VIRULENCE FACTORS OF *Escherichia coli* AS ANTIGENS IN VACCINES AGAINST URINARY TRACT INFECTION

Data de aceite: 01/03/2023

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KEYWORDS: *Escherichia coli*; virulence factors; vaccine antigens; urinary tract infections.

SHORT DESCRIPTION OF THE TOPIC TO BE PRESENTED IN THE CHAPTER/SUMMARY:

Escherichia coli is the main agent of urinary tract infection (UTI) among uropathogens isolated of community-acquired or hospital infections around the world.

Because, in general, UTI treatment is empirical, it is a medical condition in which both antibiotic prescription and consumption are high, generating strong social, economic, and people health impact.

As the antibiotic therapy of UTI is directly associated with the increase of antimicrobial resistance rates of uropathogens, including *E. coli*, alternative strategies to antibiotic use to treat or prevent UTI, are urgent.

A promising option is the development of vaccines against UTI using *E. coli* virulence factors as antigens.

THE PROBLEM OF THE RESISTANCE OF UROPATHOGENS TO ANTIMICROBIAL AGENTS: WHY URINARY TRACT INFECTION THERAPY CAN FAIL?

Urinary tract infection (UTI) is among the most common infectious diseases, characterized by an excessive number of medical consultations in public and private health services (Erdem *et al.*, 2018; Reis *et al.*, 2016; Flores-Mireles *et al.*, 2015; Foxmann *et al.*, 2014; Gupta *et al.*, 2011).

In cases of symptomatic UTI, the number of medical visits can exceed 7

million in the U.S. and 100,000 of hospitalizations, annually. In hospitals, UTI is the most common hospital-acquired infection and the second most common cause of bacteremia (Stamm, 2002). Therefore, UTI generates a large economic impact with annual cost to the American Health Care System of around $1.6 billion (Foxman *et al.*, 2003).

UTI is more common in elderly and women (Hooton *et al.*, 2012). Etiological agents causing UTI in community can differ of the uropathogens of hospital environment (Wilson *et al.*, 2004; Foxman, 2003). Gram-negative bacteria belonging to the *Enterobacteriaceae* family are generally involved with UTI cause, being *Escherichia coli*, mainly the uropathogenic pathotype (UPEC), the most frequent and common uropathogen both in community-acquired or at the hospital, complicated or uncomplicated UTIs (Terlizzi *et al.*, 2017; Flores-Meirelles *et al.*, 2015; Foxman 2014; Lo *et al.*, 2013). Beyond *Escherichia coli*, other Gram-negative bacteria such as *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, the yeast *Candida* spp. and Gram-positive bacteria as *Staphylococcus saprophyticus* and B *Streptococcus* can be involved in UTI cases (Flores-Meirelles *et al.*, 2015; Foxmann *et al.*, 2015)

Antibiotics are often prescribed in the treatment of UTIs, although the resistance dissemination is not a new and it being widely reported, particularly in developing countries. The increased number of uropathogens resistant to antibiotics is related with the way by UTIs are treated, usually happening before of the urine cultures results and susceptibility tests be obtained and analyzed. Antibiotic therapy is based on epidemiology data, main etiological agents and antimicrobial susceptibility profile obtained in surveillance studies. So, the therapeutic regimens using different antibiotic may vary according to country and region, hospital, or community due the access to local epidemiology data (Cuba *et al.*, 2014; Terlizzi *et al.*, 2017).

The World Health Organization (WHO) recognizes bacterial resistance to antimicrobials as a growing threat to global health (WHO, 2014).

Studies show the exponential growing of antimicrobial resistance for all infections caused by Gram-negative bacteria, including the UTI (Mydock-McGrane *et al.*, 2017; Erdem *et al.*, 2018).

Antimicrobial resistance is the main cause of fail of UTI treatment. Indiscriminate use of antimicrobials can exert selective pressure under bacteria and favor the predominance of multidrug-resistant microorganisms (Galindo-Mendez, M. 2018). Multidrug-resistant bacteria produce a series of resistance mechanisms that make the antibiotic inactive (Seki *et al.*, 2013; Chagas *et al.*, 2018; Galindo-Mendez, 2018).

NEW ANTIBIOTICS OR NEW ALTERNATIVES TO TREAT URINARY TRACT INFECTION?

UTI can be considered challenging infections, mainly because the large number of

occurrences each year and antimicrobial resistance (Wilson et a., 2004; Gupta *et al.*, 2011; Flores-Mireles *et al.*, 2015; Terlizzi *et al.*, 2017; Bader *et al.*, 2020).

