VIRULENCE FACTORS OF *E. COLI*, ASSOCIATED WITH URINARY TRACT INFECTIONS

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ABSTRACT

Urinary tract infections (UTIs) are the most common type of infections second only to respiratory tract infections. Millions of UTI cases are reported each year, affecting in- and outpatients. The most frequent causative agents of UTIs are the enteric Gram-negative bacteria, among which *Escherichia coli* (*E. coli*) dominates. While most strains of *E. coli* are harmless and indeed play a beneficial role in gut health, some strains (uropathogenic *Escherichia coli*, UPEC) can cause infections when they are translocated to generally sterile body areas, such as the urinary tract.

This review presents the wide range of virulence factors of UPEC, involved in the urinary tract colonization, infection development and host tissue invasion. Cell-associated and extracellular key virulence factors such as adhesins, invasins, iron acquisition factors, factors mediating serum resistance, toxins and structural components are discussed in detail. Also, the review focuses on the process of biofilm formation, another crucial virulence factor in UPEC, responsible for UTI persistence, reoccurrence and antimicrobial therapy failure. The regulatory mechanisms involved in biofilm production are also discussed.

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Escherichia coli (E. coli) is a Gram-negative bacterial species, a member of the order *Enterobacterales.* It is an opportunistic organism, part of the human microbiota, with the potential to cause intestinal and extraintestinal infections, including severe invasive complications [1]. Members of the species belong to several phylogenetic groups, A, B1, B2, C, D, E, F and clade I [2, 3]. Nonpathogenic strains of *E. coli* are most commonly from A and B1 groups, while those causing extraintestinal infections are usually assigned to B2 and D groups [4, 5]. Extraintestinal pathogenic *E. coli* (ExPEC) can cause a variety of infections usually associated with penetration in primary sterile anatomical areas or with severely immunocompromised patients. The ExPEC group is currently represented by UPEC (uropathogenic *E. coli*), NMEC (neonatal meningitis-associated *E. coli*), SEPEC (sepsis-associated *E. coli*) and AREC (avian pathogenic *E. coli*) [4, 5].

Urinary tract infections (UTIs) are among the most common bacterial infections in both community and hospital settings, with over 150 million cases diagnosed annually worldwide [6]. In over 80%, UTIs are associated with *E. coli* [7]. Most UTIs are caused by the highly heterogeneous UPEC group. UPEC strains due to their multiple virulence factors, usually encoded on pathogenicity islands, plasmids and other mobile genetic elements, migrate from the intestinal tract, colonize the urinary tract and cause different types of UTIs [4, 5]. The virulence factors related to UTIs pathogenesis are extremely diverse and serve different functions (adhesins, invasins, iron acquisition factors, factors mediating serum resistance, toxins, structural components and etc.) [8]. They are divided into two major groups - cell-associated and extracellular virulence factors.

Cell-associated virulence factors

1. Outer membrane: lipopolysaccharides and proteins

Lipopolysaccharides

Lipopolysaccharides (LPS) are a major component of the outer membrane of *E. coli*. Due to strong immunogenicity, they play a key role in the activation of host immune system. LPS are composed of lipid A,

responsible for the toxicity of the structure, R antigen and the O antigen, which is the major bacterial somatic antigen [9]. Based on the O antigen specificity, there are more than 180 different *E. coli* serotypes, with UPEC belonging mainly to O1, O2, 04, O6, O7, O8, O14, O15, O16, O18, O21, O22, O25, 075 and O83 [10-15]. Amongst them, there is an *E. coli* subtype, which belongs to O25 serogroup and a widely spread and hypervirulent sequence type 131 – *E. coli* O25b/ST131. It is often associated with UTIs and subsequent complications such as sepsis. Furthermore, the majority of the isolates are multidrug-resistant and render the therapy with beta-lactams, fluoroquinolones and aminoglycosides ineffective [3]. Typically, uropathogenic serotypes of *E. coli* have the ability to inhibit the induction of certain cytokines in the epithelial cells, specifically IL-6 and IL-8 [16, 17]. In addition, the resistance to serum bactericidal effect (serum resistance) is also associated with specific O antigens typical for UPEC (O6, O8) [18].

