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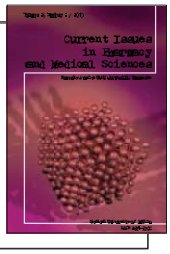

Involvement of biofilm-like intracellular bacterial communities of uropathogenic *Escherichia coli* in pathogenesis of urinary tract infections – a mini review

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ABSTRACT

This paper presents a precisely defined question about the role of the biofilm-like intracellular bacterial communities in pathogenesis of the urinary tract infections. According to the recent literature, uropathogenic *Escherichia coli* is one of the leading etiologic agents of the urinary tract infections. Although *E. coli* is regarded as an extracellular pathogen, some experiments have revealed a multi-step infection cycle, which involves adhesion, invasion, proliferation within invaded urothelial cell in the form of biofilm-like intracellular bacterial communities and dispersal, leading to infection of next neighbouring cells. Therefore, the prevention and treatment of the urinary tract infections must include intracellular stage of infection.

Keywords: urinary tract infections, uropathogenic *E. coli*, intracellular bacterial communities

INTRODUCTION

Urinary tract infections (UTIs) are a highly common, serious clinical and epidemiological problem of public health, occurring especially in 40-50% of young women between the age of 20 and 40 years and in postmenopausal age and in about 5% of men [6, 13]. Majority of UTIs first concerns the bladder (cystitis), while in more invasive infections – kidneys (pyelonephrithis) and thus lead into renal insufficiency [13]. Uropathogenic *E. coli* (UPEC) strains hardly ever penetrate to the bloodstream (inducing urosepsis) and to the central nervous system (being the occasional cause of meningitis), so bacteriuria, cystitis or pyelonephrithis are a usual UTIs symptoms. UPEC strains were found to be an etiological agent in almost 90% of community-acquired UTIs and about 40% of nosocomial UTIs. The main reservoir of UPEC seems to be the intestinal tract and rectal or vaginal niches from where bacteria migrate through urethra up to the bladder or further, to reach ultimately even the kidneys [6].

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UTI seems to be the result of complex interactions between two elements – a host defence mechanisms and UPEC virulence. Genome of UPEC strains encodes a numerous virulence factors that facilitate their colonization within the urinary tract as adhesins, toxins, capsules (including K1 capsule), biofilm formation and iron uptake factors or heme receptors (Tab.1).

Table 1. Some virulence factors of uropathogenic *E. coli* [6]

Biological function	Virulence factor	Gene
Adhesion	S pili	*sfa*
	P pili	*pap*
	type 1 pili	*fimH*
Biofilm formation	antigen 43 (Ag43)	*agn43*
Toxins	α-hemolysin	*hlyD*
	cytotoxic necrotizing factor 1 (CNF1)	*cnf1*
	cytolethal distending toxin	*cdt*
Iron uptake	aerobactin siderophore receptor	*iutA*
	salmochelin siderophore receptor	*iroN*

