Experiment 14	**Bacterially induced inflammation of the urogenital tract: Virulence and pathogenicity of uropathogenic *Escherichia coli* (UPEC)**
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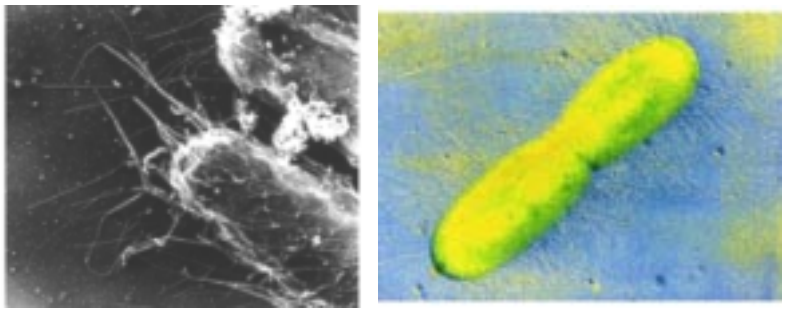
Reading	Chapters 13.10, 19.4,19.6, 19.7, 19.8, 19.11 in BBOM (9th ed.): Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition, Prentice Hall, 1999. ISBN: 0-13-085264-3
Objectives	To learn about adhesion of bacteria to epithelial surfaces as one example of virulence factors of bacterial pathogens infecting humans.
Background	**Virulence factors** Macro-organisms live intimately together with micro-organisms throughout their entire lives, and to their mutual benefit in the overwhelming number of cases. Infection is usually just an interruptive abberration of the balance. Nevertheless, the experience can be anything from mild to extremely dramatic or even fatal. In order to exert damage, bacteria either invade normally sterile compartments of the body where they multiply, or they produce toxins with general or highly specific effects. Bacteria express various virulence factors. Such factors can be (i) toxins, (ii) factors for adherence on surfaces such as epithelia, (iii) degrading enzymes for mazeration of tissue, (iv) factors promoting endocytosis, or preventing phagocytosis, (v) factors to overcome or survive mechanisms of the host defense, or combinations of factors of the 5 groups. **Urinary tract infections (UTIs)** UTIs are among the most frequent infections in both the general community and in hospital. Worldwide, ca. 150 million people contract UTIs per year, costing approximately $ 6 billion. Women are more often affected than men, although the gender differences vary with the age of the patient. Apart from the lowermost part of the urethra, which is usually colonized with various species of bacteria, the urinary tract is normally sterile. This is mostly due to the fact that bacteria moving upwards are regularly washed out by urination. Depending on the location of infecting bacteria, distinct diseases can be defined, such as cystitis (infection of the bladder) or (pyelo-) nephritis, when the kidneys are involved. Interestingly, a normal inhabitant of the human intestine, *Escherichia coli*, is by far the most frequently encountered cause of UTIs, namely in 70-95% of community-acquired, and in about 50% of hospital-acquired cases. What is the reason for this bias? **Adhesion** UTI-causing *E. coli* need to be able to adhere to the urinary tract epithelium in order to prevent being washed out. They do so by expressing specific virulence factors - in this case adherence factors. These factors are proteinacious filaments anchoring in the cell envelope. They are called pili or fimbriae:

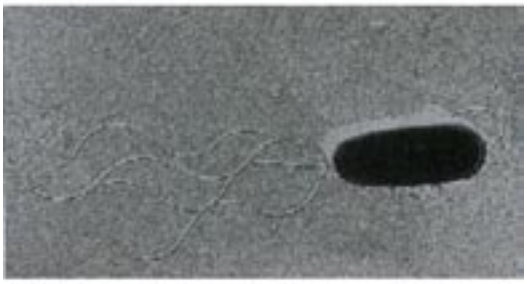


(Sources: http://www.uhmc.sunysb.edu/microbiology/dtrespix.jpg
http://telem.openu.ac.il/courses/2001a/c20237/gifs/permission/pili.jpg)

Strains of *E. coli* that express pili for adhesion on human uro-epithelia are called uropathogenic *E. coli* (UPEC).

