory we observed that biofilm cells are able to cause more renal tissue damage compared

to planktonic counterparts possibly through evasion of phagocytosis and production of free radicals in mouse model of ascending UTI (Fig. 2) [74]. Hence formation of biofilms is the most important virulent trait of *P. aeruginosa* which enables this pathogen to cause recurrent and chronic UTIs by evading host immune defense mechanisms.

Quorum-sensing in *P. aeruginosa*

A variety of gram-negative and gram-positive bacteria 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* [75] and *rhl* [76] 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 $3OC_{12}$-HSL) [77] and *N*-butanoylhomoserine lactone (BHL or C_4-HSL) [78,79], respectively. The *lasIR* encoded quorum-sensing system has been shown to modulate expression of *lasI* itself [80], *lasB* (elastase) [81,82], alkaline protease [83], secretion pathway [84] and *rhlR* [85,86]. The *rhlIR*-encoded quorum sensor modulates expression of *rhlI* itself [85], *rhlAB* (rhamnolipid biosynthesis) [76,82], *lasB* [78,82,87] and *rpoS* [85]. Both these quorum-sensing systems are involved in the differentiation of planktonic cells to biofilm mode [88]. Role of these quorum-sensing signals in virulence and pathogenicity of *P. aeruginosa* has been demonstrated in models of respiratory tract infections, burn wound infections and keratitis [89—94]. However, very limited studies highlighting the role of these signal molecules in the pathogenesis of urinary tract infections are available. Stickler and co-workers [69] reported production of AHLs by *P. aeruginosa* isolated from urethral catheters using cross-feeding assay. These workers demonstrated production of AHL molecules in biofilms *in vitro* as well as *in vivo* in the patient's bladder. Relatively recently from our laboratory, we reported that quorum-sensing signals play a crucial role in ability of *P. aeruginosa* to cause urinary tract infection [95]. Single mutant harboring mutated *las* I gene and double mutant harboring mutated *las* I and *rhl* R as well as quorum deficient clinical strain of *P. aeruginosa* were cleared from the renal tissues much earlier than parent strain possessing functional las and rhl quorum-sensing systems highlighting central role of quorum-sensing signals in virulence of *P. aeruginosa*. Recently some new types of quorum-sensing systems like PQS and have been identified in *P. aeruginosa* however their role in UTIs has yet to be elucidated [96—98].

Environmental factors and urovirulence of *P. aeruginosa*

P. aeruginosa has been reported to continuously sense and respond to various environmental stimuli. While establishing in the urinary tract, presence of urine, which is a complex medium, exposes invading organism to conditions like varied osmolarity, pH and Tamm—Horsfall protein (THP) as well as variability of ions such as iron [99—101]. Urine is subject to change in pH and osmolarity depending on host's diet and clinical situation. Environmental conditions prevalent in the host mileu may bring about certain changes in organism like change in outer membrane protein (Omp) profile, porin size [102,103] and adhesive ability operative through lectins [99,102,104] which may play an important role in deciding the ultimate outcome of an infection.

Iron

Iron-limiting conditions have been reported to be prevalent in the milieu of urinary tract [100], therefore the ability of uropathogens to sequester iron from the host becomes a significant factor in determining their growth, metabolic process and pathogenicity [105]. *P. aeruginosa* has been reported to produce two siderophores, pyochelin and pyoverdin, which help this pathogen to obtain iron from host's iron binding proteins like lactoferrin and transferrin. In relation to *P. aeruginosa* some *in vitro*, studies are available where iron has been shown to regulate production of toxin A [106], alkaline protease [107], elastase [99,107] and siderophores [99], the recognized virulence factors of this opportunistic organism. Iron concentration of the culture medium employed for growth of *P. aeruginosa* was also shown to have the potential to influence pathogenicity of this organism in corneal [108] as well as in the acute respiratory tract infection model [109]. Recent studies from our laboratory demonstrated that *P. aeruginosa* grown in iron deplete medium were more virulent as compared to iron replete grown bacteria as indicated by higher production of virulence factors and lodgement of bacteria in the urinary tract of experimental animals [110]. Hence, existing literature indicate that levels of iron dictate vir-

ulence of *P. aeruginosa* and are thus critical for its pathogenicity. Extrapolation of available information may help in developing alternative preventive approach against UTIs based on iron supplementation with far reaching consequences.

