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Review

Alternative treatment approaches of urinary tract infections caused by uropathogenic *Escherichia coli* strains

Beata Zalewska-Piątek✉ and Rafał Piątek

Departments of Molecular Biotechnology and Microbiology, Gdańsk University of Technology, Gdańsk, Poland

Urinary tract infections (UTIs) are the most widespread and annoying infections affecting millions of people every year annually. The biggest problems of urinary diseases are recurrences, increasing resistance of uropathogens to commonly used antibiotics, as well as the high health care costs of afflicted persons. Uropathogenic *Escherichia coli* strains (UPECs) are the most dominant etiologic agents of community-acquired infections of this type. During UTI pathogenesis, UPECs utilize various virulence factors, especially mono- and polyadhesive appendages of the chaperone-usher secretion pathway (CUP) required for adhesion, invasion and biofilm formation. Commonly used antibiotics for UTI treatment are usually effective, but their long-term utility may affect gut microbiota of the treated individuals and cause selection of drug resistant uropathogenic variants. Due to increasing resistance of UPEC strains to antibiotics *via* the evolution of specific defense mechanisms, there is a need to develop alternative methods and therapeutic strategies to fight UTIs (vaccines, receptor analogues, pilicides and curlicides, bacterial interference or phagotherapy). Such therapeutic approaches usually target processes enabling uropathogens to survive within the urinary tract and cause recurrent infections.

Key words: uropathogenic *E. coli*, urinary tract infections, chaperone-usher pathway, adhesins, vaccines, pilicides

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✉e-mail: beazalew@pg.edu.pl

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Abbreviations: ABU, asymptomatic bacteriuria; CAUTIs catheter-associated infections; CUP, chaperone-usher secretion pathway; DAF, decay-accelerating factor; DSE, donor strand exchange; DSC, donor strand complementation; ECs, uropathogenic *E. coli*; FyuA, yersiniabactin ferric uptake receptor; Gal, galactose; GalNAc, N-acetylgalactosamine; Hma, haem acquisition protein; HlyA, haemolysin; HSV-1, *Herpes simplex* virus type 1; IreA, iron-responsive element, IroN iron uptake receptor; IutA, aerobactin iron transport receptor; TLR4, toll-like receptor 4; TF, Thomsen–Friedenreich; UPIBCs, intracellular bacterial communities (IBCs); UTIs, urinary tract infections; QIRs, quiescent intracellular reservoirs

INTRODUCTION

Urinary tract infections (UTIs) are one of the most dominant bacterial infections among humans, accounting for up to 150 million registered cases per year worldwide. UTIs cause a wide-ranging health problem and economic burdens for the countries resulting from the need to treat or hospitalize individuals affected by the infections of this type (Stam & Norby, 2001; Cassini *et al.*, 2016). In the United States, these infections constitute the reason for 100 000 hospital admissions and 11 million medical visits annually. They also represent at least 40% of infections directly acquired in the hospitals (Foxman, 2014).

UTIs affect women more often than men. It is estimated that 50% of women suffer from UTI at least once in their lifetime and above 30% undergo antibiotic therapy due to UTI before the age of 24 (Foxman, 2002; Foxman, 2014). A total of 20–40% of the afflicted women also experience one or more recurrent infection within months of the first episode of UTI. In turn, frequent recurrences of infection require repeated antibiotic therapy. Such UTI treatment promotes the development of drug resistance among uropathogens (Franco, *et al.,* 2005; Terlizzi *et al.*, 2017).

UTIs are caused by a range of various pathogens but the most frequent causative agents for complicated and uncomplicated UTIs are uropathogenic *E. coli* strains (UPECs) (about 80% of cases) (Foxman, 2014; Flores-Mireles *et al.*, 2015). UPEC encodes many virulence factors required at various stages of UTI pathogenesis, including adherence and colonization, invasion and persistence, biofilm development, formation of intracellular bacterial communities (IBCs) and quiescent intracellular reservoirs (QIRs), kidney colonization and epithelial damage. However, the most important stage during UTI pathogenesis is bacterial adherence and colonization of the host cells and tissues (Flores-Mireles *et al.*, 2015).

Many UPECs initiate infection using pili or fimbrial appendages belonging to the surface-exposed polymeric adhesive structures whose biogenesis in Gram negative bacteria is driven via the highly conserved chaperone-usher pathway (CUP). Among these adhesive factors, the most dominant are pili type 1 and P (functioning as the monoadhesins) and Dr/Afa family of polyadhesins. Genes required for the bioassembly of pili/fimbriae are located in the CUP pilus operons (e.g. *fim*, *pap*, *dra*, *afa*) with conserved genetic organization. The above polymeric structures are often capped with various adhesins responsible for the recognition of different receptors coating the surface of epithelium within the lower and upper urinary tract. Such bacterial interactions with receptors can be used to mediate a distinct tissue tropism (Thanassi *et al.*, 1998; Soto & Hultgren, 1999; Zavialov *et al.*, 2007; Terlizzi *et al.*, 2017). Thus, the tip FimH adhesin of type 1 pili enables *fim*$^+$ *E. coli* to cause cystitis by binding the mannosylated receptors of the bladder epithelium (Sokurenko *et al.*, 1997; Spaulding *et al.*, 2108). In turn, the PapG of P pili adhesin, recognizing globosides in the kidneys, allows the *pap*$^+$ *E. coli* strains to develop pyelonephritis (Leffler & Svanborg-Edén, 1981; Roberts *et al.*, 1994; Dodson *et al.*, 2001). Importantly, *E. coli* strains harboring Dr/Afa adhesins (*dra*/*afa*) which recognize a decay-accelerating factor (DAF) as the main

