Catheter-associated urinary tract infections and bacterial biofilm formation on urinary devices

PhD Thesis

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Communications connected with the thesis

I. **Tenke P.**, Kisbenedek L. :
Az urológiai beavatkozások perioperatív antibiotikus profilaxisa.
Magyar Urológia, XI./3., 231-244.,1999

II. Kovács G., **Tenke P.**:
A húgyúti infekciók terápiája – farmakológiai szempontok.
Magyar Urológia, XI./2., 145-155.,1999

III. **Tenke P.**, Kisbenedek L., Kovács G., Pataki L., Fél T. :
A felső húgyúti infekciók levofloxacin kezelésével szerzett első hazai eredmények.
Magyar Urológia, XII./4., 375-383.,2000

IV. **Tenke P.**, Kisbenedek L.:
A húgyúti infekciók diagnosztikája és kezelése az urológus szemszögéből.
Magyar Egészségpiac, III./5., 125-132., 2000

V. **Tenke P.**, Szalka A., Mészner Zs. :
Húgyúti bakteriális infekciók : diagnosztikus és terápiás irányelv.
Magyar Urológia, XIV./3., 237-297.,2002

VI. **Tenke P.**, D. Ashaber, G. Hartmann
Húgyúti infekció terhességben.
Háziorvos Továbbképző Szemle, VII./7., 509-513., 2002

VII. **Tenke P.**, Bálint P.:
Az állandó katéterezéssel kapcsolatos teendők.
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A heparinos felületkezelés megakadályozza a biofilm képződését a húgyúti idegentestek felszínén.
Magyar Urológia XV./3.,172-182., 2003

IX. P. Schneede , **P. Tenke,** A.G Hofstetter:
Sexually transmitted diseases – a synoptic overview for urologists.
European Urology, 44./1-7., 2003 **I.F.: 2,47**

X. A.J. Schaffer, W. Eidner, G. Barbalias, H. Botto, T E. Bjerklund, W. Hochreiter, J.N.Kriege, B. Lobel, K.G. Naber, J.C. Nickel, J.M. Potts, **P. Tenke,** C. Hart :
Summary Consensus Statement : Diagnosis and Management of Chronic prostatitis / Chronic Pelvic Pain Syndrome.
European Urology, Suppl.2.,1-4., 2003

XI. **P. Tenke**, C.R. Riedl, G.Ll. Jones, G.J. Williams, D. Stickler, E. Nagy :
Bacterial biofilm formation on urologic devices and heparin-coating as preventive strategy.
International Journal of Antimicrobial Agents, 23S1., S67-S74.,2004
I.F.: 1,95

XII. **P. Tenke**, M. Jackel, E. Nagy :
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EAU Update Series, Vol. 2/3., 106-115., 2004

XIII. **P. Tenke**, D. Ashaber, L. Kisbenedek
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Poster at the 5th European Congress on Chemotherapy and Infection
17-20 October, 2003, Rhodes / Greece

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Poster at 6th European Congress on Chemotherapy and Infection
1-3 December, 2004, Paris / France

Abbreviations

UTI	urinary tract infection
Ca^{2+}	calcium ion
Mg^{2+}	magnesium ion
NUTI	nosocomial urinary tract infection
EPS	exopolysaccharide
pH_v	voided urinary pH
pH_n	crystallization pH
ESWL	extracorporeal shock-wave lithotripsy
MBC	minimal bactericidal concentration
PIPC	piperacillin
CAZ	ceftazidime
PAPM	papipenem
AMK	amikacin
CPFX	ciprofloxacin
LVFX	levofloxacin
PC	phosphoryl-choline
DPPC	dipalmitoylphosphatidylcholine
REM	rastelectron microscopy
EDAX	energy dosing X-ray microanalyser
UPJ	ureteropelvic junction
Ch	Charier
PCN	percutan nephrostomy
HPLC	high-performance liquid chromatography
MIC	minimum inhibitory concentration
SEM	scanning electron microscopy
SDS	sodium dodecyl sulfate
TBAA	tetrabutil ammonium acetate
ACN	acetonitril
PUR	polyurethane
STD	standard deviation

1. INTRODUCTION

Bacterial adherence and the growth of bacteria on solid surfaces as biofilm are both naturally occurring phenomena. Bacteria form biofilm in a wide variety of environments and play a crucial role in many disciplines including medicine, dentistry, immunology, biotechnology and process engineering. In medicine biofilm infections have a major impact on temporary and permanent implants or devices placed in the human body. The most frequently used foreign bodies in urology are urethral catheters, ureteric and prostatic stents, penile, testicular implants and artificial urinary sphincters. Very often they have potentially serious consequences. Biofilms can have a positive impact as well, namely lining healthy intestine and female genito-urinary tract. Biofilms have significant implications for clinical pharmacology, particularly related to antibiotic resistance, drug adsorption onto and off of devices, and minimum inhibitory concentrations of drugs required for effective therapy.

The fact that bacteria grow mostly on surfaces has been known for more than 150 years. During this time microbiologists have used direct methods to examine natural populations of these organisms growing in real ecosystems. In 1547 Antonie van Leuwenhock used his primitive but effective microscope to describe aggregates of 'animalcules' that he had scraped from human tooth surfaces. Almost 100 years later, in 1934 Claude Zobell examined natural marine populations by direct microscopy and concluded that these bacteria are attracted to the surfaces to which they sometimes adhere, to form sessile populations. Steadily, throughout the history of microbiology, very few microbiologists have chosen to examine their subjects directly by microscopy and have found that bacteria grow differently after they adhere to a surface and initiate biofilm formation. In 1964 Ralph Mitchell and Kevin Marshall examined the first stages of biofilm formation by bacteria in pure cultures, and distinguished between the reversible adsorption of bacteria to surfaces and the subsequent irreversible attachment that constitutes the first stage of biofilm formation. Since then, extensive studies have been carried out to identify and understand the bacterial microenvironment-termed biofilm.[1] The definition of biofilm initially consisted of microorganisms and extracellular substances in association with substratum. Today, the definition not only includes aggregations of microorganisms and their extracellular products on surface but also those at some distance away and in dense and single layers.

1.1 Mechanism of biofilm formation

The formation of biofilm generally consists of four steps (Figure 1) [2-8]:

1. deposition of the microorganisms
2. attachment of microorganisms followed by microbial adhesion
3. anchorage to the surface by exopolymer production
4. growth, multiplication and dissemination of the organisms

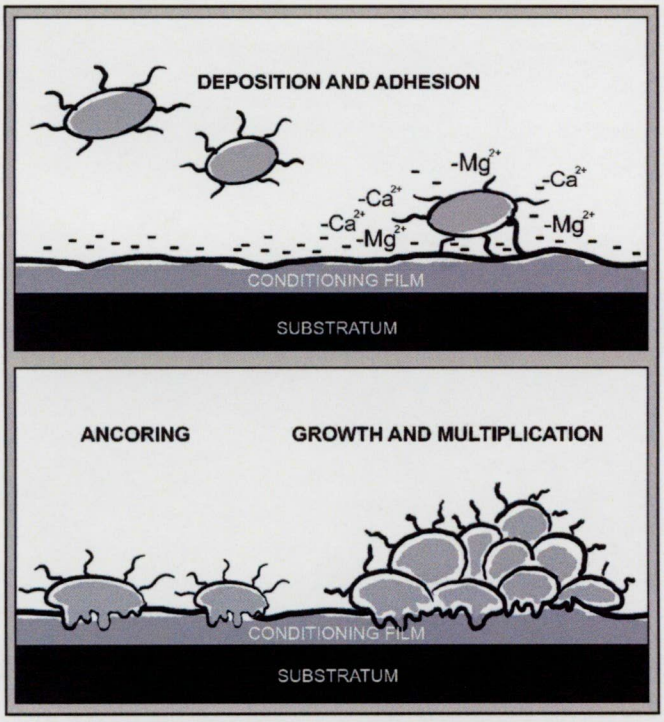


Figure 1. Formation of biofilm

1.1.1 Conditioning film deposition

Prior to being placed within the body, an implantable medical device is a clean, sterile surface, often composed of various polymers termed biomaterials. Immediately after insertion of the device into the body, the material surface comes into contact with body fluids (such as blood, urine, saliva and mucus) around the implant. In the urinary tract Tamm-Horsfall glycoprotein, various ions, polysaccharides and other components diffuse within minutes toward the implant surface from the bulk urine.[9]

Macromolecular components from these body fluids adsorb extremely quickly on to the material surfaces to form a conditioning film, prior to the arrival of the first organisms. Many of the molecules are proteinaceous, such as serum albumin, fibrinogen, collagen and fibronectin, and some have been shown to effect subsequent bacterial adhesion.[10] The types of components that will adhere to an implant depend on the surface characteristics of the substratum, including its surface chemistry, charge, and hydrophobicity. The conditioning film itself may not completely cover the entire implant surface but may form a mesh-like covering.[11,12,13] Thus, the creation of a conditioning film alters the surface characteristics of implants. It is for this reason that many implants with altered surface characteristics are ineffective, as mechanisms of preventing microbial attachment, including hydrophilic gels and antmicrobial coatings.[14] In vitro, these devices are effective in the absence of conditioning film components. However, in vivo, the formation of a conditioning film diminishes their efficacy allowing microbial adhesion.[15] The diminished efficacy may be a result of binding sites being created by film components, allowing microorganisms to adhere and colonise these surfaces. For example, the common presence of sodium or magnesium ions in the urine, act as bridges between a negatively charged implant surface and negatively charged microbial surface (Figure 2). The role of the conditioning film is vital, as many pathogens do not have mechanisms allowing them to adhere directly or strongly onto bare implant surfaces.[16]

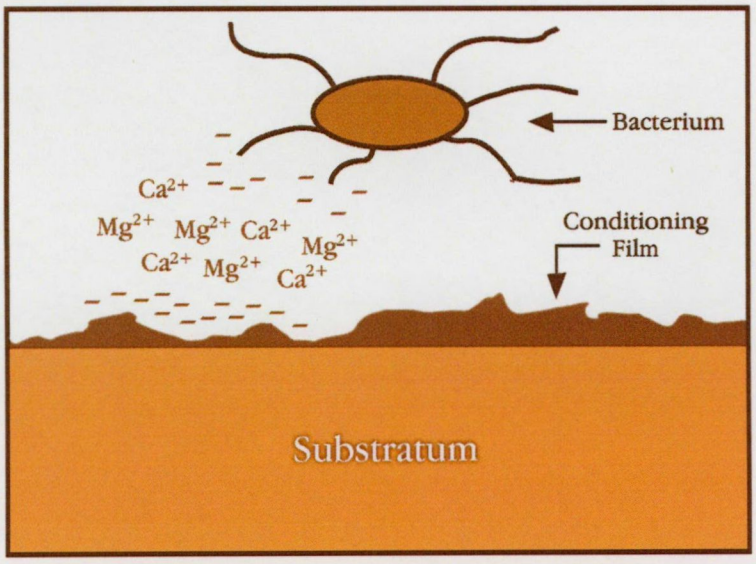


Figure 2. A bacterium adhering to conditioning film components on a substratum via a bridge of positive ions (magnesium and calcium ions).

1.1.2 Initial microbial approach and attachment

The next step in the development of a biofilm is the approach and attachment of microorganisms. Several theories have been put forth to explain the complex interaction that occurs as a microbe approaches and then attaches to a surface. However, the precise mechanisms of attachment to biomaterials are still under study.

In order for bacteria to react to a surface or an interface like an air-water interface, these cells must be able to 'sense' their proximity to these surfaces. The planktonic 'free-floating' bacterial cells release both protons and signaling molecules as they move through the bulk fluid. These protons and signaling molecules must diffuse radially away from the floating cell, if not adjacent to any surface or interface. But a significantly higher concentration of either protons or signaling molecules can develop on the side of the bacterial cell close to any surface. This allows the cell to sense that it is near a surface because diffusion is limited on this side.[4] After the planktonic bacterial cell has sensed the surface, it may commit to the active process of adhesion and biofilm formation.

1.1.3 The adhesion process

There is no single process or theory, which can completely describe microbial adhesion. The main reasons for this are the complexities of the microbial cell surface and their surrounding environment. According to Busscher and Weerkamp the microbial adhesion process consists of steps occurring at various distances from a surface.[16] Initially a distance of more than 50nm, only attractive Van der Waal's forces function. At this distance the microbial and solid surfaces are too far apart for any recognition of surface features, including surface charge, hydrophobicity and appendages. At 10 to 20 nm from solid surface, the secondary minimum occurs due to electrostatic repulsion between the bacterial and solid surfaces. At this stage, adhesion is reversible because no true bonds are attaching the bacterium to the solid surface. Hydrophobic interactions are needed to remove layers of water from between the bacteria and the solid surface so that the microorganisms can reach the solid surface, which allows the bacterium to come close to it (<1,5 nm). There, a wide variety of short-range interactions can take place, directly attaching the bacterium to the solid surface.[16] These interactions include specific receptor-mediated adhesion via bacterial surface appendages. The final result of this process is an irreversibly bound bacterium (Figure 3).

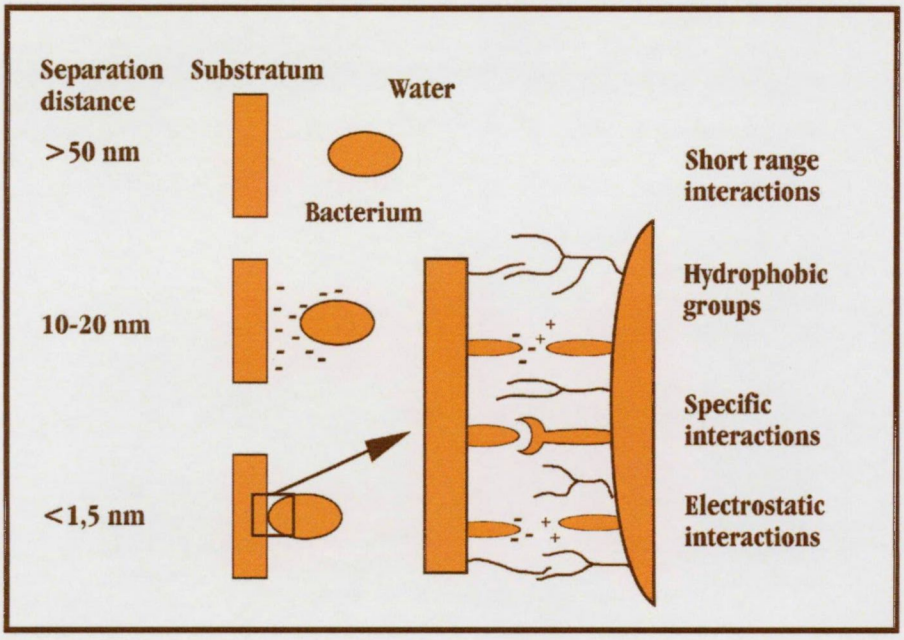


Figure 3. The adhesion process: at separation distances of >50 nm, only attractive Van der Waal's forces occur. At 10 to 20 nm, Van der Waal's and repulsive electrostatic interactions influence adhesion. At < 1,5 nm, short range interactions can occur, irreversibly binding a bacterium to a surface.

1.1.3.1 Effect of the microbial cell surface and environment on attachment

The microbial cell surface plays a crucial role in adhesion. Two factors that have been extensively studied are cell surface and hydrophobicity. Studies have correlated these cell surface properties in an attempt to predict bacterial adhesion to a wide variety of surfaces, including various polymers.[17,18] Factors in the fluid environment can vary adhesion profiles that have been based on in vitro experiments. Reid et al and Hawthorn at al found that the adhesion of uropathogens to biomaterials in the presence of various urinary compounds (including Tamm-Horsfall glycoprotein, urea, and creatinine) as well as collected whole urine did not follow any predicted trends based on thermodynamic principles.[19,20] Studies by Fletcher and Zheng et al have shown that bacterial concentration, time, temperature and flow velocity, all play roles in positively or negatively influencing the attachment of microorganisms to surfaces.[21,22] Overall these studies indicate that environmental conditions play an important role. Thus, it is impossible to make any generalisation of all the possible factors that play a role in microbial adhesion to biomaterials.

1.1.4 Growth and colonisation

Following initial attachment, bacteria can begin growth and colonisation to establish themselves firmly on a surface. The attached bacterial cells must synthesise new exopolysaccharide materials in order to fasten their adhesion to the surface, and to other bacterial cells in the developing biofilm, in order to progress from the reversible 'attachment' stage to the irreversible 'adhesion' phase of biofilm formation. Studies of some species prove that the attached cells upregulate all of the genes that produce enzymes responsible for the synthesis of exopolysaccharide (EPS) itself and for several cell envelope changes.[4] Costerton et al found 30-40% of difference in the important protein molecules, when they compared the proteins in the cell envelope fraction of cells in the biofilm phenotype, with proteins in the same fraction of planktonic cells.[4] Therefore, in at least one organism and probably in most bacteria, bacterial cells that attach to a surface at the end of the process of biofilm formation the biofilm cells are significantly different from their planktonic counterparts.[23]

The growth rates of organisms on a surface as well as the strategies used by microorganisms to spread over a surface are important for colonisation. These strategies are species specific which can influence the distribution of a biofilm on a surface. Potential colonisation strategies include mother cells that attach to a surface releasing daughter cells, which migrate to another location and become mother cells themselves. Another method known as rolling, occurs when a microorganism is loosely attached, so that it can roll along the surface as a growing and dividing cell.[24]

The final stage of microbial colonisation of a surface is the formation of a biofilm structure. At this point, the microorganisms have created for themselves a microenvironment protective against many antimicrobial agents and host immune defence mechanisms.

Biofilm has been described as having a heterogeneous structure with a rough surface.[25] Biofilm consists of groups of microorganisms, sometimes in mushroom-like forms, separated by interstitial spaces that are filled with the surrounding fluid (Figure 4). [26,27] The microcolony is actually the basic structural unit of the biofilm, similar to the tissue which is the basic unit of growth of more complex organisms. Depending on the species involved, the microcolony may be composed of 10-25% cells and 75-90% EPS matrix.[4] The biofilm contains 'water channels' which allow transporting of essential nutrients and oxygen for the growth of the cells.[28]

In summary, the biofilm is usually built up of three layers (Figure 5) [29]:

1. the linking film which attaches to the surface of tissue or biomaterials
2. the base film of compact microorganisms
3. the surface film as an outer layer, where planctonic organisms can be released free-floating and spreading over the surface.

