Anja Tušar, Ruđer Bošković Institute (Croatia),
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URINARY TRACT AND ITS UNWANTED INVADERS

There are beneficial bacteria that live in and on our bodies as members of our microbiota. Microbiota is the name given to a community of microbes (bacteria, viruses and fungi) that colonise a particular environment and have important effects on our health. These microbiota communities are mainly found in our gastrointestinal tract, but all surfaces that come into contact with the environment are also colonised with bacteria, e.g. our skin, upper respiratory tract and genital tract. Microbiota co-exist with our body and can benefit us in various ways. Helpful bacteria prevent harmful bacteria from infecting us by neutralising toxins. Bacteria also play an important role in our defence against infections by colonising the surfaces of our body and protecting these surfaces from invading pathogens. Furthermore, they aid in the digestion of food, help our bodies absorb nutrients and produce various vitamins such as folic acid, niacin, vitamin B6 and B12. Nevertheless, there is a good reason why bacteria have a bad reputation. It is well deserved, because bacteria are responsible for a number of diseases, including urinary tract infections. Urinary tract infections (UTIs) are one of the most common bacterial infections that occur when unwanted bacteria enter the urethra and infect the urinary tract (Figure online). If you are a woman, there is around 50% chance you will get an UTI once in your lifetime. If you are a man, you are a little luckier, because the chance that you will experience at least one UTI in your life is about 15%. The symptoms of urinary tract infection that may be already familiar to you are burning sensation and a frequent urge to urinate often.

WONDERING WHY WOMEN ARE MORE LIKELY TO DEVELOP UTIS?

Women are far more likely to develop UTIs because of the anatomical differences, female body has a shorter distance between the bladder and the outside world than male body. UTIs in men are less common, but if the infection happens, it is typically more complicated because treatment is more difficult treatment and prognosis is worse.

WHO IS THE UNWANTED INVADER OF OUR URINARY TRACT?

Escherichia coli is the predominant bacterium responsible for the majority of UTIs (approximately 80% of all UTI cases). We will focus on a specific bacterium with UTI in its name, *Escherichia coli* UTI89 (*E. coli* UTI89). This bacterium is present in our healthy body as a member of the microbiota. It colonizes the gastrointestinal tract and causes no harm. The harmless *E. coli* UTI89 can, however, emerge from the colon and become incorporated into the periurethral microbiota before entering the urethra and bladder. Upon entering the urinary tract, the bacteria first multiply in the lower parts of the urinary tract, leading to various unpleasant symptoms: pain when urinating, pain in the lower back and abdomen, fever, etc. Untreated symptoms can develop into life threatening illness if the bacteria spread to the upper urinary tract cause a kidney infection or exaggerated body reaction to the infection with septic shock and organ failure (called urosepsis). *E. coli* UTI89 has been studied extensively to understand its behaviour in urinary tract infection. This bacterium has garnered significant attention in research

community due to its two different possible lifestyles during infection and its high *virulence*. Namely, *E. coli* UTI89 is able to exist both in the urinary tract lumen (extracellular lifestyle) and inside the cells of urinary tract wall (intracellular lifestyle). *High virulence* means that the bacteria are very successful in causing disease, and *virulence factors* are molecules that enable bacteria to infect and persist within the urinary tract. Our urinary tract is a hostile environment for *E. coli* UTI89, which means that the bacterium must be prepared for the fight with plethora of different virulence factors.

WHY IS THE INVADER OF URINARY TRACT SO SUCCESSFUL?

This is due to the existence of virulence factors. *E. coli* UTI89 possesses a variety of virulence factors that contribute to its ability to cause UTIs. Uropathogenic *Escherichia coli* (UPEC) have virulence factors that favour their adaptation to the urinary tract and allow the bacteria to break the barriers of a strong immune system. There are two main categories of *E. coli* virulence factors: cell surface virulence factors (such as adhesins, curli, pili, flagella and capsule) and secreted virulence factors (such as toxins, and iron acquisition systems) (Figure online).

Virulence factors attached to the surface of the bacteria, such as fimbriae and pili, serve to adhere to the epithelial cells of the urinary tract and subsequently colonise them. Without them, the bacterial cells would float in the lumen of the urinary tract and would be easily flushed out of our urinary tract with the flow of urine. Since one of the crucial steps during infection is the attachment of a bacterium to the surface of a urinary tract cell, therapeutics have been developed that prevent bacterial adhesins from binding to receptors on the surface of the urothelium.