receptor, cause pyelonephritis (30%), cystitis (30–50%), chronic diarrhea in children (10–15%), and recurrent infections in young women (50%). These strains are also a significant problem for pregnant women during the third trimester of pregnancy, mainly because they are responsible for 40% of cases of pyelonephritis (Nowicki *et al.*, 1984; Nowicki *et al.*, 1994; Goluszko *et al.*, 1997; Goluszko *et al.*, 2001).

Antibiotic therapy of UTIs is effective in many cases (Terlizzi *et al.,* 2017; Giancola *et al.,* 2017). However, an increasing number of antibiotic resistant UPECs is still observed worldwide. A good example is the increase in resistance to fluoroquinolines among UPECs to around 50% in the European countries, and even up to 70% in China, India and Vietnam (Zowawi *et al.,* 2015). In case of women (especially during pregnancy) suffering from UTI recurrences, the increment of UPEC strains resistant to ampicillin (96.42%), tetracycline (85.7%), amikacin (71.42), ciprofloxacin (67.85%) and gentamycin (58.71%) is also observed (Habibi & Khameneie, 2016). In addition, a broad spectrum of antibiotics has limited utility in long-term therapy (Zowawi *et al.*, 2015). Due to the alarming and rapid overgrowth of antibiotic resistance among UPECs, there is a need to develop new, alternative and specific forms of UTI treatment and prophylaxis. Here, we briefly reviewed the most promising innovative methods and strategies for therapy and prevention of UTIs caused by UPECs (Table 1).

VACCINES BASED ON ADHESINS AND BACTERIAL EXTRACTS

In the light of increasing resistance of uropathogens to antibiotics, alternation of physiological gut microbiota during treatment, allergic reactions to standard antibacterial medicaments and difficulties in prevention or limitation of UTI recurrences, there is a need to develop novel remedies to fight them. Therefore, it can be assumed that vaccines are a promising tool for preventing UTIs caused by UPECs in humans. The design and preparation of vaccines are based on different strategies, including key virulence factors and pathogenicity mechanisms of UPECs (Foxman, 2014; Flores-Mireles *et al.*, 2015).

Pili and fimbrial adhesins

Bacterial adherence mediated by the highly organized adhesive systems such as pili or fimbriae is a key stage in the pathogenesis of UTIs (Flores-Mireles *et al.*, 2015). Therefore, Gram-negative bacterial adhesins, especially those assembled via the conserved CUP, and adherence itself represent an excellent target for potential vaccine candidates. Characteristic features of the surface-exposed adhesins, as the structural and functional elements of the linear homopolymers (polyadhesive fimbriae) or heteropolymers (monoadhesive pili/fimbriae), are prone to antigenic diversity and amino acid sequence variability and thus are used by pathogens as defense mechanisms against immune response of the infected organism (Zavialov *et al.*, 2007; Zav'yalov *et al.,* 2010; Werneburg & Thanassi, 2018). Polyadhesins mainly consist of only one adhesive subunit that occurs in hundreds of copies within the whole core of the fimbrial structure. However, monoadhesins are composed of up to seven different subunits and in comparison to polyadhesive fimbriae, they are tipped with only one adhesive subunit; this is important for the design and synthesis of vaccines, especially the chimeric ones (Hung *et al.*, 1996; Thanassi *et al.*, 1998; Soto & Hultgren, 1999; Remaut *et al.*, 2006, 2008; Zavialov *et al.*, 2007; Fronzes *et al.*, 2008; Waksman & Hultgren, 2009).

Generally two therapeutic strategies dominate, one based on the bacterial adherence and the other on ad-

Table 1. Alternative therapeutic and preventive strategies to fight UTIs.