Outer membrane proteins

Outer membrane protein A (OmpA) is one of the major outer membrane proteins of *E. coli.* It is associated with multiple structural and physiological functions, including maintenance of the cell shape and stability, biofilm formation, adhesion, colicins, bacteriophages receptor and F-dependent conjugation [19]. OmpA has been documented in all ExPEC strains [20]. It is believed that OmpA plays a key role in the intracellular bacterial colony formation and long-term persistence of *E. coli* in the bladder [21]. Another important outer membrane protein is associated with the *traT* gene – a transfer surface exclusion lipoprotein, which confers serum resistance and prevents bacterial death [3].

2. Flagella

E. coli is a motile organism with motility, mediated by peritrichial flagella. These surface structures, composed of the protein flagellin, are of particular importance for the UPEC virulence. The flagella are directly related to the bacterial cell adhesion and colonization of the urinary tract and biofilm production during the course of the UTI [22, 23]. A direct link has been demonstrated between the flagella production and the fimbriae production in UPEC strains, and the regulation of expression of these structures are highly linked and coordinated [24]. This is mediated by the Pap X repressor localized in the P fimbrial operon of UPEC, with Pap X overexpression leading to inhibition of the *flhDC* gene and to a reduction in flagella production and respectively to decreasing the bacterial cell motility [25, 26].

3. Adhesive structures

The bacterial attachment to the uroepithelial cell is critical for the onset and subsequent development of UTI [27]. The ability of *E. coli* to adhere to the uroepithelium, to resist to urinary flashing and colonize the urinary tract is associated with the expression of specific adhesins. These thin and extracellularly located protein filamentous organelles are called fimbriae or pili. The loss of the ability of bacterial cell to attach to and, respectively to colonize the anatomic region by adhesin-mediated recognition of specific receptors is often sufficient to render it avirulent [28]. Of note, adhesive structures (type 1, P, S fimbriae) mediate both the bacterial cell attachment process and cytokine production by T-lymphocytes, play an important role in tissue invasion and inflammation [29, 30].

Type 1 fimbriae, P, S and F1C fimbriae and some afimbrial adhesins are the most common adhesion factors found in UPEC [31-33]. They occur more frequently in UPEC than in commensal strains of *E. coli* [34].

Type 1 fimbriae

Type 1 fimbriae are extremely prevalent among both UPEC (86-100%) and commensal isolates [35-37]. They are considered to be one of the most important and critical virulence factors [38]. Type 1 fimbriae are encoded by the highly conserved *fim* operon including 9 genes (*fimA, B, C, D, E, F, G, H, I*) and its expression is under phase variation control [39, 40]. Products of the *fim* operon are structural (FimA, FimI, FimF, FimG), adhesion (FimH) and regulatory proteins (FimB, FimE) [41]. The *fimH* gene mediates the binding of the fimbriae via the FimH adhesin, located at the end of the structure, to specific D-mannose-containing receptors, found mainly on epithelial cells of the lower urinary tract and less in the renal parenchyma [42, 43]. An additional function of type 1 pili, particularly in cases of pyelonephritis, is to aid bio-

film formation by binding to urothelial uroplakin [44-46]. Studies involving targeted inactivation of FimH adhesin conducted in human and mouse models have shown a significant reduction in the potential of UPEC strains to colonize the urinary tract [47-48].

Besides type 1 fimbriae (mannose-dependent), *E. coli* also possesses type 2 fimbriae (mannose-resistant), represented by P, S and Dr fimbriae.