Different kinds of pili of *E. coli* cause different kinds of disease: Type 1 pili account for cystitis, and P pili account for pyelonephritis. This is because the two types of pili each bind to specific receptors on the respective epithelia.

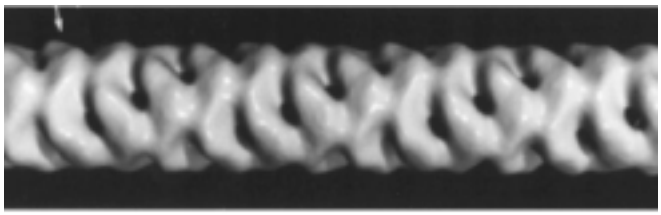
Pili are not to be confused with the longer and thicker flagellae that serve locomotion of the bacteria:



(Source: http://images.google.ch/imgres?imgurl=telem.openu.ac.il/courses/2001a/c20237/gifs/permission/pili.jpg&imgrefurl=http://telem.openu.ac.il/courses/2001a/c20237/pictures.htm&h=253&w=354&prev=/images%3Fq%3D%2Be.%2Bcoli%2Bpili%26num%3D20%26sa%3DG)

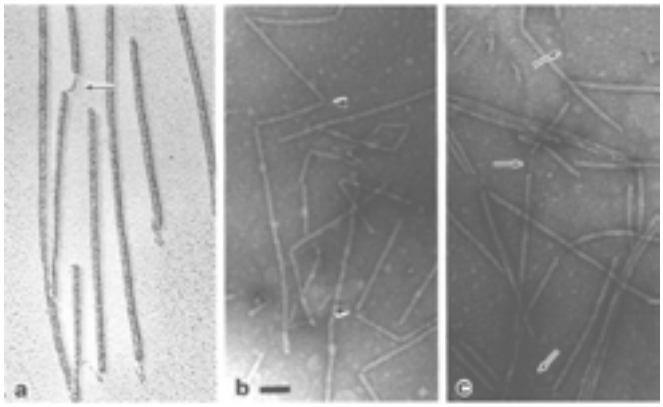
Structure of pili

Pili are helical polymerization products of one peptide subunit. The diameter of pili is 6.8 nm. One helical turn consists of 3.28 subunits, and the helical rise per subunit is 0.78 nm.



(Source: http://www.bumc.bu.edu/www/Busm/By/bullitt/newwebsite/Avg10-2.gif)

Pili are sometimes bent or otherwise damaged (see arrows in image below). However they still serve their purpose, attaching the bacterium to the host cell:



(Source: see above)

Structure and function of pili are the subjects of research, since, in light of the ever increasing antibiotic resistance that troubles the therapeutic management of UTIs, investigators seek alternative treatments, such as vaccines against or specific blockage of pilus-binding to host cells.

Literature	http://www.academicpress.com/medmicro/pdf/medmicro_81.pdf
www. Links	see above
Practical work	You will infect the human bladder epithelial cell line T24 with either a non-pathogenic strain of *E. coli* or with a UPEC strain. This infection takes place on a chamber slide with 8 wells. After incubation of 2 - 4 hours you will wash off unbound bacterial cells with buffered saline containing EDTA. This washing step will leave pilus-determined binding of bacteria to the cell surface untouched. In order to render the results visible, the assays are fixed with Me-OH followed by staining with Giemsa stain. After removal of the chambers from the slides, the latter can be examined under the microscope, and bound bacteria can be seen and counted. Moreover, the health condition of the T24 cell layer can be judged.
Material and Experimental Protocols	**Materials** Eucaryotic cells and bacteria: - Human uro-epithelia cells T24 - *Escherichia coli* DH5α: non pathogenic - *Escherichia coli* 536: UPEC Other materials: - Chamber-Slides: 

- Sterilized Phospho-buffered saline with EDTA (PBS)
- Mehanol (Me-OH) 100%
- Staining solution: Giemsa diluted 1:20 in water.
- Bright-field microscope