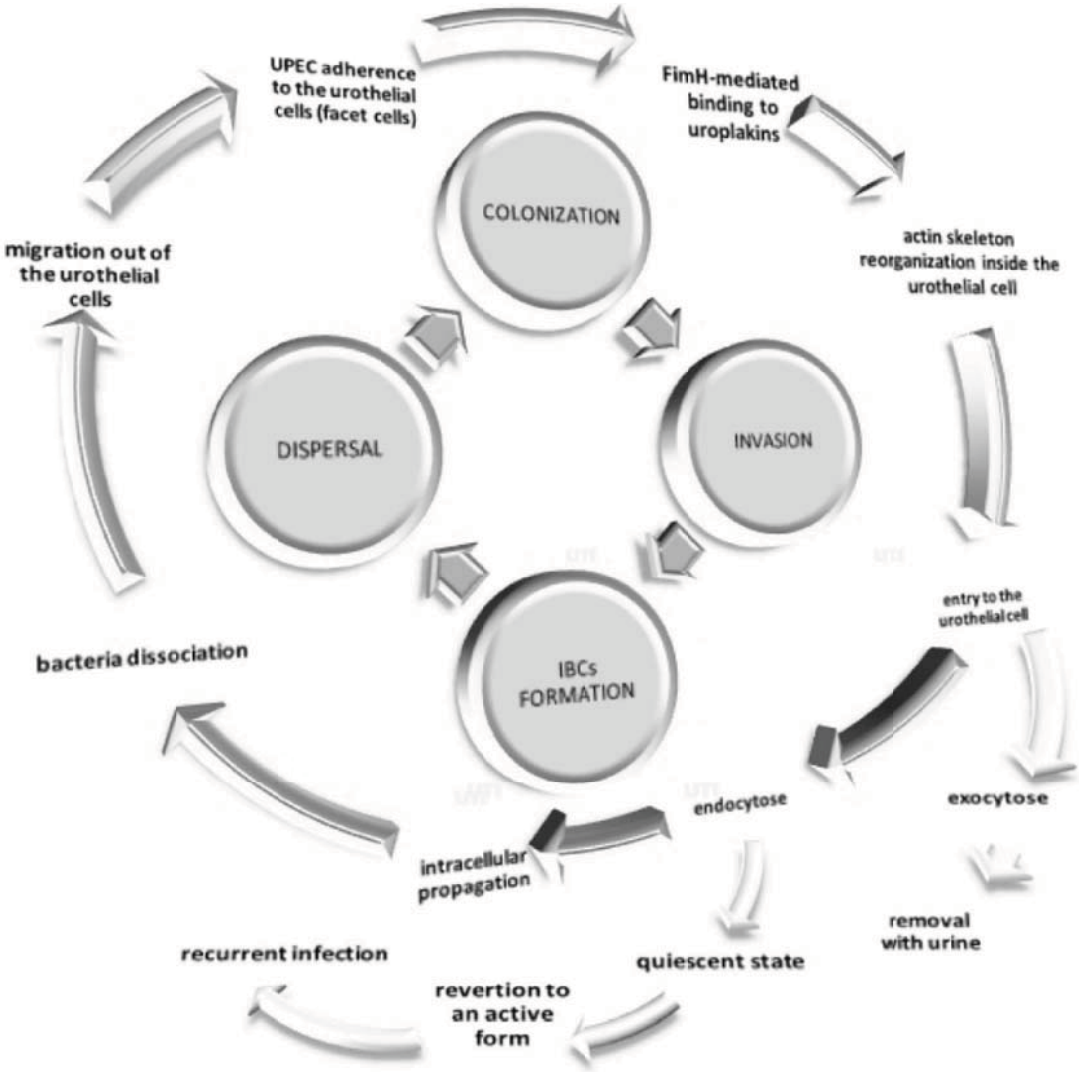
Referring to the classic virulence factors, an iron uptake is mediated by a production of some toxins – α-hemolysin is a fine example; it provides the host cell apoptosis throughout its lysis what contributes to the iron release needed in UPEC growth [13]. It should also be mentioned that polysaccharide capsules are important virulence factors of UPEC. Most of these strains contain K1 capsule being a lectin capsule subtype that inhibits

phagocytosis and protects UPEC against the host response during UTI development. Moreover, it plays the major role in nonspecific adhesion, intracellular proliferation, multiple-surfaces biofilm and intracellular bacterial communities (IBCs) formation being an important component of its matrix and determining morphology [2]. Among other recently discovered UPEC pathogenic factors there are also TcpC – the Toll-like receptors inhibitor, dynamin2, autophagy protein Atg16L1, nitric oxide synthase or cyclooxygenase-2 production [13].

UPEC infection cycle comprises four major steps which include colonization, invasion, formation of IBCs and dispersion. They are presented in Fig. 1.

dens (family members suffering from UTIs, blood group antigens), anatomic deviations (lack of normal flora etc.) [6]. The urinary tract is typically an aseptic environment due to functioning of various host defence mechanisms preventing urothelial cells against bacterial bladder epithelium colonization.

Therefore, various defence mechanisms must function as physical factors, like urine flow, systematic voiding and epithelium exfoliation, removal of intrusive bacteria not attached to the bladder facet cells or with a weak adherence abilities. The urine low pH values, decreased osmolarity and setting up a reactive nitrogen/oxygen mechanisms inhibit or diminish bacterial growth and per-



Abbreviations: IBCs – intracellular bacterial communities [2]
Fig. 1. Infection cycle of uropathogenic *E. coli* during urinary tract infections