P fimbriae

Similar to type 1, P fimbriae are also extracellularly located, however, they do not associate with D-mannose-containing receptors but exhibit specificity for glycosphingolipid-containing structures (α-D-galactopyranosyl-1,4-β-D-galactopyranoside) [49]. Such receptors are found on epithelial cells in the kidney and renal tubules [45, 50, 51]. This explains why P fimbriae are more frequently reported in cases of ascending UTIs and pyelonephritis (61 - 91%), and much less frequently in cases of UTIs (< 30%) or in *E. coli*, representatives of the normal gut microbiota [33, 50, 53-58]. Some authors also report P fimbriae particularly strongly associated with pyelonephritis in childhood [52, 53, 55, 59].

The P fimbriae are encoded by the *pap* operon (*papIBAHCDJKEFG),* which contains structural (*papA, papJ, papG*) and regulatory genes (*papI, papB*) [41, 60]. The papG gene encodes the terminal PapG protein directly responsible for the adhesion process [58]. Based on its specificity, PapG exists in four variants (PapI, PapII, PapIII, PapIV), with PapII being the most common among UPEC strains [58, 60].

It was also found that nonpathogenic *E. coli* isolates, acquiring the *pap* operon, also acquire the ability to colonize kidney tissue [58].

S fimbriae

S fimbriae are adhesins that are encoded by the *sfa* operon [61, 62]. They exhibit specificity for a**Capsule**

In *E. coli*, more than 100 capsular polysaccharide antigens have been identified. The main function of the capsules is related to inhibition of phagocytosis and complement bactericidal activity [78]. The similarity between some polysaccharide K antigens in UPEC and tissue structures in the host organism explains the antigen mimicry phenomenon and their difficult recognition by the immune system as foreign antigens [79]. On the other hand, the UPEC polysaccharide capsule, coating the bacterial cell surface, can inhibit the adhesion process to the corresponding epithelial cells. However, binding of type 1 fimbriae to mannose-containing receptors has been shown to result in down-regulation of the *kps* operon, followed by reduced production of capsular substance and thus aiding the adhesion process [80].

Extracellular virulence factors

1. Toxins

Haemolysins

Hemolysin A (HlyA) and cytotoxic necrotizing factor 1 (CNF1) of *E. coli* are the two main toxins responsible for bacterial tissue invasion and dissemination, as well as for the dysfunction of local immune responses [32, 33].

HlyA is mainly associated with the destruction of host cells, thus releasing nutrients and other factors, such as iron, which are critical for bacterial growth. HlyA is a calcium-dependent toxin that in high concentrations forms pores in the cell membranes of the host organism leading to cell lysis. In low concentrations, HlyA can induce apoptosis in the epithelial cell and thus promote the spread of infection [29, 81]. Encoded by the *hlyCABD* operon, the toxin is found in approximately 50% of UPEC [33, 82, 83] and its expression is associated with increased clinical severity of UTIs [47]. HlyA genes have been detected more frequently in patients with pyelonephritis (38%) than in those with cystitis (15%) [33].

HlyE is another toxin of the haemolysin group [84]. Its production and action are mediated by the cytoplasmic enzyme HlyF, which increases the formation of outer-membrane vesicles containing HlyE. The presence of *hlyF* in the UPEC genome is associated with more severe UTIs such as pyelonephritis accompanied by urosepsis and induction of an exacerbated inflammatory response, a specificity that distinguishes the *hlyF* positive strains from the typical UPEC [84-86].

Cytotoxic necrotizing factor 1 (CNF1)

The effects of CNF1 are closely related to its ability to bind to specific cellular receptors (BCAM), inducing RHO GTPases activation, responsible for the control of multiple eukaryotic cell functions (actin structure formation, motility, phagocytosis, etc.) [87-90]. The

CNF1 production is associated with the induction of apoptosis of bladder epithelial cells with subsequent exfoliation, with bacterial invasion and persistence, and involvement of new cells [47, 91, 92]. The genes encoding CNF1 have been documented in about 1/3 of UPECs and are more frequent in uropathogenic than in commensal strains of *E. coli* [93, 94].