Osmolarity

Osmolarity is another important factor which has been reported to affect growth and virulence of *P. aeruginosa*. In order to establish and cause UTI, *P. aeruginosa* has to adapt itself to variations in osmolarity of urine. We observed that osmolarity has profound influence on urovirulence of *P. aeruginosa* [111]. There was significant increase in production of virulence factors with increase in osmolarity from 200 to 300 mOsmol/l. However further increase in osmolarity led to significant decrease in production of virulence factors. In addition, organisms grown in medium having osmolarity 300 mOsmol/l were resistant to phagocytosis and were more virulent in mouse model of ascending UTI as indicated by significantly higher neutrophil recruitment, bacterial load, malondialdehyde (MDA) production, a marker of tissue damage, and renal as well as bladder pathology. Culham et al. [112] highlighted that in addition to directly influencing bacterial growth in the urinary tract, osmoregulatory mechanisms may indirectly influence urinary tract infection by affecting the expression of virulence determinants. In case of *P. aeruginosa*, the sigma factor, Rpo S, has been shown to play an important role during exposure of this organism to various environmental stresses including osmotic stress. Suh et al. [101] also suggested importance of Rpo S in the pathogenesis of *P. aeruginosa* induced respiratory infections. There is strong possibility that similar mechanism may be operative in the urinary tract affecting the evolution of infection caused by *P. aeruginosa* although this needs further confirmation.

Tamm—Horsfall protein

In the urinary tract, complex urine provides a medium which has copious amounts of mucus. The urinary mucus predominantly has Tamm—Horsfall protein (THP) which is a polymeric glycoprotein, produced in thick ascending limb of loop of Henle in renal tissue [113]. Majority of THP is in the form of secreted protein in urine but it also exists in membrane bound form especially at the renal distal nephron cell surface [114]. Concentration of THP has been reported to be crucial in deciding the ultimate role played by this protein [115]. We observed that with increase in concentration of THP from 10 to 50 μg/ml, there was gradual rise in elaboration of all the virulence factors as compared to control (i.e. in absence of THP). However with further increase in concentration of THP from 50 to 70 μg/ml there was significant fall in production of all the virulence traits by biofilm cells of *P. aeruginosa* [116]. Decreased uptake and intracellular killing of THP (50 μg/ml) coated planktonic and biofilm cells of *P. aeruginosa* by murine peritoneal macrophages were also observed. In addition, it was observed that THP coated *P. aeruginosa* cells were more virulent *in vivo* in UTI model, showing higher level of destruction in kidney as well as in bladder tissue in comparison to uncoated organisms [117]. These results therefore bring out that THP coating provide better opportunity to this pathogen for survival *in vivo* by evading phagocytosis. Hawthorn et al. [118] while comparing adhesion of three uropathogens to THP coated renal tubular cells *in vitro*, also stressed that THP may not help to remove all uropathogens from urinary tract. It may help in renal colonization of uropathogens like *P. aeruginosa*. In the milieu of the kidney where THP is available in abundance these observations have immense relevance. Once *P. aeruginosa* reaches renal parenchyma, this ability may help this organism to colonize, get established and persist. Further *in vivo* studies using THP knock-out mice are warranted which can shed more light on the precise role of THP in *P. aeruginosa* induced UTIs.

Host innate immune system and urinary tract infections

Besides environmental factors, the host also plays an important role in the establishment of an infectious process. Microbial virulence is dependent on host factors, as exemplified by the pathogenicity of avirulent microbes in immunocompromised hosts and the lack of pathogenicity of virulent pathogens in immune hosts. In this regard the innate immunity provides a first line of defense in which macrophages and neutrophils play an important role. Macrophages, coming mostly from circulation, form one of the initial lines of defense in the urinary tract and offer resistance against infection. These macrophages interact with invading pathogen leading to elaboration of biochemical substances referred to as macrophage secretory products (MSPs). MSPs have been recognized to contain peptide hormones, complement components, enzymes, bioactive oligopeptides and lipids, reactive oxygen and nitrogen species as well as cytokines [119]. *P. aeruginosa* has been reported

to exploit these MSPs for its own growth and enhancing production of virulence traits leading to enhanced virulence in mouse model of ascending UTI (unpublished data) [120]. Utilization of MSPs by *P. aeruginosa* can have far reaching consequences including chronicity and recurrence of infections caused by this pathogen. Since MSPs contain a diverse array of biomolecules which can act in a complex manner among themselves, further studies are warranted which can throw more light on the precise role of MSPs in UTIs.