As antibiotics options for UTIs treatment are increasingly limited, consequently, there is a great necessity in the development of new antimicrobial drugs to treat UTI (Livermore *et al.*, 2004).

But, due the evolution of antimicrobial resistance, the production of new antimicrobial drugs is not profitable to pharmaceutical industries (Cole *et al.*, 2014).

Since 1940s, with penicillin commercialization, the discovery of new compoundswith antimicrobial action was accompanied by the accelerated increase of resistant bacterial pathogens, discouraging the industries (Demain *et al.*, 2011; Cole *et al.*, 2014). In 30 years, few molecules with antimicrobial activity have been approved for use in humans (von Nussbaum *et al.*, 2006; Demain *et al.*, 2011; Cole *et al.*, 2014).

The lack of new antimicrobials becomes alarming at a time when resistant infections compromise human health (Cole *et al.*, 2014; Poirel *et al.*, 2018).

New alternatives to avoid the antibiotic use have been investigated to treat or prevent urinary tract infection (Nathan *et al.*, 2012; Cole *et al.*, 2014; Mydock-McGrane et a., 2017).

Vaccines of virulence factors are an example of new alternatives to prevent UTIs (Mobley *et al.*, 2016; Mydock-McGrane *et al.*, 2017).

PATHOGENICITY AND VIRULENCE OF *ESCHERICHIA COLI*

The bacterial species, *Escherichia coli*, described by Theodor Escherich, is the most prevalent facultative Gram-negative bacillus in normal microbiota of the human gastrointestinal tract (Eisenstein *et al.*, 1988).

E. coli produces many virulence factors that allow it to resist to host immunological defenses and, to escape to body regions far from competition of other bacterial species (Johnson *et al.*, 1991; Tenaillon *et al.*, 2010).

Clinically relevant *E. coli* strains to humans can be classified into 3 major groups: *commensal strains*, *intestinal pathogenic strains, and extraintestinal pathogenic strains.* Commensal strains of *E. coli* are in fecal microbiota in most healthy humans and typically lack the specialized virulence factors present in pathogenic strains (Russo *et al.*, 2000). Intestinal or diarrheagenic strains cause diarrheic syndromes clinically variable, according to the virulence of the strain and its differences permit the classification of *E. coli* strains into pathotypes [entero-hemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC)]. Extra-intestinal strains [extra-intestinal pathogenic *E. coli* (ExPEC)] colonizes the human intestine, but they are the unique with the ability to enter and survive within normally sterile extra intestinal sites and cause disease (Vila *et al.*, 2016). Among the ExPEC, Uropathogenic *Escherichia coli* (UPEC) is the most prevalent as etiological agent of urinary tract infections (UTIs). UPEC produces several virulence

factors or determinants, a lot of them acquired by horizontal gene transfer from other Gram-negative bacterial species (Terlizzi *et al.*, 2017, Vila *et al.*, 2016).

Pathogenicity and virulence go together; pathogenicity can be defined as the bacterial ability to cause disease and virulence is intricately linked to the degree or severity with as bacteria can cause the infection in a particular host.

In *E. coli*, virulence is the result of the action of different virulence factors, which also are used to distinguish potential pathogens from harmless strains. *E. coli* is one of the most important pathogens humans associated with diarrhea and extra-intestinal infections such as UTIs and meningitis (Johnson *et al.*, 1991; Vila *et al.*, 2016).

THE ROLE OF THE VIRULENCE FACTORS IN THE URINARY TRACT INFECTION BY *ESCHERICHIA COLI*

Escherichia coli produces virulence factors, structural and secreted, that allow bacteria to colonize the urinary tract and persist in it, despite the presence of host defense mechanisms (Hannan *et al.*, 2012; Shah *et al.*, 2019).

Fimbria and non-fimbrial adhesins, lipopolysaccharides (LPS) of the outer membrane are examples of structural virulence factors; siderophores and toxins are example of secreted virulence products (Johnson *et al.*, 1991; Terlizzi *et al.*, 2017; Shah, *et al.*, 2019).

UTI starts when *E. coli* climbs through the urethra and access the bladder, after colonization of the periurethral area, coming from gastrointestinal tract (Yamamoto *et al.*, 2007). In urinary bladder, bacteria use fimbriae (type 1 fimbria and P-fimbrial), in adhesion structure to bind to epithelial cells (Hannan *et al.*, 2012). An adhesin named FimH located at the tip of type 1 fimbria promotes the specific adhesion to urinary tract cells (Tchesnokova *et al.*, 2011; Shah *et al.*, 2019; Dias *et al.*, 2010; Basu *et al.*, 2013; Tabasi *et al.*, 2016). P-fimbrial contributes to bacterial dissemination in the urinary tract, promoting bacteriuria and stimulating the production of cytokines (Stamm, 2006; Terlizzi *et al.*, 2017).