Other toxins

Spa (Serine protease autotransporter), Sat (Secreted autotransporter toxin) and Vat (Vacuolating autotransporter toxin) are also toxins found in UPEC associated with kidney injury. They exhibit cytotoxic activity against uroepithelium, cause vacuolization of renal glomeruli and tubules, exhibit marked proteolytic effects against some complement factors, degrade mucin and promote the colonization stage [95-99].

2. Iron acquisition systems

Siderophores

Iron (Fe^{3+}) is essential for life and proper functioning of all living organisms [100]. This element plays a key role in all essential processes in the bacterial cell, including the "virulence" behavior [101-103].

The human body uses several mechanisms to restrict pathogenic organisms' access to free iron: in tissues and cells, the iron is stored as ferritin, and in the blood, transferrin binds to iron with high affinity [104-106]. Stored as part of the heme (a cofactor of hemoglobin, myoglobin and cytochromes), the iron ions, are in a form inaccessible to microorganisms [101].

However, bacteria develop mechanisms that allow them to acquire iron from the host organism and thus survive and cause infections. The secretion of siderophores, iron-chelating molecules is such a mechanism. The siderophore high affinity for iron ions, especially trivalent iron (Fe^{3+}), allows its capture by ferritin and transferrin [107-108]. After iron binding, the complex is recognized and transferred into the bacterial cell via specialized transport systems. This mechanism allows bacteria to obtain the necessary amount of iron even when its level in the organism is very low. Siderophores belong to 5 main classes - catechols (cateholates), phenols (phenolates), hydroxamic acids, alpha-hydroxycarboxylates and a mixed type containing different siderophores [34]. *E. coli* has several siderophore systems - enterobactin and salmochelin (catechol siderophores), yersiniabactin (phenolate siderophore) and aerobactin (mixed type). Enterobactin is found in both pathogenic and non-pathogenic *E. coli* and its role in virulence is of less importance compared to other siderophore systems. One reason for this is that enterobactin can be inactivated by certain defense mechanisms of the host organism associated with the Lipocalin-2 protein [109]. On the other hand, the modification of this siderophore by glycosylation leads to the formation of salmochelins that manage to escape the action of Lipocalin-2 [110].

In contrast to enterobactin, the production of aerobactin, salmochelin and yersiniabactin has been demonstrated predominantly in UPEC and much less frequently in commensal *E. coli* strains [8]. Aerobactin is the most frequently studied siderophore system in uropathogenic *E. coli* and dominates as a survival mechanism [31, 111, 112]. Compared to enterobactin, aerobactin is much more efficient in iron capture and even at very low concentrations can stimulate bacterial growth [108].

The siderophores salmochelin and yersiniabactin have also been attributed an important role in the pathogenesis of *E.* coli-associated UTIs. The expression of *iroN*, encoding the salmochelin-siderophore receptor IroN, is associated with a 5- to 10-fold increase in the invasiveness in the urothelial cells [113]. Yersiniabactin has been attributed also to be important for biofilm production in *E. coli* and for the increased bacterial resistance to phagocytosis [108, 114].

In addition to siderophores, UPECs use the Hma and ChuA transporters, which are involved in the iron uptake directly from extracellular heme [8, 108]. Another transporter type that also delivers iron to the interior of the bacterial cell is SitABCD [115].

Other extracellular virulence factors

Curli protein

Curli protein is an amyloid protein encoded by the *csg* gene and secreted by many bacterial species, including *E. coli*. This protein is involved in different processes, including biofilm production, in which curli is a major component, but also in the adhesion,

colonization, invasion and in the development of an inflammatory response mediated by the release of some cytokines (IL-6, IL-8, TNF-alpha) [116-117]. Curli protein is also involved in the intercellular interaction and communication under biofilm conditions [116-117].