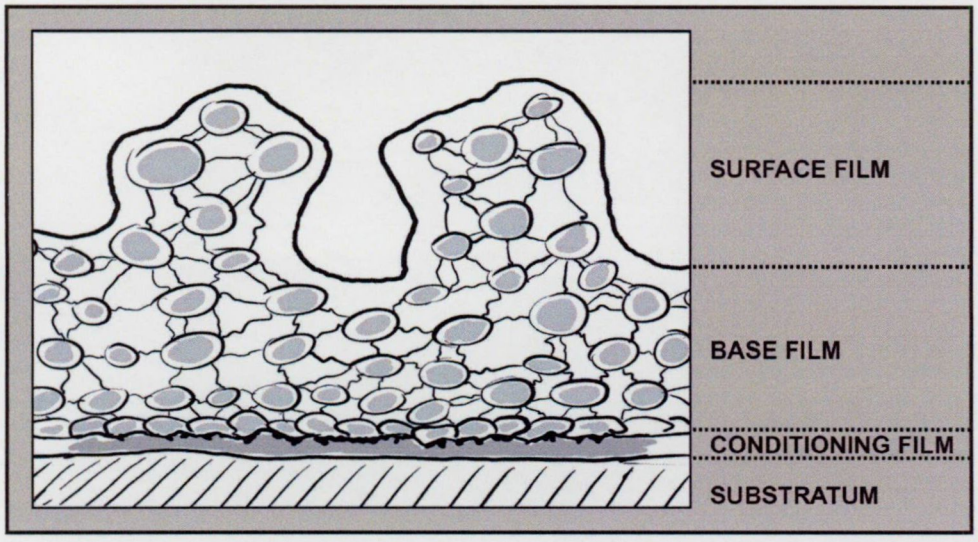


Figure 5. Composition of biofilm

1.2 Antimicrobial susceptibility of bacteria living in biofilm

The use of antibiotics is currently one of the possibilities of the prevention of biofilm formation. However, even in the presence of antibiotics, bacteria can adhere, colonise, and survive on implanted medical devices, as has been shown for urinary catheters and ureteral stent surfaces in vitro and in vivo. In addition, resistance to antimicrobial agents and other chemicals is one of the greatest problems in the age of widespread use of medical devices. The problem in conventional clinical microbiology is how best to treat patients based on bacterial cultures from samples derived from planktonic free-floating bacterial cells, which are very different from bacteria in the biofilm mode and are responsible for the clinical failure rate of treating chronic bacterial infection.

The failure of antimicrobial agents to treat biofilms has been associated with a variety of mechanisms [30-34] :

- One mechanism of biofilm resistance to antimicrobial agents is the failure of an agent to penetrate the full depth of the biofilm (extrinsic resistance). The matrix of a biofilm can delay the diffusion of antibiotics.
- The growth rates of bacteria within a biofilm vary widely. Slow-growing bacteria are particularly resistant to antmicrobial agents. In general, the faster growing, more susceptible bacteria lie more superficially, with the slow-growing less susceptible bacteria being placed more deeply. The failure of antimicrobial agents to eradicate these slow-growing bacteria may force selection pressure on the least susceptible genotype to select for a resistant population. Furthermore, antimicrobial binding proteins are poorly expressed in the slow-growing bacteria, making the antimicrobial agents ineffective.
- Bacteria within biofilm are phenotipically so different from their planctonic counterparts that antimicrobial agents developed against the latter often fail to eradicate organisms in the biofilm. Bacteria within a biofilm activate many genes which alter the cell envelope and molecular targets and the susceptibility to antimicrobial agents (intrinsic resistance). Current opinion is that phenotypic changes caused by a genetic switch, when approximately 65-80 proteins change, play a more important role in the protection from antimicrobial agents than the external resistance provided by the exopolysaccharide matrix.
- Bacteria within a biofilm can sense the external environment, communicate with each other and transfer genetic information and plasmids within biofilm.
- Bacteria in a biofilm can usually survive the presence of antmicrobial agents at concentrations 1000-1500 times higher than the concentrations that kill plankton cells of the same species.

1.3 Catheter-associated infections

Millions of urethral catheters are used annually in acute care hospitals and nursing homes across the world. According to a multicentre prevalence study carried out in eight European countries, 11% of hospital patients were undergoing indwelling bladder catheterization.[35] Another study in Denmark found that 4,9% of patients in nursing homes and 3,9% of those in home care were catheterised.[36]

Urethral catheters are widely used to provide temporary relief of anatomic or physiologic obstruction, to facilitate surgical repair of the urethra and surrounding

structures, to provide a dry environment for comatose or incontinent patients and to permit accurate measurement of urine output in severely ill patients. Unfortunately the urethral catheter is the most prevalent cause of nososcomial urinary tract infections (NUTI) and gram-negative bacteremia.

In the process of endourological development a great variety of foreign bodies have been invented besides urethral catheters like ureter stents and percutan nephrostomy. Although the tendency of foreign bodies to predispose patients to infections has been recognised since the fourteenth century, the mechanisms of device related infections are still not completely understood.

1.3.1 Incidence of catheter- associated urinary tract infections and epidemology

The presence of an indwelling urethral catheter bypasses normal host defences allowing continuous access of organisms into the urinary bladder and markedly increasing the frequency of urinary tract infections. The duration of catheterization is the most important risk factor for development of catheter-associated bacteriuria. The risk of infection with a single catheterization is 1-2%. The risk of new infection can arouse 3 to7% a day in catheterised patients while the catheter remains in situ and almost all the patients will be bacteriuric by the end of the 30^{th} day. Other important risk factors are as follows [37,38] :

1. microbial colonisation of the drainage bag and periurethral area
2. diabetes mellitus
3. female patients
4. absence of use of urinometer
5. absence of antibiotic use
6. indications other than surgery or output measurement
7. compromised status of renal function
8. errors in catheter care
9. abnormal serum creatinine
10. periurethral colonisation with uropathogens.

1.3.2 Pathogenesis of device-related infection

Device-related infection results from the multifarious interaction of bacterial, device and host factors. Of these 3 factors, bacterial factors are probably the most important in the pathogenesis of device-associated infection, whereas device factors are the most amenable to modification with the objective of preventing infection.

1.3.2.1 Bacterial factors

Bacteria can adhere to almost any surface. The mechanism by which bacteria adhere to tissues or implant surfaces is mediated by bacterial adhezins that are located either on filamentous appendages called fimbriae or directly on the bacterial surface. Adhesion permits the bacteria to resist being washed away by the flow of urine in the urinary tract and is a necessary pre-condition to growth, colonisation and subsequent infection.[28,4] Different bacteria use different adhesions to colonise medical devices. Many examples of the role of bacterial adherence to the device have been reported in the literature, and specific bacterial adherence to the device has been shown to be characteristic of many pathogenic microorganisms. For example, *Providencia stuartii* is more prevalent in the urinary tract of patients with long-term catheters than it is in catheter-free patients. Persistent adherence of *Providencia stuartii* to urinary catheters is thought to be mediated by type 3 fimbriae on the basis of the following observations [39]:

- bacterial isolates that caused long-term (>12 weeks) bacteriuria expressed type 3 fimbriae more than the isolates that caused short-term (<1 week) bacteriuria (74% v. 26%, respectively) in catheter dependent patients
- bacterial isolates that expressed type 3 fimbriae bound in higher numbers to catheters than the isolates that did not express type 3 fimbriae
- bacterial isolates that expressed type 3 fimbriae bound less to Tom Harsfall protein (an inhibitory protein) than the bacterial isolates that did not express type 3 fimbriae.

Similarly, despite the overabundance of data on the adherence of *Escherichia coli* to the uroepithelium, relatively little is known about adherence of this organism to urological devices. *Escherichia coli* isolates that caused long-term (>12 weeks) bacteriuria expressed type 1 fimbriae more than isolates that caused short-term (<1 week) bacteriuria (92% vs. 59% respectively).[40]

1.3.2.2 Device factors

The presence of the device can enhance bacterial virulence. Careful analysis of the data on bacterial adherence and surface modification of the device yields the following 5 major principles:

1. Different bacteria may adhere differently to the same device material.
2. The same bacteria may adhere differently to different device materials.
3. The same bacteria may adhere differently to the same device material placed under different circumstances, including the medium in which the device is placed (hydrophobic vs. hydrophilic medium), type of flow (dynamic vs. stationary), and temperature.
4. In vitro inhibition of bacterial colonisation of the device does not ensure anti-infective efficacy in vivo.
5. The clinical benefit of a particular surface-modifying approach may vary from one application to another. Notwithstanding these 5 major principles, there are a number of device-related factors that can affect bacterial adherence to the device, including the source of device material, surface of the device, and shape of the device (Table 1).

Type of device material:
Polyvinyl chloride favours bacterial adherence more than teflon
Polyethylene favours bacterial adherence more than polyurethane
Latex favours bacterial adherence more than silicone
Silicone favours bacterial adherence more than polytetrafluoroethylene
Source of device material:
synthetic favours bacterial adherence more than biomaterial
Surface of device:
Irregular favours bacterial adherence more than regular
Hydrophobic favours bacterial adherence more than hydrophilic
Shape of device:
Polymeric tubing favours bacterial adherence more than wire mesh

Table 1. Device-related factors that may favour bacterial adherence

1.3.2.3 Host factors

These factors can be divided into 2 groups [4]:

1. host factors that can affect bacterial adherence of the bacteria to the device, including tissue ligands that can mediate adherence of some bacteria
2. host factors that can either promote or inhibit the persistence of already adherent bacteria on the surface of the device

1.3.3 Pathogenesis of catheter-associated infection

The ways in which bacteria infect the catheterised bladder are well established. Bacteria can enter the urinary tract in catheterised patients in three ways [37,41-43]:

- Introduction of organisms into the bladder at the time of catheter insertion: This is especially common in patients who may have inadequate cleaning of the perineum and distal urethra.
- Periurethral route: This route of entry is especially important in catheterised women. Organisms colonising the periurethral epithelia and skin can enter the urinary tract via external surface of the catheter in the mucous sheath between the catheter and urethral mucosa.
- Intraluminal route: In males the predominant route is intraluminal, suggesting an exogenous source. Microorganisms can ascend through the lumen of the urinary catheter into the bladder. Nickel et al demonstrated in their experimental work that the intraluminal ascent of bacteria is faster (32-48 h) than the extraluminal route (72-168 h). Bacteria contaminating the drainage system can also gain access to the bladder through the lumen of the catheter. The taps of the urine drainage bags commonly become contaminated during use and their regular opening to drain the urine also affords the bacteria access to the bags. Bacterial multiplication in the urine produces dense populations of contaminating organisms in the bags and from these reservoirs of infection can migrate to the drainage tube, the catheter and bladder. Disconnection of the catheter from the drainage tube has also been shown to lead to contamination of the system. In wards where high standards of catheter hygiene are not maintained, the spread of organisms to the bladder via a contaminated drainage system will be the more significant and rapid route.

1.3.4 Microbiology of catheter-associated infection

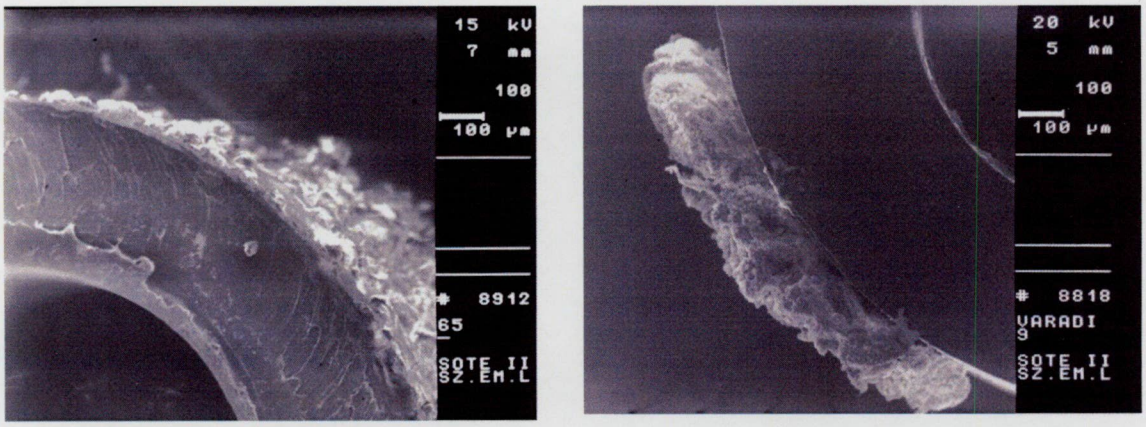
Most episodes of short-term catheter-associated bacteriuria are asymptomatic and are caused by single organisms. The most common species is *E.coli.* Other common organisms are *Pseudomonas aeruginosa, Klebsilella pneumoniae, Proteus mirabilis, Staphylococcus epidermidis, and Enterococcus spp.* and candida species.[37,42,43] The patient with a long-term indwelling catheter is at high risk of morbidity due to this procedure. With prolonged catheterization of 10 days or longer, bacteriuria with at least one bacterial strain is common. But most patients have at least 2 bacterial strains. The first common uropathogene is *E. coli* and the second is another kind of organism, namely *Providencia stuartii* which is rarely found outside the catheterised urinary tract. *Pseudomonas aeruginosa, Proteus mirabilis, Morganella morganii, Acinetobacter* however, can occur as well.[37,42,43] These phenomena result in polymicrobial bacteriuria in up to 95% of urine specimens from long-term catheterised patients. During long-term catheterization, besides symptomatic UTI, we can frequently observe encrustation and blockage of catheters.

1.3.5 The structure and features of catheter biofilms

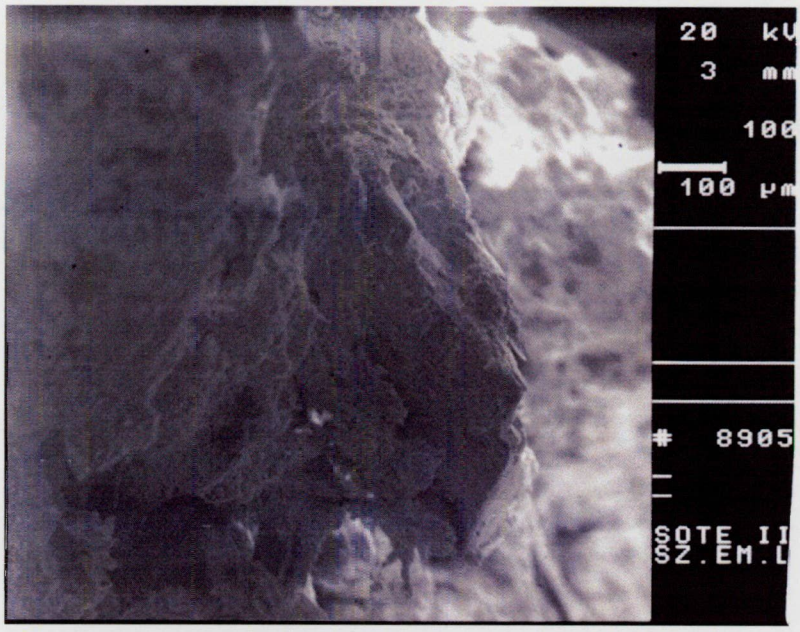
Using scanning electron microscopy, Nickel observed that the external and luminal surfaces of catheters removed from patients with symptomatic *Pseudomonas aeruginosa* urinary tract infections were heavily colonised by viable bacteria built into an extensive polysaccharide matrix.[44] Subsequent studies have revealed that catheters are commonly colonised, particularly on the luminal surfaces by these bacterial biofilms. Ohkawa et al also observed that the surfaces of catheters removed from patients after just 1-3 days were coated by fibrinogen.[45] They suggested that this proteinaceous layer originated from host tissues injured during catheterization and initiated biofilm colonisation. Ganderton et al found biofilms on 44 out of 50 catheters that had been indwelling from 3 to 83 days.[46] They extended along the whole length of the catheters, and bacteriological analysis revealed the presence of *Pseudomonas aeruginosa* of luminal surface area. It was shown that after inoculation of the species, biofilm developed locally within a lag phase of 2 hours, then the biofilm ascended very rapidly within the lumen against the urine flow in the creep phase (1-2cm/h). Biofilm has also been seen throughout catheter drainage systems. The surfaces of the drainage bag taps, the internal

surfaces of the drainage bags, the 'non-return' valves of the drainage tubes can all become colonised by bacterial biofilm. Nickel and co-workers presented evidence from laboratory, experimental animal and clinical studies that the adherent biofilm can initiate bladder infections by creeping from the drainage tap into the bag and from there progressing along the internal surfaces of the drainage system and catheter.[47] It is also probable that the columns of urine that develop in the drainage tubes above the bag facilitate the ascent of bacteria towards the catheter.

Bacterial biofilm can even develop on ureteral stents that are lying completely within the urinary tract (Figure 6 a, b, c). Reid at al found that 90% of indwelling silicone double J stents were colonised by adherent bacteria, however the incidence of urinary infection detected clinically was only 27%.[48] But 45% of adherent pathogens were present in low numbers (10^1-10^2 per 1 cm^3) and 55% were in small and large microcolony biofilms (>$2x10^2 - 10^7$). The isolated organisms were Gram-positive cocci (77%), Gram-negative rods (15%) and *Candida sp.* (8%). The difficulty in detecting biofilm formation by using conventional laboratory procedures was confirmed in a large study of 237 ureteric stents in which it was shown that 68% of stents were actually colonised but only 30% of patients were found to have bacteriuria.[49] Therefore, a negative urine culture does not rule out the possibility that the stent itself is colonised. In the study there was a correlation between the duration of stent implantation and infection.



a. **b.**



c.

Figure 6 a, b, c. Scanning electron microscopy picture of a developing biofilm (Pictures are taken during our own studies.)

1.3.5.1 The role of biofilms in the encrustation and blockage of catheters

An additional problem of medical biomaterials in the urinary tract environment is the development of encrustation and consecutive obstruction. When the drained urinary tract becomes infected by urease producing bacteria such as *Proteus mirabilis*, the bacterial urease generates ammonia from urea and elevates the pH of the urine. The enzyme urease from *Proteus mirabilis* hydrolyses urea six to ten times faster than the urease enzymes from other species (*Morganella morganii, Providencia stuartii, Klebsiella pneumoniae, Proteus tettgeri, and Proteus vulgaris).* In the alkaline environment, crystals of magnesium ammonium phosphate (struvite) and calcium phosphate (hydroxyapatite) are formed and trapped in the organic matrix surrounding the cells.[50,51] Progression of these encrustations eventually blocks the catheter lumen (Figure 7 a, b, c, d). Examination of these infection-induced stones by scanning electron microscopy confirmed the presence of microbial cells throughout their structure. Similarly, the scanning electron microscopy study of Cox et al showed the presence of large numbers of bacilli associated with catheter encrustations and substantiated the view that they are generated by the process responsible for the formation on infection-induced stones.[52] It is now clear that catheter encrustations are mineralised bacterial biofilms.

