

*Review*

# Importance of biofilm in medical sciences: With special reference to uropathogens

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**Biofilm formation is a well organised, genetically-driven process, well characterized for numerous bacterial species which plays important role in urinary tract infections (UTIs). Adherence is a key event initiating each step in UTI pathogenesis. Such UTIs are difficult to treat owing to increased drug resistance within the biofilm cells. The review is mainly focused on biofilm-growing microorganisms because this form of growth poses a threat to chronically infected or immunocompromised patients and is difficult to eradicate from medical devices. Biofilm formation process and mechanisms to its increased resistance to various antimicrobials is also discussed together with newer prophylactic and therapeutic approaches like catheters coated with hydrogels or antibiotics, nanoparticles, iontophoresis, biofilm enzyme inhibitors, liposomes, bacterial interference, bacteriophages, quorum sensing inhibitor, combining antimicrobial photodynamic therapy and antiadhesion agents. The review justifies the need for new antibiofilm drug.**

**Key words:** Biofilm, uropathogens, catheter, urinary tract infection, catheter-associated UTI (CAUTI).

## INTRODUCTION

Various defence mechanisms of the body prevent the infection of urinary tract. One of the most important defence mechanism is the outward flow of urine that can clear 99% of the organisms experimentally inoculated in the bladder. The acidic pH (5.5) and low osmolarity of the urine also discourage the bacterial growth. However, there are a number of factors that increase the risk of developing urinary tract infections (UTIs). Some of these are sex, age, pregnancy, catheterization, kidney stone, tumours, urethral strictures, neurological diseases, congenital anomalies of bladder, suppressed immune system diabetes mellitus and ureteric stresses (Ramzan

et al., 2004).

A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Although, urine contains a variety of fluids, salts and waste products, it usually does not have bacteria in it. When bacteria get in to the bladder or kidney and multiply in the urine, they cause a UTI. The most common type of UTI is a bladder infection which is also often called cystitis. Another kind of UTI is a kidney infection, known as pyelonephritis, and is much more serious.

UTI is a serious health problem affecting millions of people each year. The recurrence rate is high and often

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the infections tend to become chronic with many episodes. UTI usually starts as bladder infections but often evolves to encompass the kidneys and ultimately can result in renal failure or dissemination to the blood. UTI is the most common infection in patients with a chronic indwelling bladder catheter and bacteriuria is essentially unavoidable in this patient group (Foxman, 2002).

Most UTIs are thought to be caused by organisms originating from the patient's own bowel. Normally, UTIs are caused by a variety of Gram-negative and positive bacteria. The Gram-positive bacteria includes *Staphylococcus* sp, *Streptococcus* sp and *Enterococcus* sp. Gram-negative includes a large number of aerobic bacilli such as *Escherichia* sp., *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Serratia* sp., *Salmonella* sp. and *Pseudomonas* sp. Among this, 80-90% of UTI is caused by *E. coli* (Rushton, 1997) and in ambulatory patients and nosocomial infections, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Enterococcus faecalis* are the most frequently isolated.

Urinary tract infections pose a serious health threat with respect to antibiotic resistance and high recurrence rates. Uropathogenic *E. coli* forms intracellular bacterial communities with many biofilm like properties within the bladder epithelium. These intracellular biofilm like pods allow bacteria to outlast a strong host immune response to establish a dormant reservoir of pathogens inside the bladder cells. Re-emergence of bacteria from this reservoir might be the source of recurrent infections (Suman et al., 2008).

A biofilm is a sessile community of microorganisms which are attached to an interface or to each other and are embedded in an exopolysaccharides matrix or to each other and alter growth rate and transcribes genes free for floating organisms (Thomas and Day, 2007). Biofilms are currently estimated to be responsible for over 65% of nosocomial infections and 80% of all microbial infections (Forster et al., 2010). Biofilm formation occurs in various stages as described below:

1) Reversible attachment of planktonic bacteria to surfaces: The first attachment of the bacteria is influenced by attractive or repelling forces that vary depending on nutrient levels, pH and the temperature of the site (Donlan, 2002). In this step, flagella (Lemon et al., 2007; Toutain et al., 2007) and chemotaxis play an important role avoiding the action of the hydrodynamic and the repulsive forces as well as selecting the surface (Schmidt and Kirsching, 2012), respectively.

2) Irreversible attachment to surfaces: In the case of *E. coli*, it is mediated by type 1 pili, curli fibres, and antigen 43 that also favours the interbacterial interactions (Danese et al., 2000; Anderson et al., 2003; Beloin et al., 2008; Cegelski et al., 2009). In the case of *P. aeruginosa* as well as other *Pseudomonas* species, transition from

reversible to irreversible attachment has been well studied. It has been observed that *Pseudomonas fluorescence* requires an ATP binding cassette [ABC] encoded by the *Lap* genes for carrying out this process (Hinsa et al., 2003). On the other hand, *P. aeruginosa* requires the Sad B Protein and the two component regulatory systems; BfisR for irreversible attachment (Caiazza et al., 2012; Petrova et al., 2010).