Type of UTI treatment/ prevention	Therapeutic components	Biological effect	References
Vaccines	Native or chimeric pili/fimbriae and adhesins of CUP	Reduction in bacterial adhesion and colonization	Asadi *et al.*, 2013; Goluszko *et al.*, 2005; Roberts *et al.*, 1984; Zalewska *et al.*, 2003, 2007; Savar *et al.*, 2014
	Chaperone-adhesin complexes		Langermann *et al.*, 1997, 2000; Roberts *et al.*, 2004; Spaudling & Hultgren, 2016
	Killed uropathogens (Solco Urovac, Uro Vaxom)		Beerepoot, 2013; Kochiashvili *et al.*, 2014; Naber, 2009; Neto *et al.*, 2016; Wolski *et al.*, 2017
	Asymptomatic bacteriuria isolates	Interference with bladder colonization by uropathogens	O'Brien *et al.*, 2018; Stork *et al.*, 2018
	Toxins and proteases	Protection of the host tissues from bacterial damage	O'Hanley *et al.*, 1991; Sivick & Mobley, 2010
	Siderophores	Inhibition of iron acquisition systems and reduction in bacterial colonization	Alteri *et al.*, 2009; Brumbangh *et al.*, 2013; Mike *et al.*, 2016; Russo *et al.*, 2003
Small inhibitory compounds	Receptor analogues: mannosides, galactosides, glycodendrimers, galactosides, galactosaminosides	Blocking of bacterial adherence	Brumbaugh & Mobley, 2012; Conover *et al.*, 2016; Cusumano *et al.*, 2011; Guiton *et al.*, 2012; Han *et al.*, 2010, 2012; Hu *et al.*, 2016; Mousavifar *et al.*, 2018
	Derivatives of 2-pyridone: pilicides	Inhibition of CU pili/fimbriae or curli biogenesis	Chorell *et al.*, 2010; Dang *et al.*, 2014; Green *et al.*, 2014; Piątek *et al.*, 2013; Svennson *et al.*, 2001
	Curlicides		Cegelski *et al.*, 2009; Chorell *et al.*, 2011
Phage therapy	Bacteriophages	Lysis of uropathogens	Leitner *et al.*, 2017; Sybesma *et al.*, 2016; Ujmajuridze *et al.*, 2018

hesive proteins. The first approach is associated with isolated pili or fimbrial fractions, by using their strong immunogenic properties and their presence on the surface of bacterial cells in a large number of copies (~ 500 copies per cell) (Klemm & Schembri, 2000). Investigations carried out with a mouse model (C3H/Hej) had shown that vaccination with purified wild type Dr fimbriae (of *E. coli* Dr$^+$ IH11128) induced an excellent humoral response leading to the production of specific serum antibodies. Additionally, antibody production (as a result of a post-vaccination response) protected animals that underwent experimental UTI from increased mortality but did not decrease colonization of the bladder and kidney (Goluszko *et al.*, 2005).

The second strategy is based on the adhesin-chaperone complexes. Experimental studies with mouse and cynomolgus monkey models of UTIs using PapD chaperone–PapG adhesin or FimC chaperone–FimH adhesin protein complexes, confirmed a high efficacy in inhibiting host-pathogen interactions of adhesion vaccines of this type, and thus their protective function against UTIs (such as pyelonephritis or cystitis) (Langermann *et al.*, 1997; Langermann *et al.*, 2000; Roberts *et al.*, 2004; Asadi *et al.*, 2013; Savar *et al.*, 2014). The potential use of PapD–PapG and FimC–FimH interactions in vaccine development was largely associated with the production of antibodies against FimH and PapG (blocking the function of FimH and FimG adhesins, respectively) during bacterial colonization of the bladder or kidneys (Roberts *et al.*, 1984; Roberts *et al.*, 2004; Langermann *et al.*, 1997; Langermann *et al.*, 2000). The purpose of current research is to modify the FimC-FimH based vaccine to make it a better stimulator of cellular and humoral immune response in the immunized host (Asadi *et al.*, 2013; Savar *et al.*, 2014). One possibility is to obtain a fusion protein composed of a FimH adhesin and a FliC flagellin in order to stimulate an acute inflammatory response associated with the Toll-like receptor 4 (TLR4) signaling cascade (Valore *et al.*, 1998; Savar *et al.*, 2014). TLR4 activity is associated with production of bactericidal peptides (mainly β-defensin-1 and cathelicidin) by epithelial cells that leads to the destruction of bacteria (Chromek *et al.,* 2006; Nielsen *et al.,* 2014). In January 2014, a first phase of clinical human studies was started to assess the usefulness of immunization with the FimC–FimH vaccine containing a synthetic monophosphoryl lipid A analogue as an adjuvant (Spaudling & Hultgren, 2016).

Another possibility of using pili/fimbriae in the context of a potential vaccine is their application as the carriers of heterologous antigenic determinants of bacterial or viral origin. A very serious limitation of this strategy is the size of the epitope used, which should not affect the structure of adhesive subunit (carrier) and interfere with bioassembly of adhesive hetero- or homoassociates into a complete pili/fimbrial structure (on the surface of bacterial cells) (Klemm & Schembri, 2000). A good example of this approach are homopolymeric Dr fimbriae (consisting of hundreds of repeating DraE adhesive subunits) of uropathogenic *E. coli* Dr$^+$, which were applied as a carrier of a model antigenic determinant of glycoprotein D derived from the *Herpes simplex* virus type 1 (HSV-1). Insertion of a heterologous epitope included the N-terminal region of the surface-exposed domain 2 within the DraE fimbrial subunit which is responsible for the invasive properties of bacterial cells (Das *et al.*, 2005). In this region of the DraE adhesin, the selected antigenic determinants can be inserted without interfering with the fimbriae biogenesis, which determines the display and amplification of a given epitope (in thousands of copies per bacterial cell) in the form of polyadhesive homopolymers on the surface of bacteria. Immunogenicity of the Dr-HSV chimeric fimbriae was determined by immunization of rabbits in which a protective immune response was stimulated leading to the production of antibodies specific for both, the DraE adhesin and the HSV epitope (Zalewska *et al.*, 2003). All analyses highlight the high efficacy of chimeric Dr fimbriae as the vaccine candidate against UTIs and other diseases, depending on the inserted antigenic determinant (Zalewska *et al.*, 2007).