Bacteriological analysis has confirmed that the urease producer *Proteus mirabilis* is the predominant organism in encrusted catheter biofilms.[53] While urease is the main driving force behind mineralization of the biofilm, there is evidence that the bacterial capsule polysaccharide is also involved in the process. In vitro studies have indicated that the capsule produced by *Proteus mirabilis* creates a gel which stabilises the growing crystals and that the purified exopolysaccharide from the capsule was capable of binding magnesium and accelerating struvite formation.[54,55] McLean et al observed that the first stage in the process was the deposition of organic material on the test surface.[53] Crystals of struvite then started to form slowly on this conditioning film. Aggregates of cells attached to the surface and growth of these cells produced microcolonies that eventually developed into complete biofilm. Crystals of struvite then formed rapidly in the biofilm matrix. Stickler et al used a simple physical model of the catheterised bladder to produce encrusted catheters. Transmission electron micrographs of sections through the mineralised biofilms revealed elongate struvite crystals lying in the body of the biofilm and also in direct contact with catheter surface, where they had obviously become heavily colonised bacteria. The amorphous calcium phosphate particles were seen distributed throughout encrusted biofilms and many of them appeared to have formed around bacterial cells. These observations suggest that in catheterised patients infected by *Proteus mirabilis,* bacteria and crystals present in the urine adhere to the luminal surface of the catheter. Further colonisation and growth of the cells on catheter and crystal surfaces produces the alkaline biofilm, which provides the conditions for continued encrustation and catheter blockage.

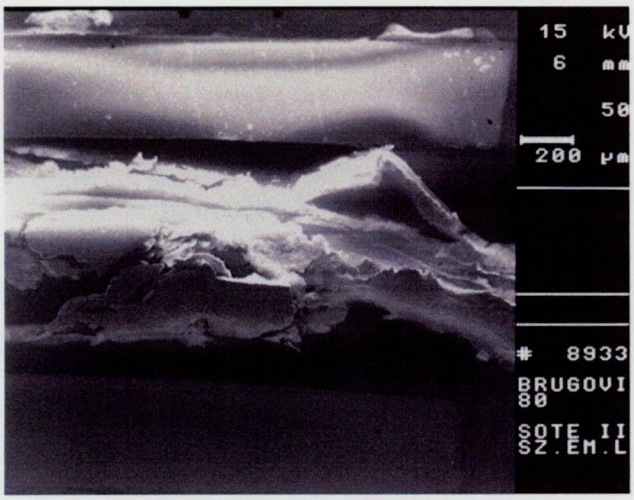


Figure 7. Encrustation blocks the lumen of the catheter

In summary, the current evidence suggests that catheter encrustation is brought about by

- the infection of the urinary tract by *Proteus mirabilis* or other urease-producing species
- the formation of an organic conditioning layer on the catheter surface
- the adherence of bacterial cells onto the catheter
- multiplication of the attached cells and the development of the biofilm community within a polysaccharide matrix
- the elevation of the pH of the urine and the biofilm by the action of urease on urea
- the attraction of calcium and magnesium ions from urine into matrix
- the gel-stabilised crystallisation of calcium and magnesium ammonium phosphates.

Clinical experience and laboratory studies have shown that all types of catheters currently available are vulnerable to blockage by crystalline *Proteus mirabilis* biofilms.[37] The complications resulting from catheter encrustation seriously compromise patient care. The crystalline deposits can be hard and abrasive and can traumatise the bladder mucosa and urethra. Obstruction of urinary flow through the catheter may cause either incontinence due to leakage of urine around the catheter or painful distension of the bladder due to urinary retention. Bacteriuria is always found in these patients, so retention and vesico-ureteral reflux may facilitate ascending infection of the urinary tract, culminating in episodes of pyelonephritis, septicaemia and shock. Thus, undetected catheter blockage may lead to life-threatening complications. Several studies have reported that up to 50% of patients undergoing long-term catheterization will require unscheduled catheter replacement because the flow of urine has been blocked by crystalline deposits.[42] Cools and Van der Meer, for example, recorded that only 18% of catheters in nursing homes were replaced according to a once-a-month protocol.[56] In all other cases, early unscheduled replacement was necessary because of obstruction, persistent leakage around the catheter or removal of the catheter by the patient. Kunin et al characterised nursing home patients with catheters as 'blockers' or 'non-blockers', defining blockers as individuals whose catheters became totally clogged or grossly occluded, leaving only a narrow channel for urine. They reported that 55% of long-term catheterised patients were blockers.[57] Getliffe found that 43% of the population of catheterised patients being cared for in the community suffered from recurrent catheter encrustation and that catheter status was significantly associated with female sex and poor

mobility.[58] Kunin, in attempting to identify patients at special risk of developing catheter encrustations, found no significant differences between blockers and non-blockers with respect to age, activities of daily living and mental status.[59] Examination of the constituents of urine revealed no significant differences between the two groups for protein, sodium, calcium, potassium, chloride, uric acid, oxalate and osmolality. Blockers excreted more alkaline urine containing less magnesium, urea and phosphate. They were also more often colonised by *Proteus mirabilis* and *Providencia stuartii* than non-blockers.

Choong at al examined the relationship between urinary pH, UTI and catheter encrustation.[60] A large component of catheter encrustation is composed of calcium salts and this study focused on the relationship between urinary pH and ionic calcium concentration in patients with long-term catheters. The non-blocker group shows that there is a wide safety margin between the pH in the bladder (pH_v), and the crystallisation pH (pH_n). The blocker with urease producing organisms group shows that this margin of safety has been significantly reduced and therefore, crystallisation and precipitation are more likely to occur in this group. The blocker and urease negative group shows that the voided pH of the blockers without urease-producing organisms lies within the crystallisation zone, which favours encrustation and blockage of the urinary catheters. The relationship between urinary pH and Ca^{2+} concentration markedly different in catheter blockers and non-blockers. Non-blockers had a significantly more acidic voided urine pH as compared to blockers and did not have urinary infection. Catheter non-blockers had a wide safety margin between the voided pH and the value above which crystallisation occurred, which was absent in the catheter blockers. Blockers with urease producing organisms had nucleation pH values similar to those of non-blockers, the main difference being the value of the voided urine pH, which was significantly more alkaline (Figure 8).

Similarly encrustation of ureteric stents is influenced not only by the shape, surface properties and composition of the catheter but also by the physicochemical environment in which it is placed. There exist several individual variations among patients, irrespective of the type of material used. With identical ureteric stents, some patients show no evidence of encrustation and blockage whereas other patients show variable degrees of the phenomenon. This implies that encrustation is at least partially dependent on the composition of the urine in which the stent dwells.

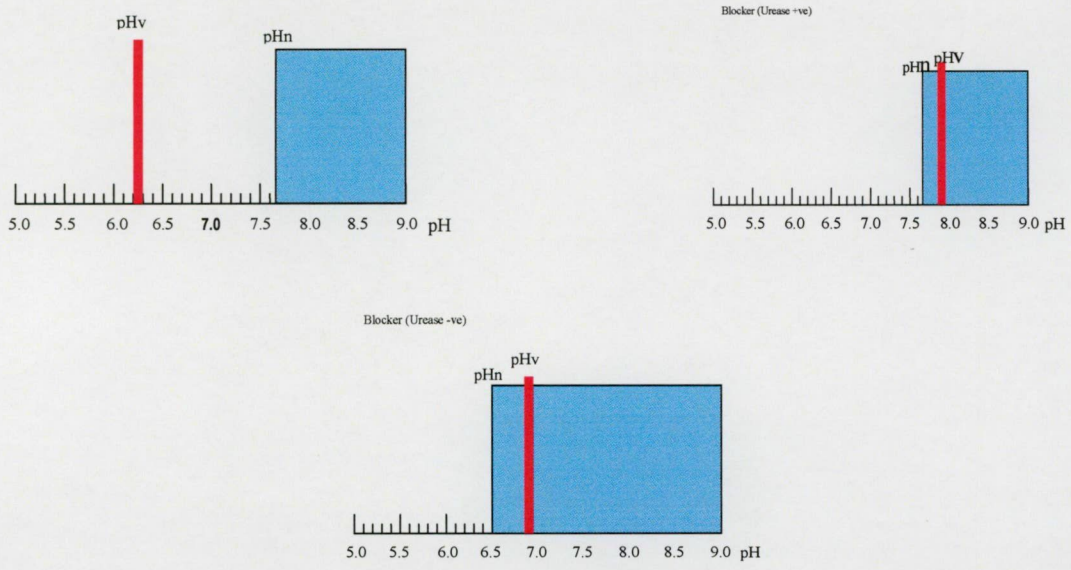


Figure 8. The relationship between the voided urinary pH and pH_n among catheter non-blockers and blockers with and without urease activity.

(adapted from Choong S., Wood S., Whitfield H.F. Catheter-associated urinary tract infection and encrustation. Int J of Antimicrobial Agents 2001; 17: 305-310)

Keane at al examined ureteric stents from 40 patients and identified a profuse biofilm on 28% and encrustation on 58% of the stents.[61] They concluded that newer materials must be used if effective long-term stenting is to be achieved. Schulze et al also reported severe encrustation and stone formation on indwelling ureteric stents in two patients with a lithogenic history in the presence of sterile urine.[62] He observed that patients who are stone-formers are at an increased risk of stent encrustation. The problem of encrustation has been exacerbated by increased use of extracorporeal shock-wave lithotripsy (ESWL) in combination with double J stents. These stone-forming patients have been shown to develop encrustation rapidly.

1.3.6 Management of catheter-associated bacteriuria

Most patients who have bacteriuria immediately after catheter removal have accompanying pyuria and are asymptomatic. The majority of bacteriuria will have cleared on repeat culture 1-2 weeks later. The optimal antibiotic regimen in this setting is unclear. The optimal period of antibiotic treatment varies between 4 to10 days. For older women,

patients with upper tract symptoms and males certain authors favour 10 to14 days of antibiotic treatment. In patients who have been catheterised for short period of time urine culture should be obtained at the time the catheter is discontinued or within 24 to 72 hours of the event.

Antibiotic irrigation of the catheter or bladder does not appear to be as effective as closed drainage any more.[43] Asymptomatic bacteriuria should not be treated as long as the catheter remains in place. Because complications of long-term catheterization are primarily infectious in nature, treatment during catheterization is not helpful in eradicating infection for prolonged periods of time and serves only select populations of organisms that are resistant to the antibiotics being used. There are some exceptions [7,37,42]:

a) when therapy is part of a plan to control infections by particular organism in medical unit
b) patients who have a high risk of serious complications (e.g. granulocytopenic patients, pregnant women)
c) patients undergoing urologic surgery or prostheses implantation
d) patients with recurrent obstructions of the catheter which are mostly associated with infections by *Proteus mirabilis*.

Generally after the catheter is removed the urinary tract will clear bacteria spontaneously. Among catheterised-patients dysuria, pain, irritative symptoms are not significantly different between those who develop bacteriuria and those who do not. However, in some cases catheter-associated complications can develop.

Systemic antibiotics should be used for catheterised patients who are febrile and ill-appearing, presumably for UTI, with signs or symptoms suggesting a possible UTI-related bacteremia. Causes for fever other than the urinary tract have to be evaluated and catheters are assessed for partial or complete obstruction. The patient is examined for periurethral complications of urethral catheterization. Urine and blood cultures are performed. Catheters should be replaced or removed during the therapy of symptomatic catheter-associated bacteriuria because of the likelihood of bacteria sequestered in a biofilm on the catheter surface. Definitive antibiotic therapy is to be adjusted based on microbial susceptibility studies. If bacteremia is suspected or known, broad-spectrum antibiotics are indicated (ampicillin or cephalosporin and aminoglycoside or fluoroquinolone), while one is waiting for the results of urine and blood cultures. It is advisable to exclude enterococcal bacteraemia by unspun urine gram stain. If urine gram

stain shows no gram-positive cocci an aminoglycoside can be used alone. When culture results are available, initial empiric therapy can be tailored based on susceptibility data. These patients usually require a full 10 to 14-day therapy. The mortality rate of nosocomial catheter-associated bacteremia varies between 9-13%.[63] The risk factors for death include severity of comorbidities, shock, inappropriate antibiotic therapy, presence of infection in other sites and presence of unrecognised urological abnormalities.
If no bacteremia is suspected or documented, these patients should be treated with short (3 to 5 days) courses of antibiotics based on susceptibility data. This will usually sterilise the urine without selecting more resistant bacteria and may allow a prostatic focus to persist. Prolonged therapy can only select out resistant organisms. For patients who have a low-grade temperature and are clinically stable, observation might be reasonable as the low-grade temperature may be transient.

Candiduria may occur in patients depending on the duration of catheterization. It is usually asymptomatic and resolves without treatment. In case of complications systemic therapy by amphotericinB B or fluconazole is indicated. The chronic antibiotic suppressive therapy is not effective because the catheter acts as a foreign body, the urine of these patients cannot be sterilised for a prolonged period of time.

1.3.6.1 The role of antibiotics in the treatment and prevention of catheter-associated biofilm formation

In the case of urinary catheters, there is evidence from clinical observations that within protection of the biofilm, bacteria defined as sensitive to antibiotics by conventional laboratory test procedures can survive the urinary concentrations of antibiotics generated by conventional treatment regimens. During an in vitro test Nickel et al showed that when strains of *Pseudomonas aeruginosa* produced biofilms on catheter materials they acquired resistance to tobramycin, amikacin, ceftazidime, ciprofloxacin and meropenem.[64,65] For example, when tobramycin was added to the artificial urine, the lag phase was prolonged for almost 12 hours. Despite antibiotic treatment, biofilm developed and ascended the catheter surface very slowly, at a rate of approximately 0,2-0,3 cm/hour. Goto and co-workers investigated in vitro and in vivo models of catheter-associated infection, biofilm formation of *Pseudomonas aeruginosa* in artificial urine and bactericidal activity of several classes alone or in combination with macrolide antibiotic. [66] According to these studies the beta-lactam antibiotics and aminoglicosids can prevent

the formation and the extension of 'young' biofilms in growth. Papipenem showed more potent activities than piperacillin and ceftazidime, and 128 times the MBC of amikacin showed almost comparable bactericidal activity with that of 64 times the MBC of papipenem against *Pseudomonas aeruginosa* biofilms (Figure 9,10) [66]. On the other hand, fluoroquinolones, like for example, levofloxacin and ciprofloxacin were effective in cases of both 'young' and 'older' biofilms because the biofilm bacteria were completely eradicated by treatment with 64 times the minimal bactericidal concentration (MBC) of ciprofloxacin and levofloxacin for 24 h. (Figure 11). However, the effect of fluoroquinolones is limited, and it appears that none of the commercially available drugs are sufficiently active against the cells in a mature biofilm. There were few combinations of antibiotics which showed synergistic activities against *P. aeruginusa* biofilm. Tsukamoto et al studied the clinical effect of ciprofloxacin, which has high antimicrobial activity against gram-negative bacteria, in combination with antibiofilm agent clarithromycin in complicated urinary tract infection.[67] The study indicated that the combination was clinically superior in terms of complete bacterial elimination and clinical efficacy in patients without an indwelling catheter. But in patients with an indwelling catheter the combination achieved a higher clinical efficacy than ciprofloxacin alone, although the complete bacterial elimination rate was not different between the two groups.

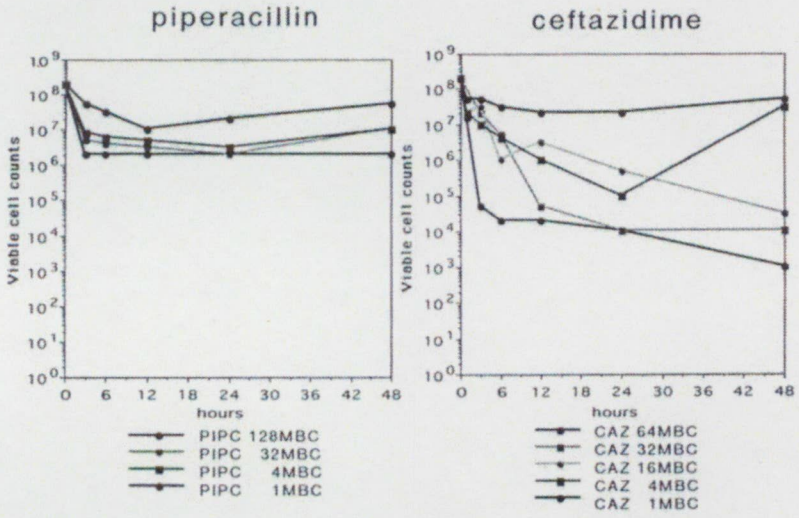


Figure 9. Time-kill curves of piperacillin and ceftazidime against the biofilm bacteria of P. aeruginosa

(adapted from Goto T., Nakame Y., Nishida M. , Ohi Y. Bacterial biofilms and catheters in experimental urinary tract infection. Int. J of Antimic. Agents 1999; 11: 221-231.)

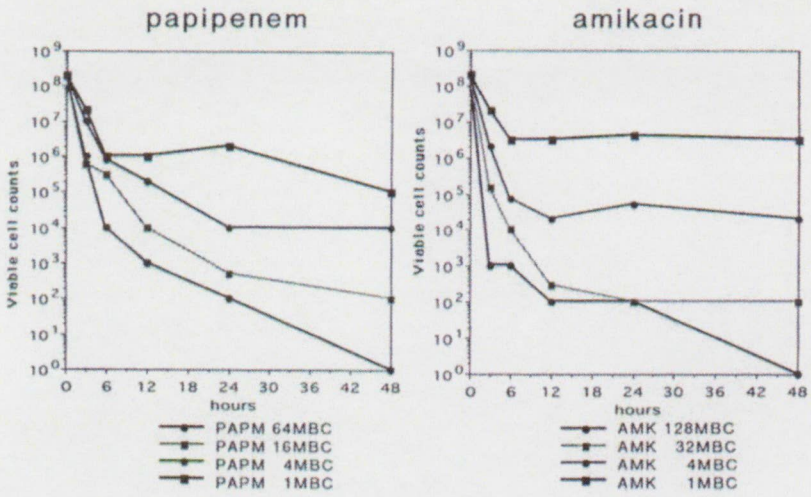


Figure 10. Time-kill curves of papipenem and amikacin against the biofilm bacteria of *P. aeruginosa*

(adapted from Goto T., Nakame Y., Nishida M. , Ohi Y. Bacterial biofilms and catheters in experimental urinary tract infection. Int. J of Antimic. Agents 1999; 11: 221-231.)