3) Formation of a complex layer of biomolecules (Lappinscott 2001) and exopolysaccharides secretion that constitute the external matrix. The production of polysaccharides in biofilm forming strains facilitates aggregation, adherence and surface tolerance, allowing better surface colonization (Laue et al., 2006). The nucleic acids, such as DNA, proteins, surfactants, lipids, glycolipids, membrane vesicles and ions such as calcium ions can also be found forming the part of the matrix composition and may play an important role in the characteristics that biofilm structure confers to the cells.

4) When biofilm are fully matured, detachment may occur. The detachment allows cells to again take on a planktonic state and can thereby form biofilm in other settings. It has been proposed that bacterial detachment could be caused by active mechanism initiated by the bacteria themselves such as enzymatic degradation of the biofilm matrix and quorum sensing in response to environmental changes related to nutrition level and oxygen depletion (Karatan et al., 2009) and by passive mechanisms mediated by external forces and erosion (Costerton et al., 1987; Kaplan, 2010; Hong et al., 2010; Rowe et al., 2010).

Consequences of Biofilm producing infections:

a) Detachment of the cell: The cell may get detached from the biofilm. This may cause blood stream and urinary tract infections (Meluleni et al., 1995)

b) Resistance to the host immune system: Biofilm coated bacteria escape from the damaging effect of the antibodies produced by the infected host cells (Holland et al., 2000)

c) Production of endotoxins: Gram negative bacteria which are encased in biofilms, produced endotoxins (Ethers and Bouwer, 1999).

d) The generation of resistant organisms: Bacteria can transfer plasmids by conjugation with the biofilm. So resistance factors may be exchanged through a plasmid (Christensen et al., 1985).

### Biofilm detection

The detection of the biofilms is done by following three methods:

1) Tube adherence method (TA) (Pramodini, 2012; Freeman et al., 1989). In this method, investigation of the biofilm production is done on the basis of the adherence

of the biofilm to borosilicate test tubes (Christensen et al., 1982). Suspensions of the tested strains are incubated in glass tubes containing trypticase soya broth aerobically at a temperature of 35°C for a period of 2 days. The tubes are decanted and stained with 0.1% crystal violet solution, washed with distilled water 3 times and dried. A positive result was interpreted as the presence of a layer of a stained material which adheres to the inner wall of the tubes.

2) Congo red agar (CRA) method (Niveditha, 2012; Tool et al., 1998). The isolate is inoculated into medium containing brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. The plates are incubated aerobically for 24-48 h at 37°C. A positive result is indicated by black colonies with a dry crystalline colonial morphology.

3) Tissue culture plate (TCP) method (Pramodini, 2012; Donlan, 2001). Isolate from fresh agar plates are inoculated in trypticase soy broth with 1% glucose and incubated for 24 h at 37°C in stationary condition and diluted (1 in 100) with fresh medium. Individual wells of sterile, polystyrene, flat-bottom tissue culture plates are filled with 0.2 ml of aliquots of the diluted cultures, and only broth served as control to check for the sterility and non-specific binding of media. The tissue culture plates are incubated for 24 h at 37°C. After incubation, the content of each well is gently removed by tapping the plates. The wells are washed four times with 0.2 ml of phosphate buffer saline (PBS pH 7.2) to remove free floating planktonic bacteria. 1% solution of crystal violet was added to each well, rinsed thoroughly and dried. OD of each well was measured at 578 nm using ELISA reader.

## BIOFILM ON MEDICAL DEVICES

When an indwelling medical device was contaminated with microorganism, several variables determine whether a biofilm develops. First, the microorganisms must adhere to the exposed surfaces of the device long enough to become irreversibly attached. The rate of cell attachment depends on the number and types of cells in the liquid to which the surface is exposed, the flow rate of liquid through the device and the characteristics of the surface. Once these cells irreversibly attach and produce extracellular polysaccharides to develop a biofilm, rate of growth is influenced by flow rate, nutrient composition of the medium, antimicrobial drug concentration and ambient temperature. These biofilms forms on different types of indwelling medical devices: central venous catheters, arterial catheters, mechanical heart valves and other surgical implants, endotracheal, nasal catheters and urinary (Foley) catheters, etc (Elliott et al., 1992). In the urinary tract, bacterial biofilms can develop on many living surfaces and virtually all artificial implants, producing chronic and often intractable infections

(Warren, 2001). Bacterial biofilms were reported to affect 90% of indwelling stents in patients (Reid et al., 1992). The medical consequences of device-related infections can be disastrous; they include potentially life-threatening systemic infections and device malfunction that may require device removal, often complicated by tissue destruction. A further complication that may be associated with urological medical devices is encrustation a phenomenon that frequently results in impairment of urine patency (Gorman et al, 2003).