Uropathogenic and asymptomatic bacteriuria *E. coli*

Other therapeutic strategies against UTIs involve inactivated vaccines based on the whole bacterial cells containing "killed" microorganisms and live (attenuated) vaccines containing viable ("live") microorganisms with weakened pathogenic properties obtained by reducing virulence of selected pathogens. Due to the possibility of reversion of the attenuated strains to their virulent forms, the inactivated vaccines may be more important for the prevention of UTIs in humans. From this group of vaccines, Solco Urovac is already available on the market. This bacterial vaccine is a lyophilisate consisting of six heat-killed uropathogenic *E. coli* (classified into different serotypes) and selected strains of *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Morganella morganii*. Clinical studies revealed high efficacy of Solco Urovac and thus it was approved for administering for vaginal suppository use in the treatment and prevention of recurrent UTIs of bacterial origin in women. Therefore, Solco Urovac seems to be the most promising alternative to standard antibiotics used during UTI therapies (Kochiashvili *et al.*, 2014; O'Brien *et al.*, 2018).

Among current therapeutic strategies, there is also an immunostimulant OM-89 substance known in Europe as Uro Vaxom and distributed by EurimPharm GmbH pharmaceutical company (Neto *et al.*, 2016). Om-89 is a lyophilized bacterial lysate of 18 strains of uropathogeic *E. coli* most often responsible for UTIs. In 2008, European Society of Urology recommended the use of OM-89 to prevent uncomplicated UTIs in women. Uro Vaxom may also provide supportive therapy in cases of recurrent and chronic UTIs caused by the UPEC strains (cystitis, pyelonephritis, urethritis, bladder infections and ureters after catheterization) (Neto *et al.*, 2016). In Poland, Uro Vaxom is used for treating acute uncomplicated UTIs in adults and children over 4 years old in combination with antibiotic therapy and as a prophylactic regimen to prevent recurrent infections of the lower urinary tract (Wolski *et al.,* 2017). The efficacy of Uro Vaxom has been also confirmed in treatment and prevention of UTIs in patients after renal transplantation. This immunotherapeutic formulation may also have the desired effect in people with neurogenic bladder dysfunction and undergoing catheterization (Naber, 2009; Beerepoot, 2013; Wolski *et al.*, 2017).

Recent studies have been focused on the strains of *E. coli* (with reduced virulence) responsible for asymptomatic bacteriuria (ABU) to prevent the occurrence of symptomatic, chronic or recurrent urinary tract infections in humans. This approach is based on the mechanism of bacterial interference (after asymptomatic colonization of the bladder) with uropathogens (Stork *et al.*, 2018). Bacterial interference is a promising alternative to the classic antibiotic therapy used during UTI treatment, and its main aim is to prevent colonization of the bladder by invading uropathogens. The key point in this

therapy is a selected strain of *E. coli* (ABU model isolate 83972) which is not able to express type 1 and P pili, and F1C fimbriae. A specific feature of this strain is faster rate of colonization of the bladder through the planktonic growth or biofilm development when compared to uropathogens. This in turn results in long-term therapeutic persistence of ABU bacteria in the urinary tract without significant symptoms and pathology, and the recurrent and symptomatic UTIs prevention (O'Brien *et al.*, 2018; Stork *et al.*, 2018). Current investigations have also contributed to the identification of three other ABU strains (isolates 61, 106 and 123) effectively interfering with colonization of the model ABU isolate 83972. In addition, these ABU isolates were characterized by low *in vivo* virulence, lack of marked cytotoxic activity and susceptibility to antibiotic therapy. The studies were based on a mouse model of sepsis. Therefore, the above ABU strains represent an alternative therapeutic and preventive tool (to ABU model strain 83972) against uropathogens that can function as a safe, asymptomatic competitor of the bladder colonization (Stork *et al.*, 2018).

Importantly, further examinations are still required to select a universal vaccine target (not affecting intestinal commensal microflora) that could be an alternative to the antibiotic-based prophylactic regimens in women with recurrent UTIs.