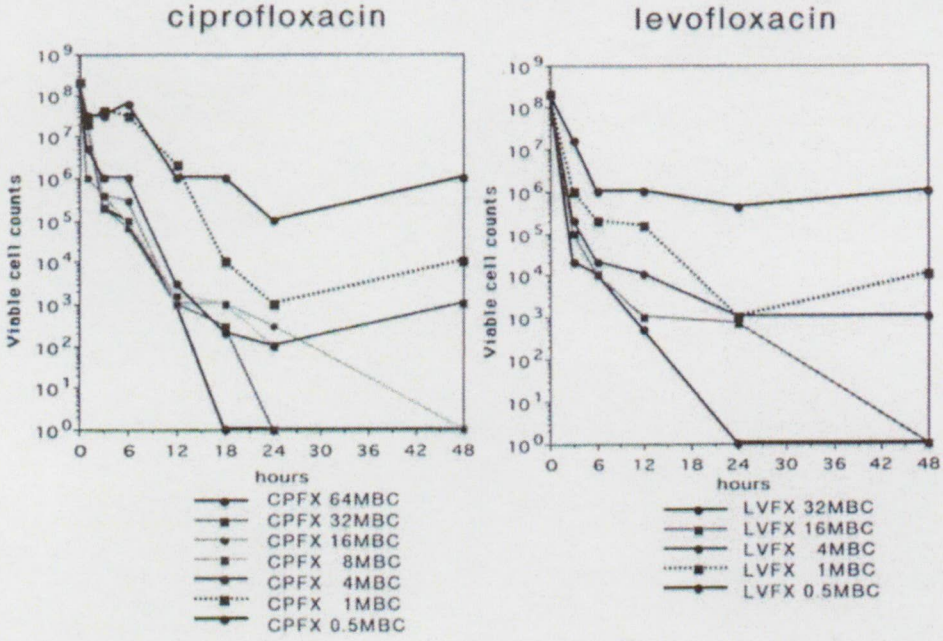


Figure 11. Time-kill curves of ciprofloxacin and levofloxacin against the biofilm bacteria of *P. aeruginosa*

(adapted from Goto T., Nakame Y., Nishida M. , Ohi Y. Bacterial biofilms and catheters in experimental urinary tract infection. Int. J of Antimic. Agents 1999; 11: 221-231.)

1.3.7 The control of catheter encrustation

A variety of methods are currently used to try and control the problem of catheter blockage. These include simply replacing the blocked catheter, changing the type or size of catheter, antibiotic treatment of the associated urinary tract infection, increasing the patient's fluid intake, dietary acidification of the urine and the instillation of acidic/antiseptic or saline solutions through the catheter into the bladder (bladder washouts). Simply replacing blocked catheters generally results in the rapid encrustation of the new catheter. Bladder instillations of acidic solutions designed to neutralise the urine and dissolve the calcium and magnesium salts are commonly used. In vitro examinations from Getliffe et al showed that it is possible to dissolve urinary catheter encrustations even using small volumes of acid bladder washout solutions.[68] It is true that struvite formation is reversible. Exposure to undersaturated urine or acidification leads to rapid dissolution of this mineral. McLean et al however, found in their flow-cell that whereas acidification of the urine medium readily dissolved struvite crystals that were in urine suspension, crystals entrapped within the biofilm remained intact.[69] A clinical trial in elderly female patients undergoing long-term catheterization also showed that bladder washouts had no demonstrable effect on the amount of encrustation occurring in vivo.[70] One has to consider that the activity of urease is dependent on the pH of the urine. The urease of *Proteus mirabilis* has its highest activity at a pH of 7. With rising pH the activity is reduced and reaches levels of only 50% at a pH of 9. Bibby and Hukins demonstrated that the acidification of the urine is not a feasible method for preventing encrustation of indwelling urinary catheters. In their experimental work, the acidification of the artificial urine in the presence of urease resulted in an initial decrease of pH.[71] This decrease in pH was followed by a rapid alkalinization of the urine despite a further supply of the acidic agent. This counteraction can be explained by an increase of urease activity in more acidic pH, so that further urea was converted into ammonia. Dilute acid solutions can remove the protective surface layer of mucus in the bladder and increase exfoliation of bladder mucosal cells, so that it should not be used clinically for bladder washouts. In contrast, there is evidence that alkaline citrate therapy can delay urease-induced crystallisation, and prevents residual fragment growth or re-aggregation after extracorporeal shock-wave lithotripsy. An antiseptic bladder washout containing chlorhexidine produced only minor and temporary effects on *Proteus mirabilis* infections in vitro.

Oral intake of ascorbic acid is commonly used as an alternative way of acidifying urine. It is clear, however that large doses are required to produce any appreciable reduction in urinary pH and that if the urine is already infected with *Proteus mirabilis,* no reduction in the urinary pH is achieved.[72] Urinary acidification alone to prevent catheter encrustation can not be recommended routinely at present, as controlled trials are lacking in this field.

High fluid intake is frequently recommended for catheterised patients but, in practice, it is difficult to maintain a high diuresis over a prolonged period by increasing water intake and urinary solute concentration is not correlated with the degree of encrustation.

Inhibitors of urease such as acetohydroxamic acid and flurofamide are much more effective in lowering pH in *Proteus mirabilis*-infected urine. Clinical trials with acetohydroxamic acid have demonstrated that it significantly inhibited the growth of struvite stones. Tremulousness and phlebotrombosis were experienced by some patients taking the drug and in one study these side-effects were judged intolerable in 22% of the patients taking the urease inhibitor.[73]

1.3.7.1 Modification of catheter material or composition

Urinary catheters are composed of various materials including rubber, latex, silicone, latex and silicone combination with or without hydrophil coating. The local host inflammatory response and tissue necroses associated with catheter use are greatest with natural rubber, less with latex and minimal with silicone catheters. Latex catheters are the least expensive but irritation and allergic reaction may occur. Silicone catheters offer no advantage over latex, although they are more comfortable and might be a better choice for long-term (>5 days) use. Silicone catheters obstruct less often than latex. Teflon or silicone-coated latex catheters in patients prone to catheter encrustation.[74]

The goal of modification of catheter material is as follows:

- to prevent bacterial adherence
- to inhibit bacterial growth
- to delay the onset bacteriuria
- to delay or prevent encrustation or blockage

Different strategies have been tried like for example, the incorporation of biocides or antibiotics into the catheter material or development of materials with surface properties, which prevent the adherence of bacterial cells.[74] Both active and passive

surface modifications can create a less infectious surface. The bactericidal surfaces are in reality employing controlled release of antiseptics or disinfectants. A thin layer of polymer matrix that covers the biomaterial surface directs the controlled drug-release approach. The mechanisms of drug release include diffusion, erosion of polymer matrix and dissociation of ionic coupling.

Concerning incorporation of biocides or antibiotics into catheter material, Nitrofurazone was examined in two clinical studies and they showed a one-week limited effect, mainly for Gram-positive and some susceptible Gram-negative organisms.[37,42] In vitro study combination of minocycline or rifampicine showed some protective effect. Recent multi-center control trials justified a delayed effect of post-prostatectomy bacteriuria for up to two weeks but this protective effect was limited to Gram-positive bacteriuria and thus could permit selection of gram-negative microorganisms as well as the development of resistant strains. Current efforts are made to incorporate ciprofloxacin into gels to achieve slow sustained release of agents.

In conclusion, catheters impregnated with antimicrobial agents have little effect in long-term patients. They can only be effective in short-term patients, especially under intensive care.[37,42,75]

Strategies to achieve the goal of bacteriostatic catheters involve coating or impregnation of the catheter material with various compounds. Catheters that are coated with silver oxide may delay bacteriuria during short-term use, but silver alloy-coated catheters seem to be more effective. The possible working mechanism is that silver surface precipitates membrane proteins of surface-associated bacteria to inhibit microorganism colonisation. On the other hand, silver ions bound to murein, produce bacteriostatic effects whereas at higher Ag+ concentrations, bactericidal effects are achieved by silver.[76,77]

In 1984, Hayward and Chapman suggested that biomaterials coated with lipids like those located on the external surface of erythrocytes would be haemocompatible and thus, suitable for use in the manufacture of medical devices.[74] It was proposed that phosphoryl-choline (PC), the major polar head group on the outer surface of erythrocytes, should be incorporated into synthetic polymers to mimic the naturally occurring membrane lipid dipalmitoylphosphatidylcholine (DPPC). Due to their poor mechanical properties, these polymers were not suitable as the base material for the manufacture of medical devices, but they have been exploited as surface coatings. It has been suggested that water molecules bind tightly to the PC head groups making it difficult for proteins

and other materials to interact with the surface. Indeed, it has been shown that proteins exposed to PC surfaces remain in their native state, whereas exposure to the base polymers induces conformational changes, which allow the proteins to bind irreversibly as a conditioning film.[74]

According to Stickler in vitro examinations the PC-coating didn't significantly reduce the encrustation of either latex or silicone. But the amount of encrustation on the PC-coated silicone was significantly less than on PC-coated latex catheters. Nevertheless, the mean blockage time of the PC-coated silicone catheter (64 h) was significantly longer than that for the latex catheters. In vivo examinations of PC-coated ureteral stents showed a lower rate of encrustation (mean encrustation score 1,64 v. 1,92) and biofilm formation (36% v. 54%) comparing to uncoated ones.[74]

Heparin with its antithrombogenicity and its strong electronegativity that repels cellular organisms is an excellent candidate for an anti-adhesive stent coating. In 1987 Ruggieri showed a 90 % reduction of bacterial adhesion on urinary catheter surfaces by heparin coating.[78] Hildebrandt demonstrated the reduction of stent encrustation by heparin coating in an experimental setting.[79]

Electric current applied to the catheter surface has been effective in vitro and animal model, but a practical clinical application of this technology awaits further studies.

1.3.7.2 Catheter cares

The following guidelines may be useful in decreasing or minimising the risk of infections:

- catheters should be used only when absolutely necessary
- insertion of the catheter should be performed under antiseptic conditions
- to minimise urethral trauma the tip should be lubricated and the smallest possible catheter size should be used
- all indwelling catheters should be attached to closed drainage
- maintain adequate urine flow at all times
- gravity drainage should be maintained
- after the catheter has been removed, a follow-up urine culture should be done
- symptomatic or persistent bacteriuria should be treated
- catheter change is suggested every 2-3 weeks.

2. AIMS OF THE STUDY

1. To examine the predisposing factors of urinary tract infections and septicaemia in patients with urinary foreign bodies. To define the strategies of preventive treatment with the help of which we can decrease the number of symptomatic urinary tract infections in patients with indwelling urinary catheters. To investigate the most frequent pathogens on urological devices.

2. To determine biofilm formation and the development of encrustation on the surface of urinary catheters.

3. To examine the advantages of heparin coating as one of the preventive possibilities of biofilm formation and encrustation on foreign bodies (indwelling urethral catheters, double J stents and percutan nephrostomy).

4. To compare the times of developing obstruction as a result of encrustation on different catheter materials. To determine the advantages of heparin coating concerning the safety of the indwelling time.

5. To define the adsorption of oral levofloxacin as the 3rd generation of fluoroquinolones to ureteral stents. To examine the adsorption level of antibiotics on stent conditioning films and their surfaces. To observe antibiotic level on stent surfaces after interrupting antibiotic treatment so that we can justify the grounds of intermittent or "pulse treatment" strategies.

3. MATERIALS AND METHODS

3.1 Methods used to investigate the predisposing factors of the urinary tract infections of patients with J ureteral stents

From January 2000 to October 2002, 56 patients were included into this study. They were inserted double J-stents with the aim of the resolution of obstructions. Before the stent insertion and on the day of the stent removal samples of midstream urine were obtained from each patient and submitted for microbiological investigation. Stents were removed in the operating room under aseptic conditions. At removal 2 to 3 cm of the J stent tips located inside the bladder were cut and sent for microbiological culture. Age, sex, previous medical history and the indication of the stent insertion were recorded for each patient. Single dose 500mg ciprofloxacin antibiotics were given to all patients at the time of the insertion for prophylaxis. Patients who had symptomatic urinary tract infection during the study were treated by antibiotics based on susceptibility results. In those cases where the symptomatic urinary tract infection failed to respond to appropriate antibiotics or who had progression to septicaemia the stent was removed. We excluded the patients from the study, if they used any antibiotics or immunsupressive drugs in the preceding week of the enrolment or simultaneous bilateral double J stents or when anterograd insertion was required. A Cook silicone 7Ch ureteral stent was used in all cases inserted in retrograde way.

Chronic renal failure was defined as serum creatinine elevated to above 200 μmol/l. Diabetic nephropathy was defined as a long-standing history of diabetes mellitus with features of chronic renal failure including macro albuminuria.

Urinalysis was performed in all urine samples using an automated Miditrone machine and those with high cell counts were confirmed by the standard manual method. The urine and stent tip samples were cultured routinely on eosin-methilen blue agar and blood agar, and incubated in air at 37

simulations with 100 000 replications were applied. A result of a statistical analysis was considered statistically significant if the result did not exceed the value of 0,05.

3.2 Methods used to prevent the urinary tract infections of patients with J ureteral stents

The most promising method of the prevention of infections connected with urinary catheters is the selection of the best catheter material that can resist to biofilm formation. Concerning vein catheters, heparin coating proved to be one of the most effective coating materials. Therefore we decided to examine the effectiveness of heparin coating materials in the urinary tract.

3.2.1. An in vitro examination of the resistance ability of heparin-coated catheters to encrustation caused by crystalline *P. mirabilis* biofilm

The ability of three catheter types to resist encrustation and blockage by crystal-generating urine cultures of *P. mirabilis* isolated from patients encrusted catheters was examined in a laboratory model of the catheterised bladder. Catheters (14 French ones) were inserted aseptically through a section of silicone tubing. They were attached to a glass outlet at the base of the vessel into a 200 ml glass chamber maintained at 37°C. The catheter balloon was inflated with water securing the catheter in position and sealing the outlet from the vessel 'bladder'. The catheter was then attached to a drainage tube and reservoir bag. Sterile pooled human urine was supplied to the bladder via a peristaltic pump. Thus, a residual volume of about 30ml was collected in the vessel below the level of the catheter eyehole. As urine was supplied to the model the overflow drained through the catheter to the collecting-bag.

The three catheters tested were a latex catheter with hydrophilic coating, a silicone catheter and a heparin-coated silicone catheter. After inoculation of the sterilised urine with the *P. mirabilis* strain the organisms were allowed to establish themselves in the model for one hour. The peristaltic pump was then switched on and fresh urine supplied to the bladder at 0.5 ml per minute. The models were operated until the catheters were blocked with encrustation. Low vacuum scanning rastelectron microscopy (REM) was performed to visually assess the extent of encrustation at catheter cross sections 1, 4, 10 and 30 cm from the tip.

3.2.2 The use of rastelectron microscopy and energy dosing X-ray microanalyser

The measuring system consists of high resolution surface electron microscope (Fa.LEO 1530 VP), with an energy dosing X-ray microanalyser (Fa.EDAX). The composition of the studied material was determined by the help of these instruments. By rastelectron microscopy electrons are diverted by the surface of the sample. The elastically and non-elastically generated primary electrons entering the matrix of the sample produce a more complex interacting effect. Depending on the volume, different signals can be seen. The most important ones are:

- secondary electrons (SE1 and SE2) give information on structure of the surface and topography
- backscatter electrons (BSE) give information on contrast of material and the thickness of sample
- X-ray quantums give information on the type and sum of atoms in the sample

The surface can be studied by low energy secondary electrons (SE1), which meet the primary electron rays, but accept these together with backscatter electrons. Backscatter electrons are in an elastic interaction with atoms of the sample. Elements with high numbers show a strong backscattering, while elements with low numbers play a role in the contrast of the sample. The interaction is dependent on the voltage and the density of the sample. The larger the energy of the primary electrons, the deeper they penetrate the sample material (Figure 12).

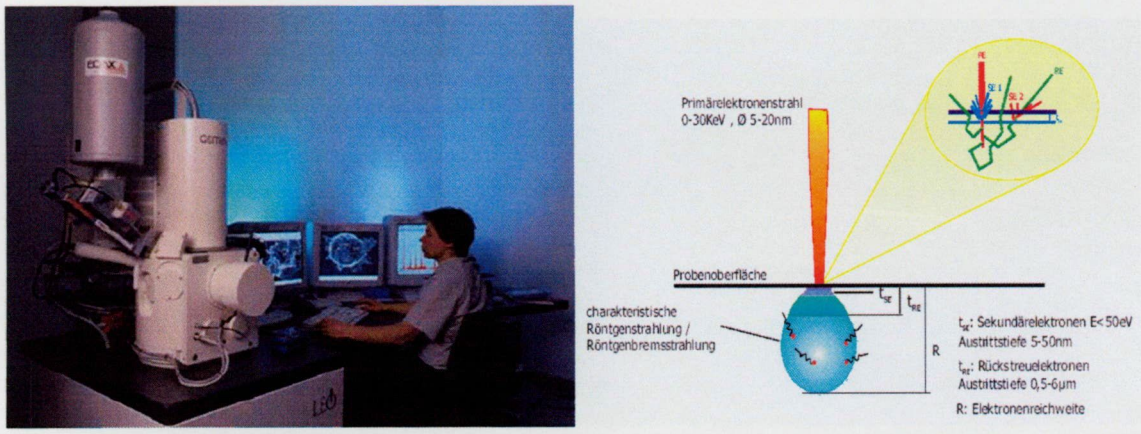


Figure 12. The rastelectron microscope and energy dosing X-ray microanalyser

3.2.3 Clinical evaluation of encrustation and biofilm formation on heparin-coated ureteral stents and nephrostomy tubes used during short period of time

In a patented procedure, the interior and external surfaces of commercially available polyurethane ureteral stents and silicone nephrostomy tubes were coated with a spacer to which heparin was bound in a covalent manner. In a pilot study the encrustation of heparin-coated ureteral stents was compared to uncoated polyurethane ones. Twenty heparin-coated and 20 uncoated stents were inserted into obstructed ureters in a prospective randomised study under sterile conditions and left indwelling for periods between 2 and 6 weeks. The stents were then removed under sterile conditions, sealed in sterile covers and sent for electron-microscopic evaluation. The average age of the patients in the group of heparin-coated stents was 52 while in the group of uncoated ones 55. Nephrostomy tubes were used in 2 male patients (average age 63 years) with permanent bilateral external urinary drainage suffering from frequent encrustation obstruction of their silicone catheters that resulted in repeated emergency visits. In these patients a heparin-coated and an uncoated nephrostomy tube were used simultaneously for either side so that direct comparison of encrustation status was possible.