LPS mediate the *E. coli* ability to colonize the bladder, participate in the bacterial reservoirs development and elicit adaptive immune responses. Então

It also works as a “barrier” against the access of hydrophobic antibiotics through bacterial cell wall (Zhang *et al.*, 2013).

Siderophores are iron chelators molecules responsible to ferric iron (Fe3+) acquisition. Since iron is critical for the bacterial survival and growth, siderophores are indispensable in environment limited to iron as the urinary tract (O’Brien *et al.*, 2016).

Most virulent *E. coli* strains produce and release toxins such as the haemolysin-α, a pore-forming toxin in target cells (Justice *et al.*, 2012; Tabasi *et al.*, 2015). Haemolysin- α is associated with kidney injury, induces Ca2+ oscillations in renal tubular epithelial cells, potentiating the rise and colonization of ureters and renal parenchyma by the rupture of

normal urine flow (Nagamatsu *et al*., 2015). Cytotoxic necrotizing factor 1 (CNF1) is a bacterial toxin commonly produced by *E. coli* strains related with extra-intestinal infections cases, as UPEC. CNF1 activate regulatory GTPases in eukaryotic cells by deamidation of a glutamine residue, promoting the gene transcription and the bacterial survival (Fabbri *et al*., 2010). This toxin enables UPEC to cause extensive tissue damage, dissemination, release of nutrients from host cells and lyses of immune cells (Fabbri *et al*., 2010; Basu *et al*., 2013; Smith *et al*., 2015).

VIRULENCE FACTORS OF *ESCHERICHIA COLI* AS ANTIGENS IN VACCINES AGAINST URINARY TRACT INFECTION

An efficient vaccine against UTI would be a good option to reduce the antibiotics consumption, considering the frequency, the severity, and costs of UTIs. Promising candidate to vaccine-antigens should present epitopes conserved exposed in cell surface, be prevalent among ExPEC strains, and produce a protective immune response (Russo *et al*., 2001).

Some of virulence genes products have been considered promising antigens to vaccine against UTI by *E. coli*: *cnf*1 (cytotoxic necrosis factor Type 1), *pap*G allele III (P-fimbrial adhesin), *sfa* (S-family adhesins), *hly*A (haemolysin), *chu*A (hemoglobin receptor), *iro*N (siderophore) and *fyu*A (yersiniabactin siderophore) (Stamm, 2006; Lloyd *et al*., 2007; Fabbri *et al*., 2010; Ellis *et al*., 2010; Tchesnokova *et al*., 2011; O'Brien *et al*., 2016; Aguiniga *et al*., 2016; Lloyd *et al*. , 2007; Basu *et al*., 2013; Tabasi *et al*., 2016; Russo *et al*., 2001; Smith *et al*., 2005; Mobley *et al*., 2016; Neto *et al*., 2016).

Although the virulence products of these genes alone can stimulate the host's immune system, more effective and lasting immune responses are observed when a combination of different virulence factors with different actions in *E. coli* pathogenicity in the urinary tract infection, is used in the preparation of the vaccine in animal model. Experimental models of vaccines against urinary tract infection have addressed the analysis of certain combinations of factors that usually include adhesins and siderophores as promising antigens in vaccines against urinary tract infection, but with the lack of clinical trials results, UTI vaccines effectiveness could not be determined, yet (Magistro & Stief, 2019).

MAIN *ESCHERICHIA COLI* TARGETS USED IN EXPERIMENTAL VACCINES AGAINST URINARY TRACT INFECTION

Adhesion and iron acquisition are essential stages during the pathogenesis of urinary tract infections (UTIs) (Hagan *et al*., 2007; Snyder, *et al*., 2004).

1. Adhesins

As the bacterial adhesion is the first step in the successful establishment of infection by *E. coli*, bacterial adhesins are prime candidates as targets in vaccines (Tchesnokova *et*

al., 2008; Tchesnokova *et al.*, 2011; Vila *et al.*, 2016).