VACCINES BASED ON BACTERIAL SIDEROPHORES, TOXINS AND PROTEASES

Systems involved in bacterial ferric uptake seem to be a novel promising target for the preparation of vaccines targeting uropathogenic strains which use iron ions as a factor necessary for bacterial colonization and their survival in the urinary tract environment which is deficient in nutrients (Garcia *et al.,* 2011; Brumbangh *et al.*, 2013). Generally, these types of vaccines are based on the ferric chelators responsible for the transport of bound iron ions into the bacterial cell (such as FyuA, yersiniabactin ferric uptake receptor, IutA, aerobactin iron transport receptor, IreA, siderophore receptor iron-responsive element, IroN, iron uptake receptor) and systems used to acquire a haem group by bacteria (Hma, haem acquisition protein) (Brumbangh *et al.*, 2013). Universally, vaccination of the tested animals with iron receptors induced protective immunity in experimental models of UTIs (Mike *et al.*, 2016). Therefore, immunization of mice with FyuA receptor and Hma protein had shown their preventive role against pyelonephritis. In turn, immunization with IutA and IreA resulted in reduced of colonization of the mouse bladder by bacteria (Alteri *et al.*, 2009; Brumbaugh *et al.*, 2013). Additionally, protective function of the IroN receptor against experimental UTI was stimulated in the rat model. In this case, a specific humoral response against the receptor was elicited (Russo *et al.*, 2003). Success of these experimental studies in animal models emphasizes the important role of selected siderophores as an alternative treatment of UTIs in humans.

Another potential targets for the rational vaccine design and development can be proteases and toxins which play an important role in the pathogenesis of UTIs. This group of vaccines includes toxoids and recombinant vaccines (Flores-Mireles *et al.,* 2015). Toxoids are purified and inactivated bacterial metabolites – toxins, with the ability to stimulate an immune response associated with the production of specific antibodies. Haemolysin (HlyA) which is a UPEC pore-forming cytolysin destabilizing the host cell membranes, arises the greatest interest in the vaccine context (Dhakal & Mulvey, 2012). Immunization of mice with HlyA contributed to reduction in renal scarring, but did not provide protection against renal colonization by UPEC strains (O'Hanley *et al.,* 1991; Sivick & Mobley, 2010). Therefore, further studies of these types of vaccines are required for the development of useful therapeutic strategies.

SMALL COMPOUNDS INHIBITING BACTERIAL ADHERENCE

Detailed studies of machinery driving the biogenesis of pili/fimbriae via the classical chaperone/usher secretory pathway in Gram-negative bacteria, as well as bacterial adhesion based on the interactions with the host receptors, allowed to design two classes of compounds. The first group of such potential antivirulence therapeutics inhibits biological functions of surface-exposed adhesive appendages and the second one blocks their bioassembly on the surface of bacterial cells (Pinkner *et al.*, 2006; Cusumano *et al.*, 2011; Piątek *et al.*, 2013; Dang *et al.*, 2014).

Mannosides and galactosides

The first strategy for protection against UTIs is based on the compounds inhibiting bacterial adherence by blocking the function of pili/fimbriae, and the subsequent stages of uropathogenesis, including: bacterial colonization, invasion and formation of intracellular bacterial communities (IBCs) (Pinkner *et al.*, 2006; Cusumano *et al.*, 2011; Piątek *et al.,* 2013; Dang *et al.*, 2014). Additionally, in comparison to treatment of UTIs with antibiotics (carrying the risk of development of bacterial resistance) the antiadhesion therapy does not generate selective pressure and induction of mutations in the adhesive subunits (Tomašić *et al.*, 2014).

A good example of compounds of this type are mannosides, the diarylated derivatives of mannose readily reaching the bloodstream, that are low-molecular weight molecules and analogues of a cellular receptor recognized and bound by uropathogens. These compounds have the ability to block the adherence of bacteria to epithelial cells mediated by FimH adhesin functioning as the tip subunit of type 1 pili (of *E. coli fim*$^+$) (Han *et al.*, 2010; Han *et al.*, 2012; Cusumano *et al.*, 2011, Klein *et al.*, 2010). The FimH receptor-binding lectin domain recognizes the mannose epitopes and mannoside glycans conjugated with the host receptor of uroplakin Ia, that coats the surface of umbrella urothelial cells lining the urethra and bladder. Therefore, the action of monovalent mannosides is based on the binding of FimH adhesin and blocking bacterial adherence to mannosylated cell receptors (Han *et al.*, 2010; Kleeb *et al.,* 2015; Sauer *et al.*, 2016).

D-mannose plays an important role in the human metabolic processes and post-translation modifications of some proteins by glycosylation (Gordon, 2000). In addition, like mannosides, D-mannose blocks the binding of FimH adhesin to mannosylated receptors, but the inhibition of the lectin pocket mediated by mannosides is 1 000 000-fold greater, which increases their potential therapeutic role (Bouckaert *et al.*, 2005; Han *et al.*, 2010). The use of D-mannose as a dietary supplement also improves the health condition of humans receiving it (Hu *et al.*, 2016). Therefore, the X-ray crystal structures of the FimH bound to α-D-mannose or its derivatives and the structural data concerning conformational conversion (between an elongated – mannose binding or compact

form) of the FimH lectin domain were the most important for the rational design and synthesis of mannosides as the bacterial FimH antagonists (Han *et al.*, 2012; Sauer *et al.*, 2016).