3.2.4 Clinical evaluation of encrustation and biofilm formation on heparin-coated ureteral stents during extended indwelling times

In 10 patients, from which 4 were women and 6 men, with the average age of 66, who had permanent ureteral stent drainage, heparin-coated stents were left indwelling for 6-8 months (Group 1). In all patients bacteriuria was demonstrable at the time of heparin-coated stent insertion, from previous stents. In 3 male patients with uretero-enteral anastomosis stricture in an ileal conduit, heparin-coated stents were left for 1 year (Group 2). The average age of the patients was 61.

3.2.5 Comparison of the heparin-coated and polyurethane stents before the development of obstruction in the long time period of indwelling stents

Between November 2001 and February 2004 18 patients were examined retrospectively and double J stents were inserted with the aim of definite resolution of the obstruction, partly because of the patients' poor general condition or basic disease. Their

average age was 67. In 66%.of cases, i.e. in 12 patients, obstruction was caused by advanced urological, gynaecological and retroperitoneal malignancy, while 2 patients had obstruction because of ureter stricture and 4 patients had obstruction of the ureteropelvic junction (UPJ). First, 7Ch Cook polyurethane stents were placed retrogradely under sterile conditions and 200mg of ofloxacin was given for antibiotic prophylaxis. From the starting point we performed biochemistry, mid-stream urine culture and abdominal ultrasound examination. These were repeated every month after the stent insertion. The stents were removed in cases when signs of increased obstruction could be detected in empty bladder during ultrasound examination or when the patient developed a symptomatic urinary tract infection. On the retrieval of the stents a1-cm-piece of bladder, ureter and kidney were sectioned, put into a 4% glutaraldehyde solution and stored at 4°C for subsequent REM analysis. Further 1cm pieces of different parts of the stent were retained for microbiological examination of adhered bacteria. The biofilm attached to the stent surface was dislodged into 25% Ringer's solution by combination of gentle scraping with sterile scalpel blade and ultrasonication, not later than 2 hours after removal. Stent biofilm suspension in 3 ml of 25% Ringer's solution was streaked onto chocolate blood agar and was incubated for 48 hours at 37°C aerobically and with 5% carbon dioxide enrichment. The REM was performed by one operator who evaluated the stent surface for the presence of microbial biofilm and encrustation. A biofilm was said to be present any time adherent bacteria were identified on the electron micrograph. The encrustation was determined in the following grading system:

Grading of stent encrustation

0. no encrustation
1. microscopic deposit on <50% of the stent section
2. microscopic deposits on > 50% of the stent section
3. macroscopic deposits on < 50% of the stent section
4. macroscopic deposits visible on > 50% of the stent section

Consequently the polyurethane stents were changed to heparin-coated ones (Heparius) in13 patients with a similar follow up. Indications of the removal of the stents were similar to the polyurethane ones, however the maximum indwelling time was extended to 12 months as long as side effects did not develop (increased obstruction, symptomatic UTI).

T-test and Wilcoxon-Mann-Whitney test were used for continuous variables in between-group comparisons. Chi-Square and Fisher exact test were used for comparison of distributions of categorical variables. Due to the small number of observations the results of Fisher exact test and Wilcoxon-Mann-Whitney test was considered as more appropriate. Paired T-test and Wilcoxon Signed Rank test were used in within-group comparisons.

A result of a statistical test was considered statistically significant if the resulted p-value did not exceed 0.05. The calculations were performed by SAS (for Windows, Ver 8.1).

3.3. The effect of levofloxacin treatment on urinary tract infections caused by obstruction

During the time between January 2002 and February 2004 we examined 24 patients who had acute urinary infection caused by urinary obstruction. For this reason, first we resolved obstruction by inserting a double J stent or percutan nephrostomy (PCN) and meanwhile, started antibiotic treatment (levofloxacin 1x 5oomg). 50 % of the patients were continuously treated by antibiotics till the day of the definitive curative operation when the foreign body was removed (Group 1). The other 50 % of the patients were given antibiotic treatment, which was stopped on the 7th day after insertion of double J stent or PCN. We started antibiotic treatment again on the day of the operation and continued it till the day of the removal of the foreign body (Group 2). On the 7th day of antibiotic treatment, due to side effects, levofloxacin treatment was abandoned in 4 patients in the continuous group, and was followed in the intermittent one. 37,5% of men and 62,5% of women in both groups with the average age of 47 in group 1 and 52 in group 2 were involved in the study. The cause of the obstruction could be definitely ceased only after treating the acute symptoms of the symptomatic urinary infection at the earliest time of 18 to 21days, i.e. after the temporary urinary deviation. Obstruction was mainly caused by ureteral stone in 100% of cases in the first group and 75% in the second group of patients. Another 2 patients had an impact large pyelum stone in the background of the obstruction. In both groups of the patients we examined and recorded clinical signs: fever, pain in the back, the number of blood leukocytes, complaints of patients with indwelling stents and the early (2 weeks after stent removal) and late (6-8 weeks after stent removal) clinical and microbiological recovery. We examined the level of levofloxacin in the conditioning

film layer and on the stent surface with the help of high-performance liquid chromatography (HPLC). HPLC analysis also allowed quantification of the drug within the biofilm and showed that the concentration of antibiotic reached the minimum inhibitory concentration (MIC). With the help of these data we could predict which uropathogenic bacteria would and which would not be able to form a biofilm. . On the retrieval of the stents a 3-cm-piece of bladder, ureter and kidney were sectioned, and sent for scanning electron microscopy (SEM). The preparates were stored in a 4% glutaraldehyde solution at 4°C. We examined biofilm formation and the rate of encrustation on 2 cross-sectional and 1 longitudinal pieces of preparation, taken from each part of the catheter and SEM was used with the same aim in both groups.

Chi-Square and Fisher exact test were used for comparison of distributions of categorical variables. Due to the small number of observations the results of Fisher exact test was considered more appropriate.

A result of a statistical test was considered as statistically significant if the resulted p-value did not exceed 0.05. The calculations were performed by SAS (for Windows, Ver 8.1).

3.3.1 The scanning electron microscopy method

During this kind of examination preparates which were fixed in 4% glutaraldehyde solution were washed in 0,1 M Na-cacodylate buffer. Postfixation in 1% OsO4. Dehydration in ethanol from 30% to absolute ethanol. Drying on 56°C in thermostat. Affixture to the sample holding by Electrodag 915 Silver Paint glue. Coating the surface with gold-film by Jeol Jee 4 B type steamer. The samples were examined by OPTON DSM 940 on 50-500x magnification.

3.3.2 Methods of in vivo assessment of levofloxacin in the conditioning film and surface of ureteral stents by High-Performance Liquid Chromatography

The removed devices were stored at –70°C prior to analysis. After thawing, different portions of the ureteral stents (kidney tip, bladder tip and ureteral part) were sectioned into 2 cm pieces (x 2 for duplication), placed in a test tube and vortexed for 30 seconds in 1 ml of HPLC water. This step was performed to recover levofloxacin from the conditioning film. The washed stent sections were then transferred to a plastic tube,

immersed in phosphate buffer saline (pH 5.5) and left on a gyratory shaker (250 rpm) for 24 hours to leach off the antibiotic from the surface of the stent. This method was found to be optimal for eluting the adsorbed antibiotic from the stent surface.

The resulting eluents were immediately re-refrigerated to –70°C. HLPC determinations of the above mentioned two eluents were carried out on the same day, not longer than 5 days after the processing of ureteral catheters.

We used an existing HPLC method developed by Hairui Liang et al after minor in-house modifications and validations. Briefly sample preparation involved addition of the internal standard (ciprofloxacin) and filtration of the eluents (SPIN-X LC centrifuge tube filter, Costar). Separations were performed on Hypersil ODS C18 column with a pH 3.5 mobile phase containing 10mM SDS, 10mM TBAA, 25mM citric acid and 45% ACN. Detection was carried out at 293 nm. Data and chromatograms were collected using Euro Chrom 2000 for Windows software. Antibiotic standards were kindly provided by the manufacturers: levofloxacin from Aventis, ciprofloxacin from Bayer.

4. RESULTS

4.1 The predisposing factors of urinary tract infections of patients with J ureteral stents

In literature a large amount of data can be found concerning the predisposing factors of urinary tract infections. Hovewer, much less is known about the cases of patients who have indwelling urethral catheters and even less about patients with ureteral stents. It is still an unsolved question when it is reasonable to use antimicrobial treatment in patients with urinary foreign bodies. For this reason during our study we tried to find the group of patients with indwelling ureteral stents for whom antimicrobial treatment was advantageous.

4.1.1 Correlation of duration of double J stenting with associated urinary tract infections

Of the 56 included patients 32 (57,1 %) were men and 24 (42,9 %) were women. The average patient age was 41 years (range 23 to 83). From the enrolled patients 17 (30,3 %) had no systemic disease, 29 had cancer of the urinary tract (51,7%), 20 (35 %) had obstruction due to cancer, 7 (12,5 %) had diabetes mellitus and 3 (5,3 %) had chronic renal failure. Table 2 shows the patient distribution pattern according to sex and systemic disease.

Group	No. Male (%)	No. Female (%)	Total No.(%)
Normal	10 (58,8%)	7 (41,2%)	17 (30,3%)
Urological cancer	16 (55,2%)	13 (44,8%)	29 (51,7%)
Diabetes mellitus	4 (57,1%)	3 (42,9%)	7 (12,5%)
Chronic renal failure	2 (66,6%)	1 (33,4%)	3 (5,5%)
Totals	**32 (57%)**	**24 (43%)**	**56 (100%)**

Table 2. Distribution of patients according to sex and underlying systemic disease

The indication for stent insertion was a previous lithotripsy in 8 cases (22%), a previous endoscopic procedure in 6 (16,6%) and the relief of ureteral obstruction in 22 (61%). The mean duration of J stent retention was 17 days (range 6 to 198 days). The urine cultures were positive (asymptomatic urinary tract infection) in 4 patients (7,14%) before stent insertion and in 15 (26,8%) at stent removal. This difference was statistically significant $p<0,001$. 15 out of all the patients with positive urine culture at stent removal 5 (33%) had a symptomatic urinary tract infection and received appropriate courses of antibiotics. One of the 5 patients was normal, while 4 had a systemic disease. Despite the adequate antimicrobial treatment 3 had septicaemia requiring premature stent removal. All patients who had septicaemia were women and also had diabetes mellitus. The bacteriuria rate was in 2 cases (10%) when stents were removed within 30 days and in 13 (36%) after 30 days ($p<0,01$). For stents removed within 30 days the positive culture rate of the stent tip was 17%. This rate increased to 42% for stents removed after 30 days ($p<0,01$) (Table 3).

Stenting duration (days)	No. Pts. (%)	No. Pos. Urine Cultures (%)	P.Value (Chi- square)	No. Pos. Stent Tip Cultures (%)	P.Value
< 30 day	18 (32%)	2 (10 %)	p<0,01	3 (17 %)	p<0,01
> 30 day	38 (68%)	13 (36 %)		15 (42 %)	

Table 3. Correlation of duration of double J stenting with positive cultures

4.1.2 Relationship of underlying systemic disease with rate of positive urine and double J stent cultures

Table 4 shows the relationship of underlying systemic diseases with the positive urine and stent tip culture rates. In almost all groups of patients the rate of J stent colonisation was higher than the rate of positive urine culture but the differences were not statistically significant.

Group	No.Pts.	No. Pos. Urine Cultures (%)	No. Pos. Stent Tip Cultures (%)
Normal patients	17	2 (12,5 %)	3 (17,6%)
Urological cancer	29	8 (27,6 %)	10 (34 %)
Diabetes mellitus	7	4 (57 %)	5 (71 %)
Chronic renal failure	3	1 (33 %)	1 (33 %)

Table 4. Relationship of underlying systemic disease with rate of positive urine and double J stent cultures.

4.1.3 Relationship of underlying systemic disease and sex with rate of positive urine and double J stent cultures

Table 5 shows the correlation of patient underlying disease and sex with the rates of positive urine and stent tip culture. In each category of patients the rate of positive urine culture and stent colonisation was higher in females. The overall bacteriuria rate in

12 cases (50%) compared to that in men in 3 cases (9,3%) ($p<0,001$). The rate of bacteriuria in patients without systemic disease was significantly lower (12,5 %) than in patients with systemic disease (33 %) ($p<0,001$). Inside this group the rate of bacteriuria was 27,6 % in patients with cancer, 57 % with diabetes mellitus and 33 % with chronic renal failure.

Group	No.Pts.	No.Pos. Urine Cultures (%)		No.Pos. Stent Tip Cultures (%)	
		man	women	man	women
Normal	17	0	2 (11,7 %)	0	3 (17,6 %)
Urological cancer	29	2 (6,7 %)	6 (20,6 %)	2 (6,7 %)	8 (27,6 %)
Diabetes mellitus	7	1 (14,2 %)	3 (42, 8 %)	1 (18,5 %)	3 (42,8%)
Chronic renal failure	3	0	1 (33 %)	0	1 (33 %)

Table 5. Relationship of underlying systemic disease and sex with rate of positive urine and double J stent cultures.

- The frequency of positive urine cultures is significantly less in men than in women in the normal group $p < 0,001$.
- The frequency of positive urine cultures is significantly less in men than in women in the urological cancer group $p = 0,044$.
- No statistically significant difference in the frequency of positive urine cultures between men and women in diabetes ($p = 0,14$)

No statistical analysis can be performed in the group of chronic renal failure because of the sample size.

- The frequency of positive stent tip cultures is significantly less in men than in women in normal group ($p<0,052$).
- The frequency of positive stent tip cultures is significantly less in men than in women in the urological cancer group $p = 0,008$

- No statistically significant difference in the frequency of positive stent tip cultures between men and women in diabetes ($p = 0,14$)

No statistical analysis can be performed in the group of chronic renal failure because of the sample size.

The colonisation rate was higher in women 66 % (16 cases) than in men 9,3% (3cases). Table 5 shows the microorganisms cultured from the urine of patients on the day of stent removal. The bacterial profile of the cultured samples from the tips of the stents was similar to that of the urine samples. The same organisms were isolated from urine and the stent tip in 11 cases (73,3%). The most frequent microorganism was *E. coli* in the urine and *Enterococcus faecalis* on the stent surface.

Organisms	No.Pts
E. coli	7
E. faecalis	4
P. aeruginosa	2
Klebsiella pneumoniae	1
Streptococcus species	1

Table 6. Microorganisms cultured from urine at stent removal.

4.2 Heparin coating as one of preventive strategies concerning infections with urinary catheters

The resistance of heparin coating to biofilm formation and encrustation in urinary catheters has been investigated only in recent times. We were also curious if heparin coating could be an advantage in patients with urinary devices. Therefore, we performed a number of in vitro and in vivo examinations, in which heparin coating and other materials of ureteral and urethral catheters were compared.

4.2.1 An in vitro examination of the ability of heparin-coated catheters to resist encrustation by crystalline *P. mirabilis* biofilm

Whereas the hydrogel-coated latex catheter was blocked after an average of 28,1 hours in 4 experiments, time until blockage was significantly longer for the heparin-coated catheter (58,2 hours) and silicone catheter (54,8 hours) in the setting described (Figure 13). However, all 3 types of catheters were vulnerable to *Proteus* blockage.

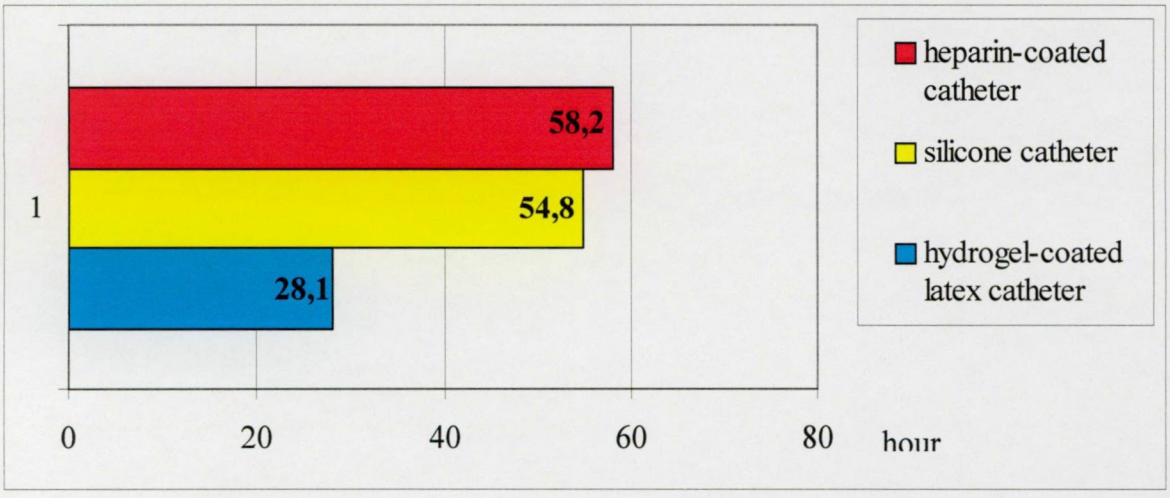
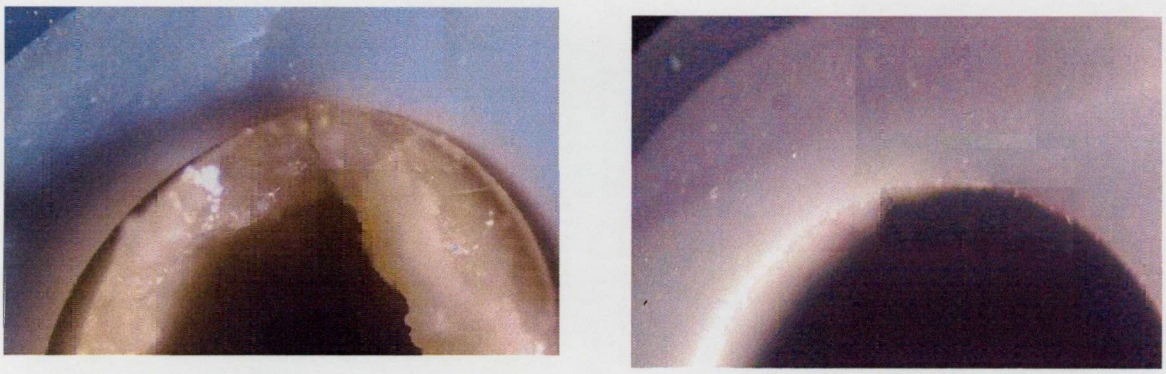


Figure 13. The average blockage time of heparin-, silicone- and hydrogel-coated catheters

The results of the in vitro examinations were as follows:

- All 3 types of catheters were vulnerable to Proteus blockage.
- There was no significant difference in time of blockage found in the 4 experiments.
- However, there was a close to significant difference between hydrogel-coated vs. heparin-coated catheters (p= 0,056).
- Encrustation (REM) was observed only on hydrogel-coated and silicone catheters (especially around eyeholes and balloon).
- No encrustation was found on heparin-coated catheters (blockage occurred by plugs of clear gel-like material).