The dynamic interaction between *E. coli* UTI89 and our immune system is a crucial determinant for the outcome of the infection. The formation of a biofilm in the lumen of urinary tract is one of the numerous strategies used by UPEC bacteria to evade or counteract the immune defences. *Biofilms* are communities of bacteria that stick together due to a self-produced matrix and adhesins. Within the biofilm, bacteria can communicate with each other, share nutrients, and avoid being killed by antibiotics or immune system cells. Recent studies provided evidence that the biofilm produced by *E. coli* facilitates the survival of UPEC in the urinary tract for a long time during antibiotic treatment. This happens because *E. coli* is protected in the biofilm by the production of a hydrophobic extracellular matrix consisting of curli and cellulose, which acts like a biofilm scaffold surrounding the bacteria. The hydrophobic matrix provides resistance to hydrophobic antibiotics. The development of a biofilm in the urinary tract is therefore often associated with complications such as pyelonephritis (kidney disease) and chronic UTIs.

The role of adhesins is not only to attach, but also invasion of the bacteria into the host cells. Special adhesins bind to the cells of the urothelium, mediate colonization, and trigger invasion into the bladder epithelial cells. When inside the epithelial cells, the UPEC bacteria hide from the host's immune response and can reach up to 10^5 bacteria per cell within hours after invasion. The high density of bacteria in our epithelial cells leads to the formation of biofilm-like intracellular communities (IBCs). The

presence of IBCs has been found to be the one to blame for recurring urinary tract infections, as the bacteria in the cells of the urinary tract have successfully escaped our immune system and antibiotics.

For colonization, the bacteria use structures made of proteins named *flagellin* to travel upward through our urinary tract system. The flagellin proteins arrange themselves into locomotion organ, called flagellum. This structure enhances the movement of bacteria from the bladder to cause infections in the kidney. Flagella participate in bacterial adhesion and invasion, and play a key role in biofilm dynamics.

Furthermore, UTI89 secretes toxins that damage the host tissue, promote infection and are a part of secreted virulence factor group. One of the most virulent secreted toxins is α-hemolysin (HlyA). HlyA is a pore forming toxin that damages the host tissue and triggers inflammation, disrupts the membranes of urothelial cells and induces cell death. Another example of a toxin secreted by *E. coli* is cytotoxic necrotizing factor 1 (CNF-1). CNF-1 production also enhances the inflammatory response of the host. UPEC strains secrete it to stimulate the production of actin stress fibers, cause scarring of the bladder epithelium, and promote bacterial invasion into the kidney cells.

Moreover, UTI89 harbours a variety of *iron uptake systems*, enabling bacteria to survive in the urinary tract by acquiring an essential nutrient that is very scarce in this environment. Lately, studies have indicated that defective production of iron-acquisition systems is associated with reduction in virulence of bacteria. The iron in our body is not easily accessible to bacteria as it is firmly attached to the proteins (for example: haemoglobin protein). Siderophores (Greek: “iron carriers”) are special molecules that have a high-affinity for binding iron and are produced by bacteria in response to iron-deficiency. When iron is scarce, the bacterium excretes siderophores from the cell. Siderophores bind the iron in the environment and are then recognized by specific receptors on the outer membrane of the bacterium. The iron-siderophore complex is then transferred into the cell with the help of these receptors, and the iron that is now inside the bacterium can be used by the bacterium.

IF WE KNOW THE INVADER, WE CAN BETTER FIGHT IT.

If our immune system is unable to fight the infection, we must seek protection through medicines, such as antibiotics. The emergence of antibiotic resistance presents a major challenge in the treatment of urinary tract infections caused by *E. coli* UTI89. Understanding the specific virulence mechanisms and their virulence factors employed by this bacterium can aid in the development of novel therapeutic strategies against this bacterial invader. Direct and indirect strategies against UPEC infections provide promising new treatment options. Direct strategies against urinary tract infection are targeting *E. coli* viability, they inhibit adhesion of the bacteria in bladder epithelium and disturb attempts of biofilm formation. Indirect strategies strengthen our immune system by stimulating infected tissues and cells to react rapidly to UPEC invasion. In addition to therapeutic approaches, the incidence of urinary tract infections caused by *E. coli* UTI89 is also reduced by preventive measures. The risk of UTIs can be significantly reduced by drinking enough water, paying attention to your personal hygiene, and avoiding harsh soaps. It holds true that it is better to prevent the invader from entering the urinary tract than to fight it. Urinary tract infections are common, but with knowledge of their causes, symptoms, and

prevention, we can be proactive and keep our urinary system healthy. Virulence factors also represent attractive targets for the development of new clinical diagnostics that can quickly and accurately detect uropathogenic *E. coli* infections in our urinary tract. The correct characterization of bacteria causing urinary tract infection is important to increase the efficiency of antibiotic treatment and reduce the rate of the bacteria acquiring resistance. By implementing advances in *E. coli* virulence research towards developing novel clinical strategies, we can keep these unwanted invaders at bay and live infection-free lives.