Most *E. coli* strains causing UTI (UPEC), express the *fim*H gene to the type 1 fimbria (Tchesnokova *et al.*, 2008; Tchesnokova *et al.*, 2011; Basu *et al.*, 2013; Tabasi *et al.*, 2016). FimH adhesin interacts with mannosylated surfaces by lectin and pilin binding- domains. FimH lectin domain possesses a ligand-induced binding site analogous to integrins (LIBS) that becomes exposed in the presence of the ligand. Epitopes of the lectin domain have been recognized by monoclonal antibodies but none of them inhibited the adhesion; in this case, antibodies enhanced FimH-mediated binding to mannosylated ligands increasing bacterial adhesion to urothelial cells. When the entire fimbria was used as an antigen, the anti fimbrial immune serum containing a significant number of antibodies against the lectin domain of FimH was also able to enhance FimH-mediated binding. These observations have implications for the development of adhesin-specific vaccines and may serve as a paradigm for antibody-mediated enhancement of pathogen binding (Tchesnokova *et al.*, 2011).

2. Siderophores

Several genes encoding iron acquisition factors have been detected and expressed in UPEC, such as, *ireA*, *hma*, *iut*A, *fyu*A (Tabasi *et al.*, 2016).

IreA is involved in iron acquisition. IreA expression is increased in human urine although there is variability in the degree of increased ireA expression among individuals. The degree of expression of IreA is similar in urine samples from individuals with and without a prior history of UTI. In mouse, during urinary infection process, IreA contributes to iron acquisition and in bladder colonization, evidencing its involvement as a virulence factor. IreA is considering a potential candidate to antigen to UTI vaccine because is in bacterial surface, is prevalent among UPEC strains, and provoke a protective immune response (Russo *et al.*, 2001). But, due the great bacterial genomic diversity, as well as the phenotypic differences among ExPEC strains, the probability that all or nearly all the strains in this group will express the same protein antigen against which protective antibodies can be developed is small; consequently, the efficacy of a vaccine with a single protein is doubtful. The development of a polyvalent vaccine is crucial to achieve a successful vaccine and IreA is a candidate protein for such vaccine (Russo *et al.*, 2001).

The siderophores IreA, Hma, IutA, and FyuA, can individually protect experimentally infected mice by UPEC colonization of the bladder and/or kidneys, when administered intranasally with cholera toxin as adjuvant. In human, to establish a multi- subunit vaccine, the combination of the four antigens (IreA, Hma, IutA, FyuA) generate antigen-specific antibodies IgG, as observed in vaccinated mice. Sera from women with and without UTI have been tested for these antigen-specific antibodies and results validated the iron acquisition as a target for vaccination against UTI (Mobley *et al.*, 2016).

CURRENT AVAILABLE VACCINES AGAINST URINARY TRACT INFECTION

Available vaccines against UTIs have presented a short-term role in the prevention of recurrent UTIs (Prattley *et al.*, 2020).

Safe and efficacy of some biological preparations aimed at immunoprophylaxis of UTIs have been widely described in the literature, but, until now, no efficient vaccine is available against UTI (Neto *et al.*, 2016; Magistro & Stief *et al.*, 2019).

The oral immunostimulant OM-89 (Uro-Vaxom; OM Pharma, Myerlin, Switzerland) is one of the forms of immunoprophylaxis to recurrent uncomplicated-UTI cases. It is a lyophilized preparation of membrane proteins from 18 different uropathogenic *Escherichia coli* (UPEC) strains (Magistro & Stief *et al.*, 2019). OM-89 stimulates T-lymphocytes, induces interferon production, increases IgA levels in urine, and activates monocyte derived dendritic cells (Schmidhammer *et al.*, 2002). OM-89 reduced need for antibiotic treatment for 6 months; but patient needs of one daily oral capsule for 3 months and an additional booster, for more 3 months (10 capsules/month) (Neto *et al.*, 2016).

Others biological available preparations are multi-strain cell lysates that are not restricted to UPEC but contain various species of uropathogens (Magistro & Stief *et al.*, 2019). Some examples are Urovac (administrated by vaginal mucosal) (Kochiashvili *et al.*, 2014), StroVac (parentally injected) (Zgoura *et al.*, 2020), Urvakol and Urostim (orally administered), but none of them has entered in clinical phase III trials. The sublingual spray Uromune (Syner-Med Ltd UK; Immunotek S.L. Spain) composed of inactivated whole bacterial lysates of four common uropathogens (*E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Enterococcus faecalis*) has been analyzed to determine its true clinical benefit in patients suffering recurrent UTIs (Yang *et al.*, 2018). Over the last decade many research groups have worked in the development of vaccines against UTI, using virulence factors, with good results in experimental murine model. However, in most cases, no clinical study in humans has been conducted (Huttner *et al.*, 2017).

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