Encrustation (REM) was observed only on hydrogel-coated and silicone catheters especially around eyeholes and balloon but no encrustation was found on heparin-coated catheters; the blockage was caused by plugs of clear gel-like material (Figure 14 a,b).



a. b.

Figure 14. (a) An uncoated ureteral stent with a biofilm formed on its inner surfaces. (b) A heaprin-coated ureteral stent which remains unaffected by biofilm formation and encrustation.

4.2.2 Clinical evaluation of encrustation and biofilm formation on heparin-coated ureteral stents and nephrostomy tubes in a short period of time

Electron microscopy showed a significant difference between heparin-coated and uncoated ureteral stents. Two weeks after the insertion, two types of deposits could be detected on the surfaces of the uncoated stents - amorph anorganic deposits consisting of mineralised crystals and the other of bacterial biofilms. Heparin-coated stents were unaffected by encrustations. After 6 weeks of indwelling time, all uncoated stents showed varying degrees and forms of deposits. (Figure15) . Within the limited observation period of this pilot study none of the uncoated stents became totally obstructed. The heparinised nephrostomy tubes remained unaffected for the whole 6 to 8 weeks indwelling periods (Figure 16), whereas uncoated tubes got obstructed within 2 to 3 weeks.

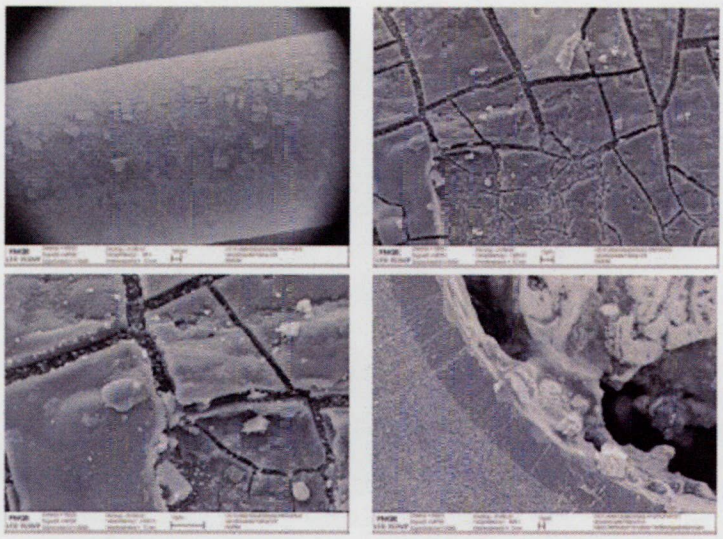


Figure 15. Developed encrustations and biofilms after 6 weeks of implantation on uncoated stents of different sections and enlargements.

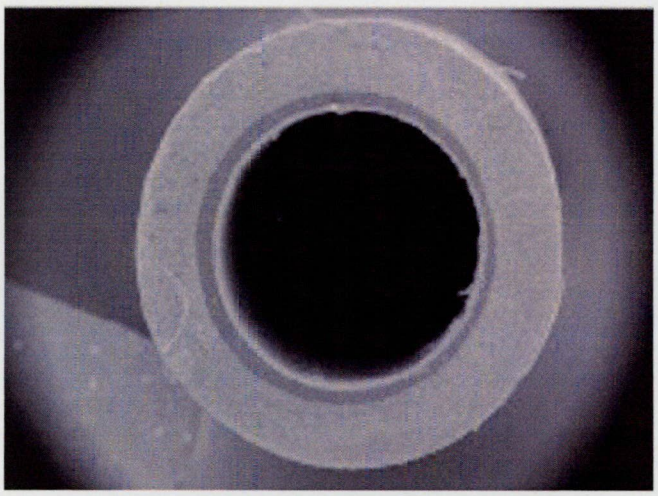


Figure 16. A cross section SEM picture of heparin-coated stent. No encrustation or biofilm detected on the stent surface after 6 weeks of implantation.

This pilot study showed that no biofilms were detectable on heparin-coated stents, whereas significant biofilms were demonstrated in 33 % of uncoated stents. Mild encrustation was observed in 10 % of heparin stents compared to significant encrustation in 50 % of uncoated stents, and encrustations/biofilms were demonstrable on uncoated stents as early as 2 weeks after implantation (Figure 17).

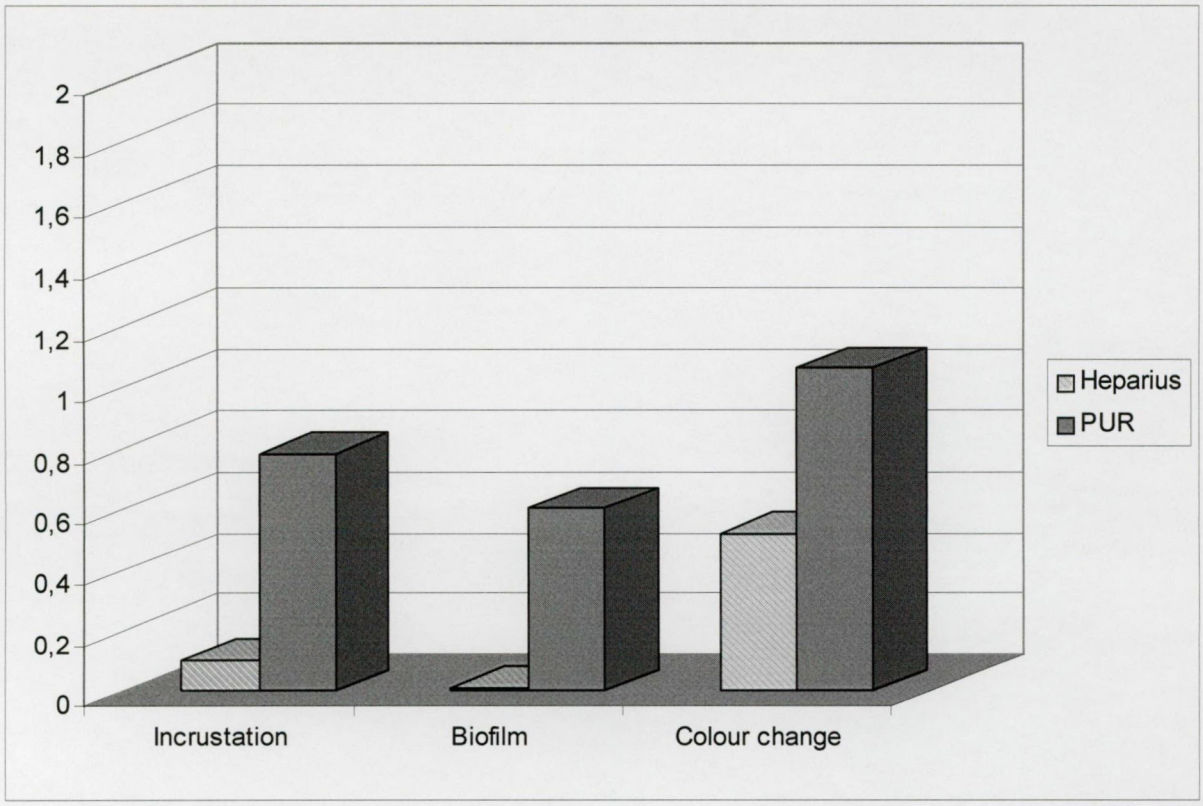


Figure 17. Encrustation, biofilm formation and colour change of heparin-coated ureteral stents and uncoated polyurethane stents (PUR) within a 6-week observation period (Y-axis is 0: no change, 1: moderate change and 2: significant change.)

4.2.3 Clinical evaluation of encrustation and biofilm formation on heparin-coated ureteral stents and nephrostomy tubes during extended indwelling time

No obstruction / blockage of the stents was observed during this time in Group 1. On REM none or only minimal encrustations were found after this prolonged indwelling time. In the difficult bacteria-exposed Group 2 situation, the silicone stents were found to be obstructed after 7 weeks while the hydrogel-coated stents in 5 months' time, whereas all of the heparin-coated stents were unaffected after 12 months of indwelling time (Figure 18).

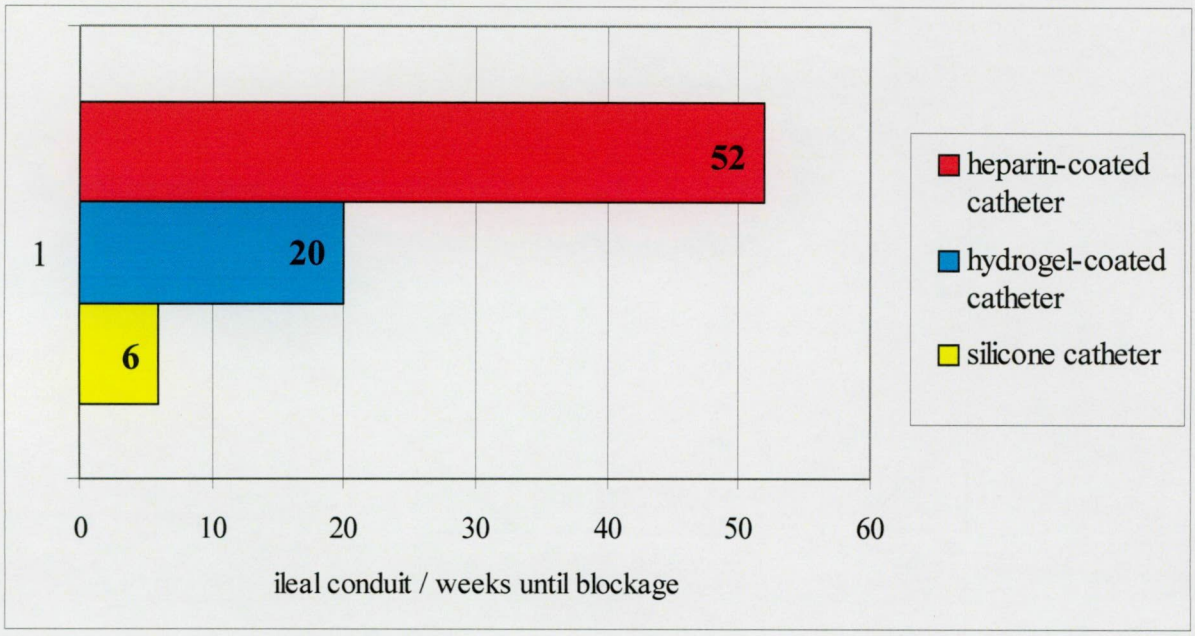


Figure 18. Indwelling times for various ureteral stents in ileal conduits

4.2.4 Comparison of the heparin-coated and polyurethane stents before the development of obstruction in the long time period of indwelling stents

The average indwelling time of the polyurethane stents was 15 weeks (varied 4 to 19). In the background of the removal of the stents was symptomatic urinary tract infection in 4 patients and signs of increasing obstruction were in 14 cases. We justified biofilm formation on the surface of the stents in 77,7 % of cases (14 patients) and we also isolated microorganisms in 66,6% of cases (in 12 patients) (Table 7). We detected encrustation in different grades in 94,4% on the inner and outer surfaces of the stents (17 patients). The average encrustation rate was the highest in the bladder followed by the ureteric and kidney portion of the stents. (Table 10). We found a significant difference between heparin-coated ureteral stents and polyurethane ones concerning biofilm formation and bacterial colonisation (Table 8, 9).

In many cases the central channels of the stents and eye-holes were completely occluded (Figure 19). In contrast the average indwelling time of the heparin-coated stents was 11 months (varied 10 to 13 months). Obstruction or symptomatic UTI were not detected in any of the cases. We observed mild encrustation in 4 cases (30,7%, grade 1-2) and biofilm formation in 2 cases (15,3% of stents) (Figure 20).

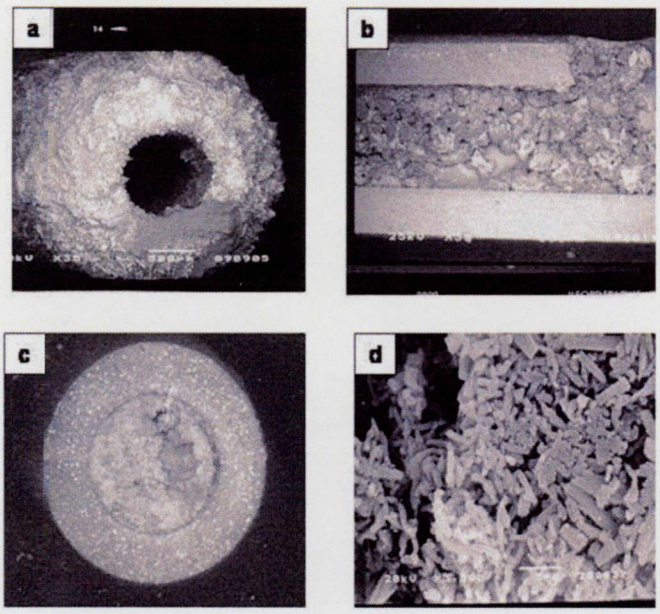


Figure 19. The central channels of the stents and eye-holes are completely occluded.

(a,b,c,)

(d – enlargement)

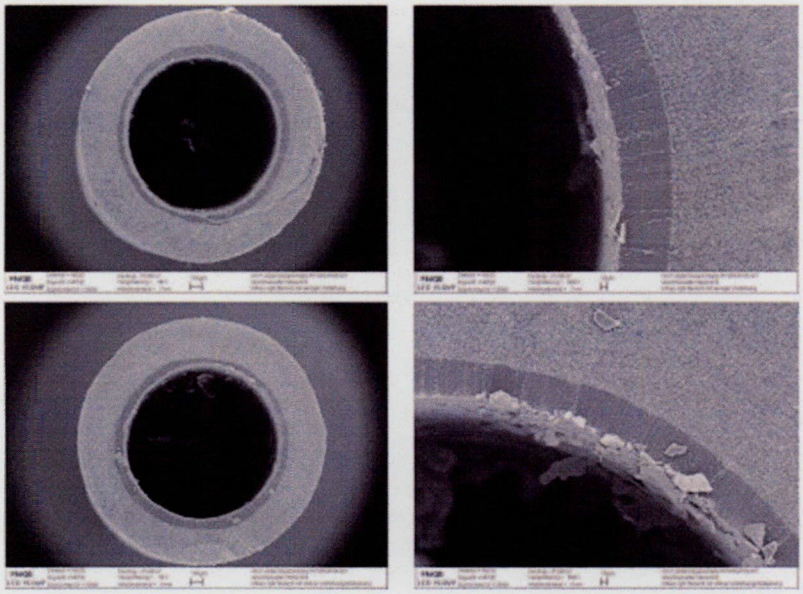


Figure 20. A heparin-coated stent with mild incrustation after 12 months' indwelling time (different sections, different enlargements).

Type of stents	Total number examined	Number (%) with visible biofilm	Number (%) from which microbes were isolated
PUR stent	18	77,7 (14)	66,6 (12)
Heparin-coated	13	15,3 (2)	15,3 (2)

Table 7. Biofilm formation and microbial colonisation of PUR and heparin-coated stents.

	Heparin-coated		PU-stent		All	
	N	%	N	%	N	%
Without visible biofilm	11	84,6%	4	22,2%	15	48,4%
With visible biofilm	2	15,4%	14	77,8%	16	51,6%
	13	***100%***	***18***	***100%***	***31***	***100%***
chi-square test: p<0,005 *Fisher-test: p<0,005*						

Table 8. Statistical analysis concerning biofilm formation between the two types of stents. The difference proved to be statistically significant.

	Heparin-coated		PU-stent		All	
Isolated microbes	N	%	N	%	N	%
Microbes were not isolated	11	84,6%	6	33,3%	17	54,8%
Microbes were isolated	2	15,4%	12	66,7%	14	45,2%
	13	***100%***	***18***	***100%***	***31***	***100%***
chi-square test: p<0,005 *Fisher-test: p=0,01*						

Table 9. The statistical analysis concerning microbial colonisation between the two types of stents. The difference proved to be statistically significant.

Stent type	Mean encrustation scores on stent sections			Mean total encrustation scores
	Bladder	Ureteral	Kidney	
PUR stent	3,1	2,7	2,4	2,7
Heparin-coated	0,53	0,38	0,30	0,4

Table 10. Summary of stent encrustation scores

The mean values quoted were calculated from the data on 18 PUR stents and 13 heparin-coated stents. The summary of the microbiological findings is given in table 11. Microbes were recovered from fewer of the heparin-coated stents than from the uncoated types (15,3% compared with 66,6%).

Organisms recovered	Number of stents colonised by each organism (%)		
	Total	PU-stent	Heparin-coated
Enterococcus spp.	5 (16,1)	4 (22,2)	1 (50)
Escherichia coli	4 (12,9)	3 (16,6)	1 (50)
Pseudomonas aeruginosa	2 (6,45)	2 (11,1)	0 (0)
Coagulase –ve staphylococci	2 (6,45)	2 (11,1)	0 (0)
Klebisella spp.	1 (3,2)	1 (5,5)	0 (0)

Table 11. Bacteria isolated from the stents

4.3 The examination of antibiotic adsorption capability on ureteral stents

During in vitro and in vivo examinations it was justified that fluoroquinolones are effective in a short- term period of time in prevention of biofilm formation on urinary catheters. It was also defined that ciprofloxacin and ofloxacin had a detectable level in conditioning film and on the surface of the stents. Data about the duration time of antibiotics on ureteral stents can be rarely found in literature. Thus, our task was to

evaluate the adsorption of levofloxacin - a 3rd generation fluoroquinolone on ureteral stents. We also searched for the answer to the question whether the intermittent or the continuous antibiotic treatment was more effective in patients wearing urinary catheters because of symptomatic urinary tract infections.