The activity of mannosides as FimH monovalent inhibitors and orally bioavailable therapeutic agents has been confirmed with mouse models (Cusumano *et al.*, 2011; Han *et al.*, 2012). These animal studies highlighted the role of mannosides as the original therapeutic strategy against UTIs, catheter-associated infections (CAUTIs), and also multi-drug resistant UPECs by decreasing the antibiotic resistance rate (Guiton *et al.*, 2012; Cusumano *et al.*, 2011; Totsika *et al.*, 2013; Kostakioti *et al.*, 2012). It is also possible to combine mannosides with the PapG adhesin antagonists which are galabiose-based inhibitors causing a reduction in adherence of uropathogenic P- and type 1-piliated *E. coli* strains that contain both, the pap and fim gene clusters (Brumbaugh & Mobley, 2012; O' Brien *et al.*, 2018).

Currently, scientific interests are focused around multifunctional inhibitors, such as glycodendrimers with high affinity to leguminous, bacterial, and mammalian lectins (Touaibia *et al.*, 2007; Lindhorst *et al.*, 2010; Cecioni *et al.*, 2015; Mousavifar *et al.*, 2018). In comparison to monovalent mannosides, multivalent compounds possess several copies of mannoside branching residues as the ideal target for blocking bacterial adherence, mediated by the FimH subunit, and hence infection. However, further studies concerning the pharmacokinetic properties of glycodendrimers are needed for their future use as the potential FimH multivalent antagonists of UPECs (Mousavifar *et al.*, 2018).

The newest group of FimH antagonists consists of modified and optimized *ortho*-biphenyl galactosides and galactosaminosides with increased potency. This is related to the fact that besides mannose, FimH adhesin binds terminal galactose (Gal) and N-acetylgalactosamine (GalNAc) residues in the core-1 and -2 O-glycans decorating glycoproteins in the bladder. The adhesive subunit capping the tip of type 1 pili is also able to recognize the Thomsen−Friedenreich (TF) antigen (Galβ1−3GalNAc) on the kidney epithelium, which contributes to kidney infections. These compounds represent low molecular-weight glycomimetics with increased inhibitory activity when compared to the initial lead GalNAc 1. Such compounds can be potentially used after further optimization for potential treatment and prevention of chronic UTIs via blocking the bacterial FimH binding to the inflamed kidney and bladder tissues (Conover *et al.*, 2016, Maddirala *et al.*, 2018).

Pilicides

The second group of novel compounds for UTI treatment includes pilicides, derivatives of the ring-fused-2-pyridone which are small molecules inhibiting the bioassembly of mono- and polyadhesive structures of the CUP (Åberg & Almqvist, 2007). These innovative compounds can be a promising alternative to commonly used antibiotics and therapeutics in the treatment of UTIs. Pilicides were originally designed and described as compounds that inhibit biogenesis of type 1 pili of UPEC *fim*$^+$ (Pinkner *et al.*, 2006; Svennson *et al.*, 2001).

The action of these compounds is targeted to periplasmic chaperones (FimC and PapD, encoded by the *fim* and *pap* operons, respectively) of the CUP due to the high degree of sequence and structure conservation. Preliminary studies based on the crystal structure of PapD chaperone protein bound to the C-terminal peptide of the PapG adhesin, allowed to identify the potential region of interaction between pilicide (mimicking the donor strand of PapG) and the chaperone. This region includes a conserved R8(Arg)-K12(Lys) motif of a positively charged anchor within a large cleft between both, the N and C-terminal domains of the chaperone (Svensson *et al.*, 2001; Pinkner *et al.*, 2006; Aberg & Almqvist, 2007; Chorell *et al.*, 2010). Further examinations using the PapD-PapK and pilicide-PapD crystal structures had shown the presence of a second region covering the F1-G1 loop outside the cleft of the chaperone. This region is required for the activity of pilicide by preventing binding of the chaperone-pilus subunit complexes to the usher *via* a reaction of donor strand exchange, DSE, and thus disrupting bioassembly of pili. However, interaction of pilicide with the F1-G1 region did not interfere with formation of the chaperone-pilus subunit complex through a donor strand complementation (DSC) mechanism. Ultimately, the action of these compounds resulted in the inhibition of type 1 or P piliation on the surface of bacterial cells and thus the decrease in adherent properties of bacteria. In this situation, unbound bacteria could be effectively washed out of the urinary tract by the flow of urine during micturition (Pinkner *et al.,* 2006).

Studies based on pilicides indicate a broad-spectrum activity of such compounds in relation to the pili/fimbriae bioassembled *via* the CUP (Greene *et al.*, 2014). Distribution of the CU system in a wide-range of Gram-negative bacteria additionally confirms their usefulness. For example, a single species of UPEC may contain up to 16 genetically complete CUP gene clusters (Wurpel *et al.*, 2013). Importantly, in UPEC strains possessing multiple CUP operons, gene expression is strictly controlled. Therefore, at a certain time, a single UPEC cell is able to expresses only one type of the CUP pili (Nowicki *et al.*, 1984).