HOST DEFENCE MECHANISMS WITHIN URINARY TRACT

The main host risk factors predisposing to the UTIs development are contraceptives (vaginal or intrauterine cap/diaphragm), frequent sexual intercourses, family bursistence in such a demanding and crude environment. The immune elements – the Tamm-Horsfall's protein and secretory sIgA, apoptosis activation, interleukins and cytokines release, granulocytes inflow - a small part of effective immunological system response, successfully

prevent bacterial adherence and invasion to the urothelium cells [6, 14]. Siderophores and lactoferrin-type proteins are required to remove necessary iron from the bacterial field of action, limiting bacterial growing and colonization abilities in this way [6]. Great siderophores affinity to iron ions protects host cell from catching it through specific receptors on bacterial membrane, initiates active transport systems for Fe^{3+} uptake and facilitates the use in intracellular metabolism - an iron acquisition system [6, 13].

FIMH/UROPLAKINS INTERACTIONS

Typical luminal bladder surface includes transitional multilayer epithelium called urothelium, specific to the urinary tract, composed with three to six layers. The luminal cells are large, umbrella-type and cover a few neighbouring cells in the same time. That structure allows the bladder to extend under the urine volume. So-called umbrella cells lay under an asymmetric unit membrane, which includes specific uroplakins covering apical surface.

The initial step in bacterial pathogenesis during UTIs is adherence of bacterial cells to urothelium due to possession of specific adhesive organelles such as type 1 pili, S or P pili or Dr family adhesins. Fimbrial operons, which undergo some specific rearrangements, allow for genes expression switching, enabling further activation. This results in appearing in definite order and getting a different pili-populations facilitating adherence to several tissues [13, 14].

The most important for bacterial cells is to avoid removal with micturition. The majority of UPEC strains possess the type 1 pili as the major adhesin connected with their adhesion and/or invasive abilities [14]. The type 1 pili is formed by the polymerized pilin subunits capped with FimH lectins possessing a binding site with a high affinity to α-mannosylated receptors called uroplakins on the host cells localized within uroplakins, which seems to be the key site where the infection begins [13-15]. Uroplakins tightly overlay the umbrella cells and among four known proteins, the uroplakin Ia is the main binding site for FimH, selectively recognized by the UPEC during the adhesion. Low urine pH inhibits bacterial FimH-uroplakin binding. In turn, as a part of huge membrane proteins family, it can regulate a "cross-talks" between one and another cell and the functioning of numerous proteins on the urothelium surface [15].

INVASION OF UROTHELIAL CELLS

FimH/uroplakins interaction can easily initiate intricate host defence mechanisms, leading to an actin cytoskeleton modification and evolve proliferation or transformation into an insidious quiescent state inside the host cell [14]. Literature shows additionally that FimH is not required only for the adherence as it was mentioned above but more obligatorily for proliferation inside the host cell and for an increase of the IBCs formation frequency [4].

Fluorescent microscopy and flow-chamber-*in vitro*-modelling confirmed that invasion of the bladder epithelial cells is induced by the rounds of phenotype transition uropathogenic cascade – firstly performed by a single rod-shape UPEC, followed by intracellular coccoid forms colonization, that lead consequently to urothelial cell destruction or death, and eventually reinvasion of neighbouring cells as a result of reversion into highly filamentous bacteria [1]. The UPEC cell invasion starts from membrane invagination and formation of endocytic vesicles, where bacteria behave in two different ways: (1) revert into quiescent state or (2) enter into a cell cytosol. Experimental UTI models have showed lasting for nearly 9 hours process of adherence and the bladder surface colonization, together with spreading or aggregation of bacterial cells [1].

INTRACELLULAR BACTERIAL COMMUNITIES

Murine UTIs models showed that after entering bladder facet cells, UPEC are unusually able to produce a kind of biofilm-like populations formally called the intracellular bacterial communities or to go into a quiescent intracellular reservoirs (QIRs) being an easy way to recurrent UTIs development [6].

After penetrating of the host cells, UPEC intensively multiply what is followed by IBCs formation, which can be characterized as a great thousands of bacteria cells inclusions inside the host facet cell cytosol. They have many typical features for biofilm, such as production of polysaccharide matrix or colonization of various surfaces and pass a series of life stages [6]. Bacterial filamentation, using mainly FimH adhesin, after IBCs formation, seems to be a crucial step because it possibly implies structural changes inside the invaded cell such as closing the cell membrane and covering/encircle bacteria inside an endosome or lysosome and what is the most significant, it allows bacteria to counter the voiding and encourages the urothelium colonization [6, 14].