4.3.1 Intermittent and continuous levofloxacin (Tavanic iv., tabl.500 mg) therapy results in drug adsorption on urinary devices and prevention of the urinary tract infections

We didn't find any significant difference between the two groups of patients, neither concerning clinical, nor microbiological recovery. In the 1st group of patients the pain in the back caused by obstruction resolved in 37,5% of cases after 1-3 days of stent insertion. After 4-10 days 87,5% of patients, after 11-17 days all the patients were complaint free. We observed similar results in the second group of patients and we did not find any significant differences between the two groups. (Table 12) We did not record any significant discrepancies concerning urge, fever and the number of leukocytes between the two groups either. (Table 13,14)

The patients of the continuous group were treated by 500mg of levofloxacin with the average time of 23 (20-25) days, but they took antibiotics during 20 days (19-22) until the stent removal (operation). On the other hand, patients of the intermittent group took levofloxacin for 7 days.

During the HPLC examination we did not find any significant differences of the antibiotic adsorption level of the different parts of the stents (kidney, ureter and bladder part)(Figure 21).

Statistical analysis showed that significantly greater amount of levofloxacin adsorbed to the conditioning film than to the stent surface in both groups of patients (Table 15,16). We didn't find significant differences in the amount of levofloxacin which was observed in the conditioning film of the both groups (Table7). Similarily, there was no significant divergence in either group concerning the detectable antibiotic level on the stent surface (Table18). The drug levels reached on all the device surfaces in both groups were higher than MIC of *Escherichia coli* (0,004-0,015 mg/l), the most common uropathogene. The drug levels in the film were higher than the MIC of other common pathogens, namely *Pseudomonas aeruginosa* (0,25-1 mg/l), *Enterococcus faecalis* (0,25-2 mg/l) and *Staphylococcus aureus* (0,12-0,5 mg/l) in 41,6% (10), 25% (6) and 66,6% (16) of cases.

No viable, adherent bacteria were recovered by sonication and culture in any of the patients, and no biofilms were seen under scanning electron microscopy in either of the groups. There were no significant differences in the early or late microbiological recovery rate in either group. Two weeks after the definitive curative treatment there was no bacteriuria in either group. On the other hand after 6 to 8 weeks we found it in one patient in each group.

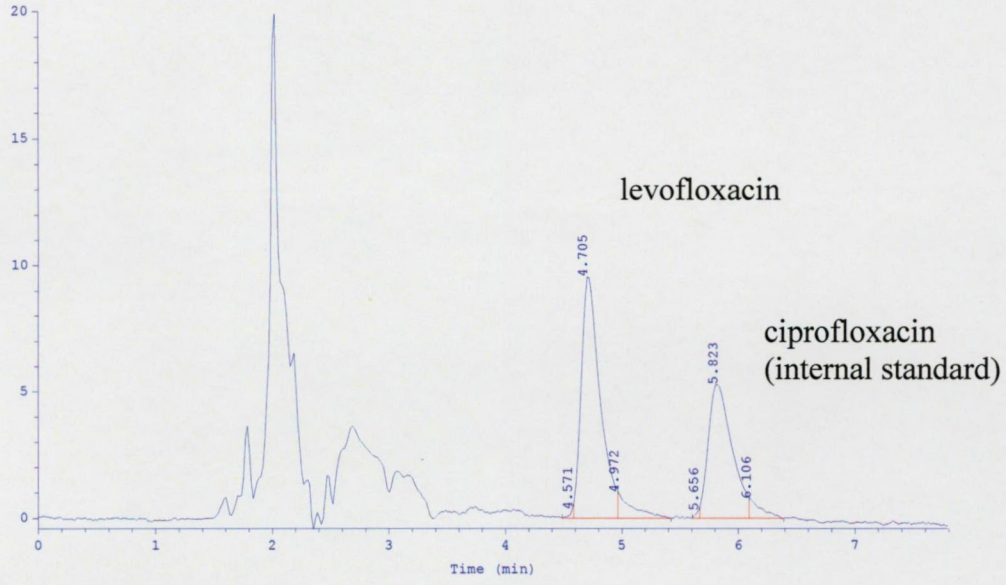


Figure 21. Representative chromatogram of a levofloxacin containing eluent (HPLC water) obtained by desolving the conditioning film of the ureteral stent

	Continuous group	Intermittent group
Before stent insertion	8 (100%)	15 (93,7%) [a]
1-3 days after stent insertion	5 (62,5%)	8 (50%) [b]
4-10 days after stent insertion	1 (12,5%)	2 (12,5%) [c]
11-17 days after stent insertion	0%	1 (6,2%) [d]

Table12. Development of back pain in the continuos and intermittent group

[a] chi-square test: p=0.47 Fisher-test: p=1 comparison of the two groups

[b] chi-square test: p=0.56 Fisher-test: p=0,68 comparison of the two groups

[c] chi-square test: p=1 Fisher-test: p=1 comparison of the two groups

[d] chi-square test: p=0,47 Fisher-test: p=1 comparison of the two groups

	Continuous group	Intermittent group
Before stent insertion	3 (37,5%)	3 (18,75%) [a]
1-3 days after stent insertion	7 (87,5%)	12 (75%)[b]
4-10 days after stent insertion	1 (12,5%)	2 (12,5%)[c]
11-17 days after stent insertion	4 (50%)	7 (43,7)[d]

Table 13. Development of irritative symptoms in the continuous and intermittent group

[a]chi-square test: p=0.32 Fisher-test: p=0,36 comparison of the two groups

[b] chi-square test: p=0.48 Fisher-test: p=0,63 comparison of the two groups

[c] chi-square test: p=1 Fisher-test: p=1 comparison of the two groups

[d] chi-square test: p=0,77 Fisher-test: p=1 comparison of the two groups

	Continuous group	Intermittent group
The average number of leukocytes before stent insertion	11,5 (6-14)	12,77 (6,9-26,7) [a]
The average number of leukocytes at day of operation	7,5 (4,4-10)	7,37 (3-10) [b]

Table 14. Development of the number of leukocytes in the continuous and intermittent groups. The average number of leukocytes before stent insertion between the two groups wasn't significant.

[a]t-test p=0,27, Wilcoxon-test p:0,54

On the day of the operation there wasn't significant difference either.

[b]t-test p=0,8 Wilcoxon-test p:0,87

Number of patients	Mean treatment duration (day)	Conditioning film (µg/ml)	Stent surface (µg/ml)
12	20 (19-22)	0,724	0,132[a]

Table 15. Mean concentration of antibiotic (µg/ml) in the conditioning film of the stent and on stent surface in the patients who were continuously treated by levofloxacin (500mg/day).

[a]paired t-test p=0,022 and Wilcoxon signed rank test, p=0,012 comparing levofloxacin concentration in conditioning film and concentration on the surface of the stent. The difference is statistically significant

Number of patients	Mean treatment duration (day)	Conditioning film (µg/ml)	Stent surface (µg/ml)
12	7	0,237	0,098[b]

Table 16. Mean concentration of antibiotic (µg/ml) in stent conditioning film and on stent surface in patients who stopped antibiotic therapy prior to stent removal.

[b]paired t-test p=0,061, and Wilcoxon signed rank test, p=0,02 comparing levofloxacin concentration in conditioning film and concentration on the surface of the stent. The difference is statistically significant

	Conditioning film (µg/ml)								
	N	Mean (µg/ml)	STD	[95% Confidence	Interval]	Min	Q25	Median	Q75	Max
Continuous group	10	0.721	0.832	[0.19	; 1.25]	0	0.102	0.419	1.327	2.497
Intermittent group	11	0.237	0.282	[0.07	; 0.41]	0	0.025	0.081	0.553	0.723
	21	***0.467***	***0.642***	***[0.19***	***; 0.75]***	***0***	***0.07***	***0.186***	***0.59***	***2.497***
t-test: p=0.08 *Wilcoxon test: p=0.14*										

Table 17. The level of levofloxacin concentration in the conditioning film in the two groups. There is no significant difference.

	Stent surface (µg/ml)								
	N	Mean (µg/ml)	STD	[95% Confidence	Interval]	Min	Q25	Median	Q75	Max
Continuous group	10	0.202	0.342	[-0.01	; 0.42]	0	0.021	0.122	0.168	1.154
Intermittent group	11	0.1	0.146	[0.01	; 0.19]	0	0.011	0.047	0.126	0.504
	21	***0.148***	***0.257***	***[0.04***	***; 0.26]***	***0***	***0.018***	***0.06***	***0.168***	***1.154***
t-test: p=0.38 *Wilcoxon test: p=0.34*										

Table 18. The level of levofloxacin concentration on stent surfaces. There is no significant difference between the two groups.

5. DISCUSSION

5.1. Factors predisposed to urinary tract infections during indwelling J ureteral catheterization

Ureteral stents have an important role in the management of upper urinary tract obstruction and prevention of postoperative complications after endoscopic urological procedures. Their use is usually associated with mild morbidity, such as dysuria, urgency, suprapubic pain and urinary tract infections. The urinary tract remains the most common site of nosocomial infection. Urinary tract infection episodes are often resolved without treatment, but sometimes in the symptomatic patients treatment may be necessary. In the treatment of such patients the correct knowledge of the types of bacteria associated with these devices and their sensitivity is very important.

Our study justfied that the risk of bacteriuria and colonisation of ureteral stents significantly depended on the duration of the insertion of stents and the underlying systemic diseases. The rate of bacteruria and J stent colonisation in patients with systemic disease was significantly higher than in those without such conditions. This finding may have been due to the fact that diabetes mellitus, urological cancer and chronic renal failure, which significantly decreased the immune system, encouraged the infection of urine and the stent tip. In almost all groups of patients the rate of J stent colonisation was higher than the rate of positive urine culture. 15 out of all the patients with positive urine culture at stent removal 5 (33%) had symptomatic urinary tract infection and 3 (20%) had septicaemia despite adequate antimicrobial treatment, causing premature stent removal. Since the patients were not on a therapeutic antibiotic regimen, this result is further proof that, as in patients with indwelling ureteral catheters, patients with J stents do not require empirical antibiotic therapy since only a small percentage had symptomatic urinary tract infection or septicaemia requiring stent removal and antibiotic treatment. All patients who had septicaemia were women who also had diabetes mellitus, indicating that patients with these systemic diseases should be closely followed for early detection and treatment of symptomatic urinary tract infection.

Female gender is associated with higher rates of bacteruria and J stent colonisation than male gender. Furthermore, this association is higher in patients without systemic disease and in the presence of cancer in the urinary tract.

This study also shows that the most common pathogens isolated from urine at stent removal were *Escherichia coli* followed by *Enterococcus feacalis* and in 73,% of cases organisms cultured from the tips of the stents were similar.

It is true that in the majority of our patients stent associated bacteriuria and stent colonisation were asymptomatic but it is prudent to recommend to give empirical antibiotic coverage to patients who are at high risk of infection. However, in normal patients the selection of resistant organisms and increased cost, argue against the routine use of empirical antimicrobial therapy for urinary tract infection in those with indwelling J stents at low risk of significant symptomatic bacteriuria or stent colonisation. Analysis of our details show that patients at high risk of infectious complications can be identified by careful follow up to reduce the morbidity associated with the device.

5.2 Heparin coating as preventive strategy for the control of catheter-associated urinary tract infections

Infection, encrustation and blockage of long-term indwelling catheters, ureteral stents and nephrostomy tubes are significant medical and health-economic problems. Because of these problems, about 50% of patients with permanent urinary drainage devices present as emergency cases during their course of disease and produce costs of up to US $ 1,5 billion per year in Western Europe.[37] Thus, effective strategies for the prevention of catheter-associated infections are necessary to interrupt the process of biofilm formation and encrustation. The prevention of bacterial adherence to biomaterial surfaces is an extremely difficult task to achieve, since a high number of adhesion mechanisms via various molecules exist. They are aspecific receptors, proteins, glycoproteins and lipopolysaccharides, that may even vary between different species of bacteria. In addition, the direct adhesions of human blood proteins (albumin, fibrinogen, and fibrinonectin) to catheter surfaces coat the modified anti-adhesive structures such as heparin and that may again facilitate bacterial adhesion. The complexity of the adhesion mechanisms is the reason why none of the anti-adherence strategies investigated hitherto proved to be optimal.

The present pilot study confirms the result of several in vitro investigations that postulated the inhibition of biomaterial encrustation by heparin coating. In a clinical

setting, we were able to show that heparin-coated ureteral stents stayed free of deposits over the indwelling time for up to 6 weeks, whereas organic biofilms or organic crystals were observed on uncoated polyurethane stents as soon as 2 weeks after insertion.

Three different ways for surface heparinisation of medical devices have been described. Whereas physically adsorbed heparin is released quickly within hours after insertion and is effective only during this limited period of time (only useful for interventional devices), heparin incorporated into polymers may not be released to a sufficient degree to prevent adhesion of molecules and cells. Covalent heparin bonding seems to be the procedure of choice, if stability is guaranteed for a longer period of time. The reduction of thromogenicity and bacterial colonisation using covalent heparin bonding has been demonstrated in a clinical pilot study on venous catheters. The present study extends these findings to catheters in the urinary tract. As a continuation of this pilot study, the indwelling times of heparin-coated ureteral stents have been extended to 3, 6, and 12 months, and bacteriological evaluation of stent biofilms is added to the protocol.

Heparin-coated ureteral stents are more expensive than standard stents. However, with longer indwelling times and reduction of the number of stent exchange procedures, the total costs for heparin stents should be reduced compared to other stents. Heparin-coated stents cost 4 times the price of uncoated stents, the ratio is 2,3 for nephrostomy tubes and 6,5 for urethral catheters. If a stent exchange procedure is calculated at 200 Euros (a very conservative estimation), the costs per year for various stents and variable indwelling times are demonstrated in figure 22.

Similar calculations have been made for nephrostomy tubes and urethral catheters and show an advantage of the heparin-coated devices. Each institution has to check its own regime with regard to cost effectiveness, but the excellent qualities of the heparin-coated urologic drainage devices add a new possibility to the urologic armamentarium. Besides the possible cost reduction for permanent urinary drainage, significant reduction of patients morbidity due to less obstruction, less emergency visits and less invasive procedures is a compelling argument.

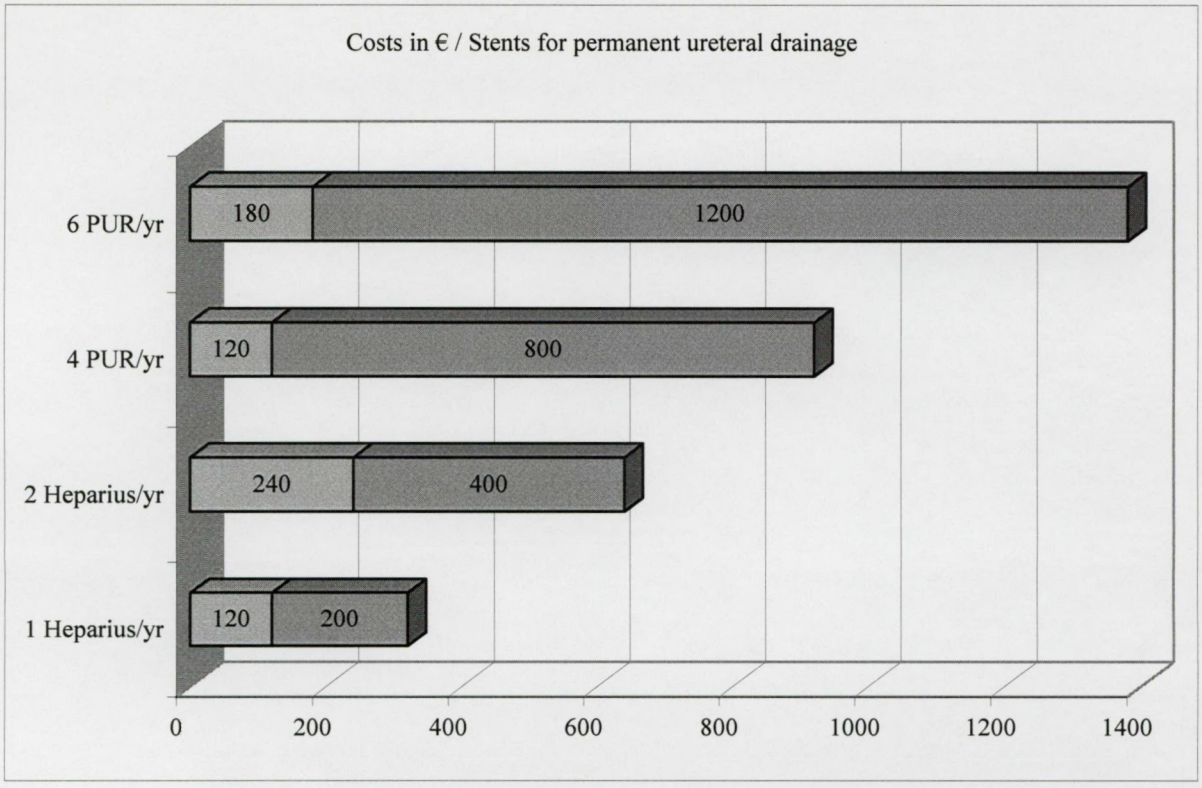


Figure 22. Costs per year for heparin-coated ureteral (Heparius) and uncoated polyurethane stents (PUR).

Our results unanimously justify that heparin coated urinary stents can resist biofilm formation and encrustation not only within short periods of time (6 weeks) but for longer terms as well (3 to 12 months). Heparin coating provides another alternative treatment of obstruction for the patients who are compelled to wear stents continuously during longer periods of time, thus preventing the frequent unpleasant and often unsuccessful change of the stents.

5.3 The place of intermittent antibiotic treatment in case of urinary tract obstruction after temporary insertion of a foreign body.

Previous *in vitro* and *in vivo* studies have shown that certain antimicrobial drugs (trimetropin-sulfamethoxazole, ampicillin, cefazolin, cephalexin, cloxacillin, cefuroxime, gentamicin, tobramycin) are able to eradicate the planktonic uropathogens associated with microbial biofilms, but they are not able to eradicate the bacteria that are adherent and

form biofilm.[64-66] *In vitro* studies have shown that ciprofloxacin can penetrate well-established biofilms on catheter surfaces.

Our study is one of the firsts, which examined the adsorption of antibiotics in vivo on stent surfaces. Reid at al and Wollin at al determined the level of ofloxacin and ciprofloxacin on the conditioning film and on stent surfaces. According to their studies both antibiotics could be detected and were observed in a significantly higher concentration in the conditioning film layer than on stent surfaces. Between the adsorption of the two antibiotics there was no significant difference.[80,81] A limited number of patients justified that on finishing antibiotic treatment after 1-2 weeks some amount of antibiotics could be still detected.