## Urinary catheter biofilms

Urinary catheters are tubular latex or silicone devices, which when inserted may readily acquire biofilm on the inner or outer surfaces. The organisms commonly contaminating these devices and developing biofilm are *Staphylococcus epidermidis*, *Enterococcus faecalis*, *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, and other Gram-negative organisms (Ghanwate, 2012; Ghanwate et al., 2014). The longer the urinary catheter remains in place, the greater the tendency of these organisms to develop biofilm and result in urinary tract infections. For example, 10 to 50% of patients undergoing short-term urinary catheterization (7days) but virtually all patients undergoing long-term catheterization (>28 days) become infected (Ghanwate, 2012; Brisset et al., 1996; Balaban et al., 2004). It was found that adhesion to catheter materials was dependent on the hydrophobicity of both the organisms and the surfaces. Mack et al. (2004) stated that no single material is more effective in preventing colonization, including silicone, polyurethane, composite biomaterials or hydrogel coated materials. According to the National Institutes of Health [NIH], biofilm forming bacteria involved up to 80% of all infections (Stowe et al. 2011), with urology being one of the main fields in which biofilm can become a serious problem.

Biofilm cannot only develop into urethral stents but they can also form on catheters causing their blockage. Thus, catheter-associated UTI (CAUTI) is one of the most common catheter-associated infections around the world (Brisset et al., 1996). Commensal perineal flora is involved in most CAUTI cases. More than 90% of these infections are monomicrobial with *E. coli*, *Pseudomonas aeruginosa*, *Enterococci*, *Candida*, *Klebsiella* or *Enterobacter* spp. being the most frequently isolated pathogens (Ong et al., 2008). The environmental conditions created on the catheter surface make it an ideal site for bacterial attachment and formation of biofilm structures (Stickler et al., 1998). In this type of medical device, microorganisms producing urease, an enzyme that hydrolyzes urea to ammonium ions, can cause encrustation, formation of infected bladder calculi, and urinary obstruction. The formation of ammonium ions increases the pH of the urine; finally causing the

precipitation of magnesium and calcium phosphate crystals (Ghanwate, 2012; Jepson et al., 2001). The pH value at which precipitation occurs is called nucleation pH (Donlan 2002). These crystals can form a layer that protects bacteria from the antimicrobial effects of compounds used for coating or impregnating the catheters (Sancher et al., 2013).

### Biofilm and persistent infections

Acute UTI caused by bacteria can lead to recurrent infection, which is defined as a "reinfection" when it involves a strain other than that causing the original infection or it is defined as a "relapse" when it is caused by the same strain as that involved in the original UTI. Several studies observed that most of isolates collected from patients with relapse infections were biofilm producers "*in vitro*" (Sano et al., 1999). Relapse by uropathogenic *E. coli* (UPEC) has been related to the ability of pathogenic strains to form biofilm. In these cases, biofilm production may be the key determinant for the persistence of UPEC in the vaginal reservoir, the bladder epithelial cells, or both.

### Genes responsible for biofilm formation

Uropathogenic *E. coli* (UPEC) are the primary cause of urinary tract infection (UTI) in the developed world. The major factors associated with virulence of UPEC are fimbrial adhesins, which mediate attachment to specific receptors, enhance persistence and trigger innate host responses. UPEC produce a range of fimbrial adhesins, with type 1 and P fimbriae of the chaperone-usher subclass being the best characterized. The prototype UPEC strain CFT073 contains ten gene clusters that contain genes characteristic of this class of fimbriae. However, only five of these gene clusters have been characterized in detail (Nickel et al., 1985). The *f9* fimbriae-encoding genes were amplified, cloned and expressed in a K-12 background devoid of type 1 fimbriae. While *f9* fimbrial expression was not associated with any haemagglutination or cellular adherence properties, a role in biofilm formation was observed. *E. coli* K-12 cells expressing *f9* fimbriae produced a dense and uniform biofilm in both microtitre plate and continuous-flow biofilm model systems. In wild-type UPEC CFT073, expression of the *f9* major subunit-encoding gene was detected during exponential growth in M9 minimal medium.

*f9* expression could also be detected following selection and enrichment for pellicle growth in a CFT073fim foc double mutant. The *f9* genes appear to be common in UPEC and other types of pathogenic *E. coli*. However, their precise contribution to disease remains to be determined (Nickel et al., 1985).

## BIOFILM AND ANTIMICROBIAL RESISTANCE

In the biofilm stage, a phenotypic change occurs in which the bacteria require generally much higher concentration of antibiotics to inhibit their growth. This biofilm effect is the mechanism responsible for the frequent failure of antibiotic treatment to cure infections of medical devices and other prosthetic materials (Sepandj et al., 2003).