Intracellular Bacterial Communities (IBCs)

A specific feature of UPEC is their ability to reproduce intracellularly [8, 118]. Only after adhesion to the urothelial cell, E. *coli* can enter the host cell and form mature IBCs as a result of active replication, followed by leaving the damaged cell, infecting new cells and spreading the infection [70, 119]. The process of IBCs formation is accompanied by a change in bacterial cell morphology from coccoid to rod-shaped and filamentous shape [119]. IBCs are mediated initially by the FimH adhesin, related to type 1 fimbriae, which recognizes specific receptors on bladder epithelial cells (uroplakin, integrin), followed by activation of RHO GTPases and a process of bacterial endocytosis [120]. The *E. coli* capsular polysaccharide antigen contributes significantly to intracellular survival and IBCs formation [121].

The ability of UPEC to form IBCs is a mechanism to evade the host immune response [56]. It is thought that IBCs are a key mechanism for the development of *E. coli* UTIs [8], including recurrent UTIs [119]. The last are associated with the Quiescent Intracellular Compartments (QICs), located in cells of the underlying transitional epithelium, containing a small number of viable but non-replicating bacterial cells that can be re-activated [56, 122].

Biofilm production

In unfavorable living conditions bacterial biofilm production is an important survival mechanism. It protects the microbial cells from the innate defense factors (complement, phagocytosis), specific (immune) defense mechanisms of the host organism and is among the most important antimicrobial resistance mechanisms [123]. Biofilm production mediates microbial colonization of various medical devices, including urinary catheters, contributing to increased morbidity from both acute and chronic infections [124].

Regarding UPEC, the biofilm production underlies the pathogenetic mechanism of UTIs and significantly contributes to the UTIs persistence and recurrence and catheter-associated UTIs (CAUTIs), accounting for about 40% of all nosocomial infections [125-129]. It is biofilm formation that is one of the most important mechanisms determining the high levels of antibiotic resistance, often accompanying the UTIs [125, 127, 130].

The biofilm is a 3D community of microbial cells embedded in a self-produced extracellular polymeric substance attached to biological or non-biological surfaces [129, 131]. Under biofilm conditions, the bacterial population differs significantly from the free-living (planktonic) cell [130, 132]. Biofilm associated bacteria reduce their motility and metabolic activity to conserve energy and nutrients [130, 132]. Besides protection, this viscous substance anchors the bacterial colony to the site of infection, and the increased release of extracellular bacterial toxins provides additional nutrient release at the site of infection [133].

According to the amount of biofilm secreted, strong, medium and weak biofilm producers are differentiated among UPEC. The biofilm formation in *E. coli* is a complex process consisting of several sequential stages: a stage of reversible attachment; a stage of irreversible attachment and early biofilm development; biofilm maturation and a stage of biofilm dispersal [123, 124]. During the first stage (the reversible attachment), which is typical for freely living bacterial (planktonic) cells, the flagella production is particularly important. It ensures the cell motility and overcoming the hydrodynamic and van der Waals forces and thus mediates the attachment to the surface of epithelial cells or foreign bodies [124]. Catheters, stents or the rough stone surfaces are ideal for biofilm attachment [123]. In the reversible attachment stage, two types of bacterial populations are found, those that can permanently form flagella and those in which the expression of genes encoding these structures is repressed [124].

When the bacterial cells find optimal conditions for a "sessile" lifestyle, the attachment becomes irreversible, and the process is mediated by a diversity of microbial structures with adhesive function (type 1 pili, F pili, curli, colanic acid) [134]. Besides to epithelial cells and/or artificial surfaces, bacteria attach

to each other, which further strengthens and stabilizes the structure, a process associated with the outer membrane protein Ag43 [135-136]. The type 1, P and S pili, the Dr and F1C adhesins are thought to be critical for biofilm formation, although various studies have shown different distribution of genes encoding adhesins in *E. coli* biofilm producers [29; 57; 64, 137-139]. A systematic review and meta-analysis on virulence factors among 1888 UPEC isolated from patients with UTIs over a 10-year period (2000 - 2019) showed a prevalence of CSH (80%) and *fimH* (75.3%) among the genes encoding the group of adhesive factors [140]. Tewawong's study also found the dominance of *fimH* (91.8%) but also of *pap* genes (79.3%) and demonstrated a very high relative proportion of UPEC isolates carrying a combination of adhesins (80.3%), with the *fimH* + *pap* combination identified in 69% [141].