In addition to macrophages, neutrophils also provide defense against UTIs operative through phagocytosis as well as elaboration of cytokines. On one hand, these cells are essential for clearance of bacteria from urinary tract, on the other hand neutrophils have been implicated in tissue damage leading to renal scarring [121]. In case neutrophils are trapped and tissue is destroyed, the kidney pathology has been reported to be progressing to the stage of chronicity and renal scarring. These cells are recruited to the site of infection in response to chemokine secretion by bladder and renal epithelial cells like IL-8. *In vitro* production of IL-8 has been studied in cultured epithelial cells from various sources [122], where it has been shown to affect neutrophil chemotaxis, degranulation and transendothelial migration. High levels of IL-8 have been demonstrated in urine of patients suffering from UTIs. Macrophage inflammatory protein-2 (MIP-2) is one of the human IL-8 homologues in the mouse. Studies from our laboratory demonstrated that although biofilm cells of *P. aeruginosa* induce higher levels of MIP-2 compared to planktonic counterparts leading to more recruitment of neutrophils but are resistant to killing by neutrophils possibly by interfering with oxidative burst capacity in mouse model of ascending UTI [9]. Thus the ultimate clearance of the organism is not based on the collection of neutrophils but the efficacy of the neutrophils to kill, especially biofilm forms of *P. aeruginosa*.

UTIs activate both mucosal and systemic inflammatory responses in which cytokines play a pivotal role [123,124]. Cytokines, both proinflammatory and anti-inflammatory, have been reported to be produced largely by macrophages. In addition, wide variety of cells including lymphocytes, endothelial cells, pulmonary epithelial cells and urinary tract epithelial cells produce these cytokines in response to bacteria [125] or their products like lipopolysaccharide (LPS) and fimbriae [126—128]. Cytokines like TNF-α, MIP-2, IL-6 and IL-1β have been reported to be produced in urinary tract following infection with uropathogenic *E. coli* which help in transepithelial migration of phagocytes from blood to the site of infection [129,130]. Increased levels of these cytokines and their receptors have been observed in urine and serum samples of patients having acute pyelonephritis [131]. However there is paucity of literature in relation to role of these cytokines in UTIs caused by *P. aeruginosa*. Since cytokines play an important role in recruiting immune cells to the site of infection, further studies in relation to UTIs are warranted, and will be of special relevance for clinicians for treating catheter- and hospital-acquired infections.

Future challenges

Despite advances in antimicrobial therapy the mortality and morbidity associated with *P. aeruginosa* induced UTIs still remains high. This unfavorable outcome is due to our incomplete understanding about the pathogenesis of the disease. Very limited studies are available in relation to the pathogenesis of *P. aeruginosa* induced UTI. This review draws attention of the researchers that there is need to understand pathogenetic mechanisms of UTIs caused by *P. aeruginosa* in order to design effective treatment strategies. Acylhomoserine lactones (AHLs) can be speculated to serve as potential target molecules for inhibition of biofilm formation. In addition, role of bacterial and host factors during evolution of urinary tract infection caused by *P. aeruginosa* needs to be looked into since such infections, which may lead to persistence and chronicity, posing a threat for a treating clinician. Recognizing how *P. aeruginosa* overrun crucial host-cell pathways by using a myriad of mechanisms may help in understanding pathogenesis of UTIs caused by this pathogen. This knowledge needs to be advanced to the point at which it can be translated into a true understanding of the disease. This remains the crucial challenge to all who are involved in this field. All this information may help in developing effective preventive strategies against biofilms of *P. aeruginosa* formed on urethral catheters which are a major cause of recurrence, persistence and chronicity.

Conflict of interest

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Competing interests: None declared.

Ethical approval: The study protocol was approved by the institutional ethical committee for animal experimentation.

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