Protocol

<u>On the day prior to the planned experiment:</u>

1) Split T24 cells that are growing in McCoy's 5A medium, supplemented with 10% fetal bovine serum, in large cell culture flasks. Dilute the cells 1:20 with fresh medium and inoculate all 8 chambers of a Chamber-Slide, each with 200 µl of the diluted cell suspension.
2) Inoculate the chamber culture in a precision incubator at 37°C with 5% CO2 supplement.
3) Inoculate two times 3 ml of 2xYT broth in a Cap-O-Test tube each with 3-5 colonies of the bacterial strains, grow on a shaker (200 rpm) overnight (o/n) in a 37°C incubator.

<u>On the day of the experiment:</u>

4) Inoculate one portion each of 20 ml 2xYT broth in a 100 ml Erlenmeyer flask with 0.5 ml of the o/n bacterial cultures. Grow at 37°C for approximately 2 h until an OD600 of 0.4-0.6 is reached (middle logarithmic growth phase). Dilute the cultures 1:5 with fresh 2xYT broth.

   (Alternatively, pre-dilute the o/n cultures with 2xYT broth approximately 1:10, until they reach an OD600 of 0.5. Dilute 1:5 as above).
5) Infect one culture chamber each with 10 µl of the bacterial dilutions. Incubate for 2 h at 37°C and 5% CO2.
6) Repeat step 5) using two more chambers. Incubate for 2 h at 37°C and 5% CO_2. (You will the have 2 identical assays, one incubated for 2, and one for 4 h).

Steps 1) to 6) are executed for you prior to the course.

7) Suck out the medium from all chambers carefully with the Eppendorf pipet. Add 400 µl of PBS, shake carefully, and remove the liquid in the same way.
8) Repeat the washing step 7) twice to remove all non-adhered bacterial cells from the tissue.
9) Add 200 µl of Me-OH to each chamber and fix the tissue for 15 min at RT°C.
10) Remove Me-OH and let dry for 2 min.
11) Add 200 µl of diluted Giemsa solution, and stain for 15 min.
12) Pour off staining solution and wash carefully under laminarly running tap water.
13) Remove chambers and rubber gasket.
14) Apply cover slides and examine under the microscope: Either x400 magnification or x1000 with oil immersion. Count bacteria attached per cell or per x1000 vision field, compare the influence of the two bacterial strains and the time of incubation, and evaluate the condition of the tissue in the 3 cases (challenged with DH5α, challenged with 536 or unchallenged).

Laboratory Rules & Precautions	*E. coli* is a normal inhabitant of the human and animal intestine. Non-adherent strains belong to risk group 1 and do not pose a health risk. UPEC strains belong to risk group 2 and pose a low hazard. Care must be taken not to contaminate oneself, fellow students or the environment. Aerosol formation has to be prevented. All waste must be sterilized before disposal. Hands must be washed and desinfected and surfaces wiped with 70 % ethanol after completion of the experiments. Remark: Infected tissue, fixed with Me-OH for 20 min can be considered inactivated and therefore harmless. Nevertheless, all solid waste has to be collected and autoclaved for disposal.
Experiences gained	- Handling of pathogenic bacteria, cell cultures and Chamber Slides. - Microscopic evaluation of the rate of adherence of UPEC compared to non-uro-pathogenic *E. coli* onto uro-epithelial tissue. - Gain some insight into the mechanisms of virulence and pathogenicity of bacteria pathogenic to humans.
Timing	- Washing, fixation and drying: 20 min (hands-on: 5 min). - Staining, washing and preparation of the microscopic slide: 20 min (hands-on: 5 min). - Microscopic examination: 10 min.
Reporting	- Drawing of healthy and damaged uro-epithelial tissue. - Table showing the number of counted bacterial cells on tissues as a function of time and strain.
Questions to be answered	- What are UPEC, and why do they pose a higher health risk than normal *E. coli*? - Is it important to use uro-epithelium for the presented experiment, and if so why? - Name two mechanisms of virulence other than the one presented in this course and give some examples to each.