The inhibition of the irreversible attachment by an antibody-mediated mechanism or by downregulation of pili-encoding genes, can dramatically reduce biofilm formation [45]. The cyclic-diguanylic acid (c-di-GMP), theconcentration oh which is increased at this early phase of biofilm development, is of great importance for the microbial transition from planktonic to biofilm (sessile) form [117]. In addition, the attached bacterial cells actively replicate and increase in number, which is associated with the induction of the intercellular Quorum sensing (QS) communication system [123].

During the maturation a matrix substance is produced, and the biofilm is differentiated into a growing, well-structured 3D microbial community, with a defined architecture and spatial arrangement [124]. The mature biofilm is a dense structure, very difficult to eradicate [130, 142]. The main components of the matrix are water, exopolysaccharides, proteins, DNA and lipids [143]. It is the exopolysaccharide component that is specific and distinguishes the microbial biofilm from the planktonic bacterial form [117]. The stability and the shape of the biofilm are primarily mediated by this component represented by poly-β-1,6-N-acetyl-D-glucosamine, cellulose and colanic acid [129, 144-147]. The matrix polysaccharides are also involved in other processes: they contribute significantly to the adhesion of the cells to each other and to various surfaces and host cells, provide protection against defense host factors, mediate resistance to antimicrobials and to desiccation, act as a mechanical barrier and a reservoir of nutrients and mediate the interactions between bacterial cells [117, 143, 148, 149]. The cellulose production is specifically responsible for the formation of a rigid biofilm, and the colanic acid forms a capsule around the bacterial cells, protecting them from adverse external conditions. However, colanic acid can also inhibit biofilm formation, which is associated with masking of Ag43 and AidA [150]. In addition, by coating highly immunogenic structures, exopolysaccharides (especially cellulose) significantly reduce the immune response against the pathogen [8]. Several authors have reported that increased production of this matrix component is associated with the development of more severe and persistent UTIs [151-154].

Although with indirect effects, the LPS and the capsular polysaccharides (O and K antigens) also contribute to biofilm formation. They mediate the interaction between the bacterial cells and the environment and especially mediate the adhesion process through interaction with cell surface structures [155].

The matrix DNA and proteins are also involved in binding to and colonization of biotic and abiotic surfaces [143] and perform a variety of functions in the biofilm maturation stage (desiccation resistance, protection, nutrient supply, exchange of virulence factors, etc.) [143, 149, 156].

Except the exopolysaccharides, the autotransporter proteins are critical for the biofilm maturation and intercellular interactions [155]. Antigen 43 (Ag43), a key autotransporter, mediates the adhesion of cells to one another, the processes of auto-aggregation and formation of the 3D biofilm structure. AidA and TibA proteins have a similar function [150].

Due to unfavorable living conditions in the biofilm during its final stage (scarce nutrients, low O_2 concentration, pH changes, accumulation of toxic products, and other stressful conditions), individual daughter cells detach from the biofilm, migrate, and adhere to adjacent, new surfaces [123, 157-160]. The process of active dispersion is mediated by proteins within the matrix, responsible for its enzymatic degradation [143, 161, 162]. Passive dispersion can also occur, but under external interference, including human influence [161].

The transformation of *E. coli* from planktonic to biofilm form and vice versa is a complex process, whose regulation is strongly dependent on 3′,5′-cyclic diguanylic acid, the two-component CpxA/CpxR signaling system, the three-component protein regulatory system RcsCDB and quorum sensing [124]. The high level of 3′,5′-cyclic diguanylic acid blocks the flagellar proteins, resulting in motility loss. In addition, 3′,5′-cyclic diguanylic acid is involved in the curli, cellulose and Poly-β-1,6-N-acetyl-D-glucosamine syntesis [163]. The CpxA/CpxR system also influences motility, and this effect is mediated by cell surface chemical composition changes via OmpC activation [164]. Additionally, CpxA/CpxR inhibits curli production [165]. The RcsCDB system regulates the gene expression of structures associated with different functions - adhesive (Ag43, curli), mediating motility (flagella) and protection (capsules) [166].