It is likely that ofloxacin remained in the conditioning film in a bigger amount after 1-2 weeks of finishing antibiotic treatment due to lasting diffusion. However, the investigators did not plan the time of antibiotic interruption and did not determine if the intermittence of the antibiotics was disadvantageous for the patients. References about levofloxacin adsorption to the foreign body surfaces cannot be found in literature yet. According to our results levofloxacin can better adsorb to stent surfaces than ofloxacin and is almost the same as ciprofloxacin comparing to Reid és Wollin's investigation results. Our data prove the hypotheses that the continuous antibiotic treatment does not have any advantages in patients with indwelling ureteral stents compared to intermittent or the so called 'pulse therapy'. In case of acute symptomatic urinary tract infections caused by obstruction it is enough to continue antibiotic treatment till the resolution of the acute symptoms in case of temporary resolution of obstruction. A repeated antibiotic treatment is satisfactory on the day of the definitive resolution of the obstruction. It is essential to pay attention to the adsorption capability when choosing the appropriate antibiotics, like for instance fluoroquinolones. Our study results back up that 'pulse therapy' can be effective in certain cases, when a three-day antibiotic treatment followed by a 7-10 days of antibiotic free period of time is more advantageous than continuous treatment by antibiotics or a wait-and-see therapy. It is also possible that stents with different material and with modified surfaces, will be quite receptive to certain drugs, and therefore the optimal patient management might use certain material-drug combination.

6. CONCLUSIONS

6.1 We were the first to investigate the predisposing factors of urinary tract infections in patients with indwelling ureteral stents in Hungary. Our results justified that the indwelling time of stents, female gender and systemic diseases that negatively influence the immune system appear to be the predisposing factors of urinary tract infections of patients with indwelling ureteral stents. Our results suggest that in case of multipredisposing factors, such as female gender, advanced diabetes mellitus) in case of a short indwelling time of stents (< 1 month) empirical antibiotic coverage should be given. In the category of patients at high risk of urinary tract infection with a long-time indwelling ureteral stent, we recommend a careful follow up. We believe that with the administration of antimicrobial prophylaxis or a short-time antibiotic treatment at stent removal, the development of bacteremia or septicaemia can be minimized. However, in normal patients the selection of resistant organisms and the increased cost argue against the routine use of empirical antimicrobial therapy in those with indwelling J stents at low risk for significant symptomatic bacteriuria or stent colonisation.

6.2 Together with Riedl and his Baden co-workers we were the first in Europe to investigate heparin–coated urinary catheters concerning biofilm formation and encrustation. Our in vitro and later in vivo studies showed that heparin coating can prevent biofilm formation and consecutive encrustation in short as well as in long periods of time. We compared the time of obstruction in patients with normal polyurethane and heparin-coated double J stents in the group of patients with similar conditions. We justified that heparin-coated ureteral stents can be safely maintained during a year's period of time with a considerable reduction of expenses.

6.3 We were also among the first investigators to determine the antibiotic level of adsorption on ureteral stent surfaces. So far there hasn't been any data in literature about the adsorption capabilities of levofloxacin on ureteral stents or its preventive effect on biofilm formation.

6.4 For the first time in Hungary we compared the results of continuous and intermittent antibiotic treatment in patients who had complicated symptomatic urinary tract infection with inserted urinary devices. Our results confirmed that the continuous antibiotic treatment does not have any advantages compared to the intermittent treatment strategy. In contrast, the continuous treatment can increase the antimicrobial resistance to pathogens as well as the expenses of treatment. Our study proved that after two weeks of finishing antibiotic treatment levofloxacin levels reached on all the device surfaces were higher than the MIC of *Escherichia coli.* In our study we also compared the above mentioned results of levofloxacin treatment to study results with ciprofloxacin treatment. We came to the conclusion that levofloxacin can reach similar antibiotic level as ciprofloxacin on stent conditioning films. However, on the interruption of antibiotic treatment, the diffusion of levofloxacin from the conditioning film is much slower than of ciprofloxacin, and therefore it has longer effect in prevention of urinary tract infections.

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References

1. Brumfitt W, Hamilton-Miller JT, Bailey R. Urinary Tract Infections. 1998 Lippincott-Raven publisher, INC. Chapter 8 Reid G, C.van der Mei H, Bussher JH. Microbial biofilms and urinary tract infections 111-115
2. Biering-Sorensen F. Urinary tract infection in individuals with spinal cord lesion. Current Opinion in Urology 2002; 12: 45-49.
3. Choong S, Whitfield H. Biofilms and their role in infections in urology. Brit J Urology 2000; 86: 935-941.
4. Costerton JW. Introduction to biofilm. Int J Antimicrob Agents 1999; 11: 217-221
5. Habash M, Reid G. Microbial Biofilms: Their development and significance for medical device-related infections. J Clin Pharmacology 1999; 39: 887-898.
6. Kunin CM, Chin QF, Chambers S. Formation of encrustations on indwelling urinary catheters in the elderly: a comparison of different types of catheter materials in "blockers" and "non-blockers". J Urology 1987; 138: 899-902.
7. Liedl B. Catheter-associated urinary tract infections. Current Opinion in Urology 2001; 11: 75-79.
8. Reid G. Biofilms in infectious diseases and on medical devices. Int J Antimicrob Agents 1999; 11: 223-226.
9. Fletcher M (ed.): Bacterial Adhesion: Molecular and Ecological Diversity. New York: Wiley-Liss, 1996.
10. Busscher HJ, Stokoos I, Schakenraad JM: Two-dimensional spatial arrangement of fibronectin adsorbed to biomaterials with different wettabilities. Cells Mater 1991;1:49-57.
11. Reid G, Tiezer C, Bailey RR: Bacterial biofilms on devices used in nephrology. Nephrology 1995;1:269-275.
12. Reid G, Tiezer C, Foerch R, Busscher HJ, Khoury AE, van der Mei HC: The binding of urinary components and uropathogens to a silicone latex urethral catheter. Cell Mater 1992;2:253-260.
13. Reid G, Davidson R, Densterd JD: XPS, SEM and EDX analysis of conditioning film deposition onto ureteral stents. Surf Interf Analy 1994;21:581-586.
14. Cormio L, Vuopio-Varkila J, Siitonen A, Talja M, Ruutu M: Bacterial adhesion and biofilm formation on various double J stents in vivo and in vitro. Scand J Nephrol 1996;30:19-24.

15. Reid G, Denstedt JD, Kang YS, Lam D, Naus C. Microbial adhesion and biofilm formation on ureteral stents in vitro and in vivo. J Urol 1992;148:1592-4.
16. Busscher HJ, Weerkamp AH: Specific and non-specific interactions in bacterial adhesion to solid substrata. FEMS Microbiol Rev 1987;46:165-173.
17. Van Loosdrecht MCM, Lyklema J, Norde W, Schraa G, Zehnder AJB: The role of bacterial cell hydrophobicity in adhesion. Appl Environ Microbiol 1987;53:1893-1897.
18. Van Loosdrecht MCM, Lyklema J, Norde W, Schraa G, Zehnder AJB: Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. Appl Environ Microbiol 1987;53:1989-1901.
19. Reid G, Lam D, Policova Z, Neumann AW: Adhesion of two uropathogens to silicone and lubricious catheters: influence of Ph, urea and creatinine. J Mater Sci Mater Med 1993;4:17-24.
20. Hawthorn LA, Reid G: The effect of protein and urine on uropathogene adhesion to polymer substrata. J Biomed Mater Res 1990;24:1325-1332.
21. Fletcher M: The effects of culture concentration and age, time, and temperature on bacterial attachment on polystyrene. Can J Microbiol 1976;23:1-6.
22. Zheng D, Taylor GT, Gyananath G: Influence of laminar flow velocity and nutrient concentration on attachment of marine bacterioplankton. Biofouling 1994;8:107-120.
23. Donald RM: Correlation between sulphate reducing bacterial colonisation and metabolic activity on selected metals in a recirculating cooling water system. Corrosion/92, paper no. 183, NACE International, Houston, TX, 1992.
24. Lawrence JR, Caldwell DE: Behaviour of bacterial stream populations within the hydrodynamic boundary layers of surface microenvironments. Microbial Ecol 1987;14:15-27.
25. Reid G, Habash MB: Urogenital microflora and urinary tract infections. In, Tannock GW (ed.): Medical Importance of the Normal Microflora. London: Chapman & Hall, 1998;423-440.
26. Lawrence JR, Korber DR, Hoyle BD, Costerton JW, Caldwell DE: Optical scanning of microbial biofilms. J Bacteriol 1991;173:6558-6567.
27. Lewandowski Z, Stoodley P, Roe F: Internal mass transport in heterogeneous biofilms: recent advances. Corrosion/95, paper no. 222, NACE International, Houston, TX, 1995.

28. Costerton J, Lewandowski Z, Caldwell D, Korber D, Lappin-Scott H. Microbial biofilms. Annu. Rev. Microbiol 1995;49:711-45
29. Busscher GJ, Bos R, van der Mei HC: Initial microbial adhesion is a determinant for strength of biofilm adhesion. FEMS Microbiol Lett 1995;128:229-234.
30. Caldwell DE. Cultivation and study of biofilm communities. In Lappin Scott HM, Costerton JW eds, Microbial Biofilms Cambridge: Cambridge University Press, 1195:64-69
31. Brown MRW, Collier PJ, Gilbert P: Influence of growth rate on susceptibility to antimicrobial agents: modification of the cell envelope and batch and continuous culture studies. Antimicrob Agents Chemother 1990;34:1623-1628.
32. Brown MW, Allison DG, Gilbert P. Resistance of bacterial biofilms to antibiotics: a growth-related effect J Antimicrob Chemother 1988;22:777-83.
33. Trieu-Cuot P, Carlier C, Martin P, Courvalin P Plasmid transfer by conjugation from *Escherichia coli* to gram positive bacteria. FEMS Microbiol Lett 1987; 48:289-94
34. Stock JB, Stock AM, Mottonen JM. Signal transduction in bacteria. Nature 1990; 344: 395-400
35. Jepsen OB, Larsen SO, Dankert J, Daschner F, Grönroos P, Meers PD, Nyström B, Rotter M, Sander J. Urinary tract infection and bacteraemia in hospitalised medical patients – a European multicentre prevalence survey on nosocomial infection. J Hosp Infect 1982;3:241-52
36. Zimakoff J, Pontoppidan B, Larsen SO, Stickler DJ. Management of urinary bladder function in Danish hospital, nursing homes and home care. J Hosp Infect 1993;24:183-99
37. Naber K.G., Pechere J.C., Kumazawa J.,Khoury S., Gerberding J.L., Schaeffer A.J. Nosocomial and Health Care Associated Infections In Urology 153-177
38. Platt R, Polk BF, Murdock B. Risk factors for nosocomial urinary tract infection. Am J Epidemol 1986; 124:977-85
39. Mobley HLT, Chippendale GR, Tenney JH. MR/K Hemagglutinin of *Providencia stuartii* correlates with adherence to catheters and with persistence in catheter-associated bacteriuria. J Infect Dis 1988; 157:564-71
40. Mobley HLT, Chippendale GR, Tenney JH, Hull RA, Warren JW. Expression of type I fimbriae may be required for persistence of *Escherichia coli* in the catheterised urinary tract. J Clin Microbiol 1987;25:2253-57

41. Sedor J, Mulholland S.G. Hospital-acquired urinary tract infections associated with the indwelling catheter. Urol.Clin. of North Am. 1999;26.4: 821-28.
42. Warren J. Catheter-associated urinary tract infections. Int J Antimicrob Agents 2001; 17: 299-303.
43. J. Warren, A. Bakke, F. Desgranchamps, J.R: Johnson, H. Kumon, J. Shah, P. Tambyah. Catheter-Associated Bacteriuria and the Role of Biomaterial in Prevention. Nosocomial and Health Care Associated Infections in Urology 2001; Chapter 6.
44. Nickel JC, Gristina AG, Costerton JW. Electron microscopic study of an infected Foley catheter. Can J Surg 1985;28:50-2
45. Ohkawa M, Sugata T, Sawaki M, Nakashima T, Fuse H, Hisazumi H. Bacterial and crystal adherence to the surfaces of indwelling urethral catheters. J Urol 1990;143:71721
46. Ganderton L, Chawla J, Winters C, Wimpenny J, Stickler D. Scanning electron microscopy of bacterial biofilms on indwelling bladder catheters. Eur J Clin Microbiol Infect Dis 1992;11:789-97
47. Nickel JC. Catheter-associated urinary tract infection: new perspectives on old problems. Can J Infect Contrl 1991;6:38-42
48. Reid G, Denstedt JD, Kang YS, Lam D, Naus C. Microbial adhesion and biofilm formation on ureteral stents in vitro and in vivo. J Urol 1992;148:1592-4.
49. Farsi HMA, Mosli HA, Al-Zemaity. Bacteriuria and colonisation of double-pigtail ureteral stents: long-term experience with 237 patients. J Endourol 1995; 9: 469-72
50. Goto T., Nakame Y., Nishida M. Bacterial biofilms and catheters in experimental urinary tract infection. Int. J of Antimicrob. Agents 1999; 11: 227-231
51. Morris NS, Stickler DJ, McLean RJ. The development of bacterial biofilms on indwelling catheters. World J. of Urology 1999; 17: 345-350.
52. Cox AJ, Hukins DWL, Sutton TM. Infection of catheterised patients: bacterial colonisation of encrusted Foley catheters shown by scanning electron microscopy. Urol Res 1989;17:349-52
53. Stickler DJ, Williams T, Jarman C, Howe N, Winters C. The encrustation of urethral catheters. In: Wimpenny J, Handley P, Gilbert P. Lappin-Scott H, eds. The life and death of biofilm. Cardiff: Bioline, 1995:119-25

54. Dumanski AJ, Hedelin H, Edin-Lijegren A, Beauchemin D, McLean RJC. Unique ability of Proteus mirabilis capsule to enhance mineral growth in infectious urinary calculi. Infect Immun 1994;62:2998-3003
55. McLean RJC, Lawrence JR, Korber DK, Caldwell DE. Proteus mirabilis biofilm protection against struvite crystal dissolution and its implications in struvite urolithiasis. J Urol 1991;146:1138-42
56. Cools HJM, Van der Meer JWM. Restriction of long-term indwelling urethral catheterisation in the elderly. Br J Urol 1986;58:683-8
57. Kunin CM, Chin QF, Chambers S. Formation of encrustations on indwelling catheters in the elderly: a comparison of different types of catheter material in „blockers" and „non-blockers". J Urol 1987;138:899-902
58. Getliffe KA. The Characteristics and management of patients with recurrent blockage of long-term urinary catheters. J Adv Nursing 1994;20:140-9
59. Kunin CM, Douthitt S, Dancing J, Anderson J, Moeschberger M. The association between the use of urinary catheters and morbidity and mortality among elderly patients in nursing homes. Am J Epidemiol 1992;135:291-301
60. Choong S., Wood S., Whitfield H.F. Catheter-associated urinary tract infection and encrustation. Int J of Antimicrobial Agents 2001; 17: 305-310
61. Keane PF, Bonner MC, Johnston SR, Zafar A, Gorman SP. Characterisation of biofilm and encrustation on ureteric stents in vivo. Br J Urol 1994;73: 687-91
62. Yachia D. Stenting the urinary system. Chapter 10. Reid.G, Denstedt J., and Tieszer C. Encrustation and microbial adhesion on stents 79-83
63. Bryan CS:, Reynolds KL. Hospital-acquired bacteremic urinary tract infection: Epidemiology and outcome. J Urol 1984;132:494-98.
64. Nickel JC, Grant SK, Costerton JW. Catheter associated bacteriuria, an experimental study. Urology 1985;26:369-75
65. Nickel JC, Wright JB, Ruseska I, Marrie TJ, Whitfield C, Costerton JW: Antibiotic resistance of Pseudomonas aeruginosa colonising a urinary catheter in vitro. Eur J Clin Microbol 1985;4:213-218.
66. Goto T., Nakame Y., Nishida M., Oh Y. In vitro bactericidal activities of beta-lactamases, amikacin and fluoroquinolones against Pseudomonas aeruginosa biofilm in artificial urine. Urology 1999; 53: 1058-62.
67. Tsukamoto T., Matsukawa M., Sano M., et al. Biofilm in complicated urinary tract infection. Int. J.of Antimicrob. Agents 1999;11: 233-236.

68. Getliffe KA, Hughes SC, LeClaire M. The dissolution of urinary catheter encrustation. Br J Urol Int 2000; 85: 60-64.

69. McLean RJC, Lawrence JR, Korber DK, Caldwell DE. Proteus mirabilis biofilm protection against struvite crystal dissolution and its implications in struvite urolithiasis. J Urol 1991;146:1138-42

70. Kennedy AP, Brocklehurst JC, Robinson JM, Faraghar EB. Assessment of the use of bladder washouts/instillations in patients with long-term indwelling catheters. Br J Urol 1992;70:610-5

71. Bibby JM, Hukins DWL. Acidification of urine is not a feasible method for preventing encrustation of indwelling urinary catheters. Scand J Urol Nephrol 1993;27:63-5

72. Murphy FJ, Zelman S, Mau W. Asorbic acid as a urinary acidifying agent. J Urol 1965;94:300-3

73. Griffith DP, Gleeson MJ, Lee H, Longuet R, Deman E, Earle N. Randomised, double-blind trial of Lithostar (acetohydroxamic acid) in the palliative treatment of infection-induced urinary calculi. Eur Urol 1991;20-243-7

74. D.J. Stickler, A. Evans, N. Morris, G. Hughes. Strategies for the control of catheter encrustation. Int J Antimicrob Agents 2002;19.499-506.

75. Tenke P, Jackel M, Nagy E. Prevention and treatment of catheter-associated infections: myth or reality? EAU Update Series 2004;vol 2/3 106-115.

76. Lundeberg T, Prevention of catheter-associated urinary-tract infections by use of silver-impregnated catheters. Lancet 1986;i:1031

77. Stickler DJ, Morris NS, Williams TJ. An assessment of the ability of a silver-releasing device to prevent contamination of urethral catheter drainage systems. Br J Urol 1996;78:579-88.

78. Ruggieri MR, Hanno PM, Levin RM. Reduction of bacterial adherence to catheter surfaces with heparin. J. of Urol.1987;138:423-6.

79. Hildebrandt P, Rzany A, Bolz A, Schaldach M. Immobilisiertes Heparin als inkrusteirungsresistence Beschichtung auf urologishen Implantaten. Biomed Techn. 1997;42:123-24

80. Reid G., Habash M. Oral fluoroquinolone therapy results in drug adsorption on ureteral stents and prevention of biofilm formation. Int. J.of Antimicrob. Agents 2001;17:317-320.

81. Wollin T.A., Chrietina T, Riddel J.V., Denstedt J.D., Reid G. Bacterial biofilm formation, encrustation, and antibiotic adsorption to ureteral stents indwelling in humans. J of Endourology 1998;12:101-111.