A modification of the pilicides by a substituent at position 2 (e.g., aryl group) in combination with a substituent at position 8 (e.g., cyclopropyl group) resulted in compounds which had reduced hemagglutination and biofilm development mediated by *E. coli fim*$^+$ or *pap*$^+$ very efficiently. The observed effect was associated with a decrease in the surface level of type 1 or P piliation, respectively (Aberg *et al.*, 2005; Aberg *et al.*, 2007; Cegelski *et al.*, 2009; Pinkner *et al.*, 2006; Chorell *et al.*, 2010; Dang *et al.*, 2014).

Further studies have shown that other derivatives of 2-pyridone (with a substituent of CH2-1-naphthyl at C7 and cyclopropyl or phenyl at C8, respectively) decreased the expression level of Dr fimbriae on the surface of bacterial cells by blocking the CUP and thus reducing the adherence of *E. coli* Dr$^+$ strains (an etiologic agent of pyelonephritis in humans and mice) to epithelial cells (Piątek *et al.*, 2013).

Recent studies revealed that ring-fused 2-pyridone ec240 pilicide was able to reduce the motility of flagella and interfere with regulation of the CU system, driving the assembly of surface-exposed type 1 and P pili, and S fimbriae, often encoded by UPEC. Examinations based on the *E. coli* UTI89 model strain of cystitis indicated a significant effect of the analyzed compound on the type 1 pilus expression. Gene expression of the *fim* operon is regulated by a *fimS* promoter element, a reversible DNA segment which can oscillate between two orientations: phase on and phase off. The action of ec240 was associated with the induction of *fimS* phase off orientation and increasing the level of S-fimbrial transcription regulators

of SfaB and SfaX, and the P-pilus PapB regulator (which also promotes *fimS* phase off) (Green *et al*., 2014).

Additional experimental studies had also shown good efficacy of pilicides in disrupting pilus bioassembly in *Haemophilus influenza* and *Klebsiella pneumonia,* highlighting their broad spectrum of activity (Kline *et al*., 2010; Flores-Mireles *et al*., 2015).

Curlicides

Another group of potential therapeutic agents is represented by curlicides which are peptide mimetic compounds exhibiting pilicide-like activity. Curli (amyloid-like aggregation fibres) are surface-exposed, long, elastic adhesive filaments with the ability to interact with host glycoproteins and stimulate autoaggregation of bacterial cells. Thus, these amyloid fibres enable biofilm formation and survival of bacteria in the external environment (Goyal *et al*., 2014). They also exhibit a typical structure and physical characteristics of the amyloid fibrils observed in the course of some human degenerative diseases, but they do not the result from misfolding of the proteins (Chapman *et al*., 2002; Wang *et al*., 2007; Dueholm *et al*., 2011).

Curli are composed of CsgA subunits (curlin) that are the major component of the fibers. The CsgB protein (the minor curli subunit) is bound to the cell surface and acts as the initiator of the extracellular polymerization of CsgA subunits forming curli with a helical configuration. Both proteins are synthesized in the form of precursors with Sec signal sequences and their secretion at the surface of bacterial cells is dependent on soluble accessory factors, such as CsgE/CsgF and CsgG lipoprotein, that form an oligomeric curli-translocation channel in the outer membrane (Loferer *et al*., 1997; Robinson *et al*., 2006; Nenninger *et al*., 2009; Goyal *et al*., 2014). In the UPEC strains, the assembly of curli requires proteins encoded by two operons, *csgDEFG and csgABC* (*curli specific genes BAC*) located next to each other but transcribed independently. The proteins encoded by the *csgDEFG* operon allow the extracellular translocation of CsgA subunits and coordinate formation of surface-exposed adhesion structures. In turn, the CsgB protein of the *csgABC* operon is essential for nucleation and incorporation of CsgA self-assembling subunits into a growing amyloid-like fiber, whereas CsgC is an oxidoreductase whose function is unknown (Hammar *et al*., 1996; Bian *et al*., 1997; Chapman *et al*., 2002; Barnhart & Chapman, 2006).

Similarly to pilicides, curlicides are bioassembly inhibitors of type 1 pili and curli fibers. Therefore, they provide protection against Aβ aggregation and formation of amyloid appendages on the surface of bacterial cells (Chorell *et al*., 2011). Studies based on the mouse cystitis model have demonstrated the effectiveness of low molecular curlicide FN075 in inhibition of the type 1 pili production and biogenesis of amyloids by UPEC strains. Ultimately, this compound is also able to reduce the formation of biofilm by bacteria and their virulent properties (Cegelski *et al*., 2009).

Although the results obtained are promising, further research is needed, especially with the mouse models of UTIs to determine the role of the above compounds in the infections caused by Gram-negative bacteria and evaluate the efficacy and bioavailability of both, the pilicides and curlicides, as the potent innovative therapeutic agents.