Epithelium invasion is made possible by lipid rafts and caveolae components or clathrin-dependent endocytose pathways or on the way of cyclic AMP-depended fusion vesicles within urothelium cells. These allow bacteria to avoid a removal within urine [2, 5]. Model experiments with cholesterol-modifying medicines such as filipin, which regulates the sequestration of cholesterol, proved that lipid rafts are necessary during invasion. Moreover, filipin markedly increases UPEC proliferation in endosomal vesicles but has no evident efficacy on adherence or invasion [4]. Next step after the bladder cells invasion is dissociation and migration of detached bacteria and re-adherence to the urothelium [2].

Urothelial cells destruction and immune system cells influx in a place of facet cells damage can result in enhanced UPEC migration into the lower layers of the bladder transitional epithelium and that is the most potential place where bacteria revert into quiescent state and resemble morphologically a little packages enclosed inside the endosomes [1]. These forms guarantee bacteria a protection against variety of antimicrobial agents, host defensive mechanisms (e.g. phagocytosis by neutrophils), long-term survival and reinvasion at a slow rate but constantly. Reversion to an active form can be encouraged by the epithelium exfoliation of facet cells in the bladder, which provokes bacteria to proliferation and entry into the next rounds of IBCs creation cycle and thereby directly lead to a re-infection [6].

ANTIBACTERIAL TREATMENT OF URINARY TRACT INFECTIONS (UTIS)

The basis of effective treatment of UTI is a rational antibiotic therapy. The main reason of UTIs treatment failure, leading to the increased frequency of recurrent or chronic infections, is the ability of UPEC to form IBCs and widespread resistance to available antimicrobials. For empiric treatment of uncomplicated urinary tract infections (cystitis) in women, several antimicrobials are recommended as the first choice drugs: trimethoprim/sulfamethoxazole or nitrofurantoin. According to the data obtained under experimental conditions, in bladder cell culture model, only a few antibiotics, including nitrofurantoin and fluoroquinolones, have been proved to eliminate UPEC from their intracellular reservoirs. However, fluoroquinolones should not be longer recommended as the drug of the first choice because of the currently increasing resistance rate among UPEC [7, 10].

Among the available antimicrobials showing activity against the most common uropathogens, including UPEC, fosfomycin continues to be active because of an extremely low incidence of resistant strains worldwide; the resistance rate of about 1% was noted in case of *E. coli*. Moreover, this antibiotic has been shown to possess promising *in vitro* activity against multidrug-resistant uropathogens. Fosfomycin, the bacterial cell wall inhibitor with bactericidal activity, is also the agent inhibiting adhesion of UPEC to urothelium. It is recommended as the first line therapy for uncomplicated UTIs. However, further microbiological and clinical studies are needed to define the role of fosfomycin in UTI treatment [8, 11].

Studies performed by Totsika et al. [12] suggested that potential FimH inhibitors may be regarded as an alternative treatment against UPEC, especially multidrug-resistant strains. Among compounds inhibiting adhesion of UPEC to urothelium via type 1 pili, D-mannose, naturally occurring simple sugar, may be useful for prophylaxis of urinary tract infections. Besides, *Vaccinium macrocarpon*, called American cranberry, has been shown to possess anti-adhesive activity against UPEC because of high content of proantocyanidins interacting with type 1 pili. Such alternative prevention or/and therapeutic strategies in UTIs may be combined with a standard antibiotic therapy [3, 9].

Another alternative strategy developed for UTIs treatment is based on forskolin – diterpene derivative, also called coleonol, obtained from the roots of *Coleus forskohlii*. It activates adenylate cyclase to promote the generation of cAMP – a cell-regulating compound, involved also in pathogenesis of UTIs. Using animal model – UPEC-infected mice, it was proved that treatment with forskolin resulted in the increase of intracellular cAMP, followed by induction of exocytosis of fusiform vesicles and reduction of the number of intracellular *E. coli* population [5].

CONCLUSIONS

Urinary tract infections are the result of multiple host-UPEC interactions, leading to adhesion of UPEC to urothelium, penetration of bacteria across the cell membrane, followed by the invasion of urothelium and formation of IBCs. Further understanding UTI pathogenesis allows for the development of new antimicrobial effective strategies acting against uropathogens, including UPEC.

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