Furthermore, during the process of biofilm formation in *E. coli*, multiple genes encoding stress tolerance, related to survival in adverse conditions and to biofilm protection are expressed. Products of such genes are the Hfq protein (promotes biofilm production in adverse conditions), YcfR/BhsA (induces indole production and makes biofilm resistant to acids, temperature and peroxide), AriR (an acid-resistance regulator protein associated with indole production), the sigma S factor (affects the expression of regulatory and structural genes associated with biofilm production and degradation) [168, 169].

Quorum sensing (QS)

QS is a bacterial communication system, mediated by the release of chemical signaling molecules called autoinducers or quormons, which allows bacteria to communicate and function as a multicellular organism, and coordinates their behavior [117, 169]. QS has a leading role on cell division control, bacterial movement, biofilm formation, upregulation of genes encoding virulence factors, as well as on the gene transfer between bacterial cells in the biofilm, and on the pathogen and host organism interactions through the immune response modulation [129, 136, 170-172]. The QS system is dependent on the cell density in the biofilm and coordinates bacterial behavior to maximize benefits to the microbial population in the biofilm, including optimal nutrient utilization, increased pathogenicity, and survival [173]. A minimum population number threshold is required for QS activation [160]. An inducer-receptor mechanism associated with the QS system is involved in the gene control mechanisms [173-176]. The Gram-negative bacteria use N-acyl-homoserine lactones (AHLs)-associated inducers and their corresponding receptors (LuxRs) [177, 178]. At low biofilm cell density, the inducers are secreted at very low, non-detectable levels, but as the bacterial population increases, their amount is sufficient to bind to the corresponding receptors, forming complexes involved in the gene expression control [179-183]. AI-2 is one of the most important autoinducers, directly related to *E. coli* biofilm production. Upon reaching optimal bacterial density, AI-2 production is inhibited, a process, related to *luxS* gene down-regulation [123, 171]. The AI-2 effect of increasing significantly the biofilm mass has been shown to be mediated by a specific QS motility regulator (MqsR) [172].

The biofilm in *E. coli not only* successfully prevents most of the humoral and cellular defense mechanisms of the host, but also through various mechanisms can render bacterial cells in the biofilm up to 1000-fold more resistant to antimicrobials than the planktonic forms [124]. The weak penetration of antibiotics in the biofilm-related bacteria, mediated by the matrix substance as a physical barrier and by other biofilm components such as polysaccharides, enzymes and DNA, binding or degrading the antimicrobial agents, is one of the most important mechanisms [185-187]. Another factor contributing to high antimicrobial resistance in biofilm conditions are the bacterial cells of "persister" phenotype, found in the biofilm, which are characterized by a very slow growth. Once the action of the antibiotic agent is discontinued, microbial cells with this phenotype can reactivate and cause infection [186, 188]. The presence of "persister cells" is associated with the chronic course of some UTIs [187]. Furthermore, the over-expression of some efflux pumps under biofilm conditions [187, 189], as well as high levels of horizontal gene transfer due to over-population and close physical contact between bacterial cells [124, 190], also contribute significantly to *E. coli* antimicrobial resistance and to the spreading of genetic resistance and virulence determinants under biofilm conditions.

In conclusion, *E. coli* is a well-studied enteric organism with a potential to cause many different types of infectious syndromes, among which the UTIs are most common. Apart from being able to become resistant to routinely used and strategic antimicrobials, this organism is also capable of harboring a wide range of genes, associated with increased virulence. In combination with the high frequency and severity of *E. coli* infections, and the related mortality, this places *E. coli* among the bacterial pathogens of highest public health importance.

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