BACTERIOPHAGE THERAPY

Due to increasing and continuing resistance of uropathogens to commonly used antibiotics worldwide, bacteriophages seem to be an excellent, alternative therapy in treating patients suffering from UTIs. It is also possible to combine phagotherapy with antibiotic therapy to increase the effectiveness of treatment. Currently, phage therapy functions as a registered medicine in several countries of Eastern Europe, including Georgia, Ukraine, Armenia and Russia (Abedon *et al*., 2011; Chanishvili, 2012; European Centre for Disease *et al*., 2017; Leitner *et al*., 2017). In the Western world, the use of phage therapy against various bacterial infections has not been approved by regulatory authorities. There is also no legal basis for the use of this type of treatment or even requirements regarding its safety and quality (Pirnay *et al*., 2011; Pirnay *et al*., 2015).

The mechanism of bacterial antibiotic resistance differs from the mechanism of resistance to bacteriophages. In comparison to antibiotics, bacteriophages have many advantages, such as self-replication, self-evolution and display a selective bactericidal activity *in vivo* and *in vitro* (without affecting the physiological bacterial microflora). Although the lytic activity of bacteriophages has been repeatedly tested as a therapy against various infections caused by antibiotic-resistant bacteria, applications of bacteriophages in the treatment of UTI are uncommon (Arshba & Bagdoeva, 1965, Danilova, 1996; Letkiewicz *et al*., 2010; Chanishvili, 2012). The main limitation of phage therapy is the low number of studied patients, lack of randomized, placebo-controlled studies and deficiency in double-blind randomized control trials that are performed in accordance with Western standards (Collins & MacMahon, 2001; Chanishvili, 2012; Ujmajuridze *et al*., 2018). However, it should be taken into account that clinical, non-randomized observational studies are also credible and have high value as randomized trails (MacMahon & Collins, 2001; Vandenbroucke, 2004).

The efficacy and safety of bacteriophage therapy was demonstrated for treating UTIs in patients with transurethral resection of prostate and after kidney transplantation to prolong the survival of the allograft, catheter associated UTIs and urosepsis (Gorski *et al*., 2003a, b; Weber-Dąbrowska *et al*., 2003; Nicolle *et al*., 2005; Hooton *et al*., 2010; Leitner *et al.,* 2017; Bonkat *et al*., 2018; Ujmajuridze *et al*., 2018). During treatment, after exceeding the threshold dose, bacteriophages were found in the urine and it did not cause their inactivation. In addition, histopathological examination of the kidneys and bladder did not show any pathological changes associated with administration of phages. Very often, antibiotic sensitivity or resistance of bacteria were not correlated with the phages tested. Thus, the bacterial strain could be resistant to all tested antibiotics but susceptible to all bacteriophages. On the other hand, the strain could be sensitive to certain antibiotics and resistant to all studied bacteriophages (Weber-Dąbrowska *et al*., 1987; Perepanova *et al*., 1995; Sybesma *et al*., 2016; Leitner *et al., 2017;* Ujmajuridze *et al*., 2018). All of these data indicate that phages may be present at relatively high concentrations, without harmful effect in the urinary tract, within which they can constitute a local anti-bacterial defense against invading uropathogens. Despite many successes associated with phagotherapy, its effectiveness and safety during treatment, further research is required to finally verify and apply this method for common therapy and prevention of UTIs in the Western word.

CONCLUSIONS

UTIs are one of the most common microbial infections affecting millions of people annually. The most serious problem with infections of this type are chronicity

and recurrences entailing huge costs from state budgets for the treatment of UTIs and complications associated with them worldwide. An additional problem is the continuously increasing resistance of uropathogens to commonly used antibiotics resulting from the need to repeat antibiotic therapy in case of recurring or chronic episodes of infection and also from the usual abuse of antibiotics (Stamm & Norrby 2001; Foxman *et al.*, 2002; Guglietta, 2017).

The major etiologic factor of complicated and uncomplicated UTIs are UPECs which account for 65% and 75% cases of infection, respectively. Up to 68% of recurring UTIs are caused by UPECs (identical to the original strain) within one year of the initial infection. UPECs are also currently the most common cause of hospital-associated infections (Foxman, 2014; Flores-Mireles *et al.*, 2015; Guglietta, 2017).

The conventionally recommended antibiotic therapy for UTIs (typically with trimethoprim, sulfamethoxazole, ciprofloxacin and ampicillin) is effective (Foxman, 2010; Kostakioti *et al.*, 2012). However, the increasing threat of antibiotic resistance mechanisms among uropathogens creates the need to develop alternative forms of treatment and prophylaxis of UTIs which would allow to reduce the suffering and improve the quality of life of afflicted persons. The targets of such antivirulence therapies, including the key virulence determinants of pathogens, differ from those used in conventional antibiotic therapy. Novel approaches are also responsible for pathogen neutralization and host protection from the disease. In this context, new therapeutic strategies and mechanisms (such as vaccines, receptor analogues, pilicides/curlicides, bacterial competitors or phagotherapy) based on the processes important during UTI pathogenesis, appear to be the most promising.

Conflict of interest statement

The Authors declare no conflict of interest

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