One of the most important advantages of biofilm status is the antimicrobial resistance shown by these structures. Biofilm can be up to 1000-fold more resistant to antibiotics than planktonic cells due to several mechanisms (Lewis, 2005; Costerton et al., 2007; Lewis, 2005, 2008; Ghanwate 2014; Ghanwate and Thakare, 2012; Morgan et al., 2009):

1. Limitation of antibiotic diffusion through the matrix-some antimicrobial agents are unable to diffuse through the matrix or sometimes the time required for the antibiotic to penetrate into biofilm is longer than the duration of treatment or the antibiotic life-time. Thus, for example, aminoglycosides penetrate more slowly through the matrix than lactams.
2. Transmission of resistance genes within the community can occur. Thus, plasmids, transposons, and other mobile genetic elements can be transmitted between cells forming biofilm by their close relationship, spreading resistance markers.
3. Expression of efflux pumps can also be considered as one of the mechanism for antimicrobial resistance not only in planktonic cells but also in biofilms structures (Van Acker et al., 2014; Lewis et al., 2001).
4. Inactivation of the antibiotic by changes in metal ion concentrations and pH values- Antibiotics able to diffuse can be inactivated by modifying the pH inside biofilm. This change in the pH could antagonize the activity of the antibiotic.
5. The persisters are dormant variants of regular cells, not mutants, which may form small colony variants that are high tolerant to extracellular stresses. They are highly tolerant to antibiotics forming a reservoir of surviving cells able to rebuild the biofilm population (Keren et al., 2004a, b 2004; Ulett et al., 2007). Persister is a problem for biofilm eradication. Proteins required for maintaining persisters may represent excellent targets for the discovery of compounds capable of effectively treating chronic infections and biofilm-related infections.

## ANTIMICROBIAL TREATMENT OF BIOFILM

Several studies recommend combination therapy as the treatment of choice in biofilm-associated infections, with macrolides being one of the first antibiotics chosen (Ethers et al., 1999). Macrolides [erythromycin, clarithromycin and azithromycin] present high "*in vitro*" and "*in vivo*" antibiofilm activity against biofilm-associated

infections caused by Gram-negative bacteria inhibiting the production of a key component of the matrix, alginate (Ethers et al., 1999). The antibiotic combination, clarithromycin plus vancomycin, demonstrated the ability to eradicate both biofilm and planktonic cells (Choong et al., 2001) as well as to eradicate biofilm on the titanium washers used in animal experiments (Davenport and Keeley, 2005). Roxithromycin plus imipenem favour a higher penetration of neutrophils into biofilm structure destabilizing the biofilm.

Another approach using antimicrobials consists of coating and impregnating the catheters with these antimicrobial agents (Ghanwate et al., 2014; Morgan et al., 2009; Brisset et al., 1996).

### Prophylaxis for CAUTI

CAUTIs are a major problem throughout the world. Catheter blockage is clinically important, as not only will the resulting bladder distension be painful for the patient, but a blocked catheter also increases the risk of serious clinical complications including septicaemia and pyelonephritis (Stickler et al., 1994). Management of urinary catheter encrustation is difficult, and occurrence is both unpredictable and extremely hard to prevent with existing strategies (Trautne et al., 2004). Most often, the approach used is catheter replacement once blockage has occurred (Stickler et al., 2014). Unfortunately, such treatment is often unsuccessful with frequent recurrence of blockage evident (Mathur et al., 2006). Prophylactic antibiotic use to prevent recurrence is not ideal due to the potential promotion of antibiotic resistance. One possible approach is to employ catheter materials that incorporate an antimicrobial agent that is either gradually released to the surface to inhibit colonisation or is utilised as an external catheter coating (Hooton et al., 2009; Jacobsen et al., 2010; Garibaldi et al., 1977).

### Chelating agents

Metal cations, such as calcium, magnesium, and iron have been implicated in maintaining matrix integrity. Consistent with this observation, chelating agents have been shown to destabilize biofilm architecture besides interfering with bacterial membrane stability. For example, sodium citrate inhibited biofilm formation by several *Staphylococci* species *in vitro* (Shanks et al., 2006). In addition, tetrasodium-EDTA eradicated biofilms in an *in vitro* biofilm model and on explanted hemodialysis catheters, whereas disodium-EDTA, in combination with tigecyclin or gentamicin, reduced biofilm formation by *Staphylococcus* species and *P. aeruginosa*.

### Antimicrobial peptides

Antimicrobial peptides are produced by the innate

immune response system and have been proposed as attractive candidates for the development of novel types of antibiotics. However, their activity spectrum and mechanism of action need to be more precisely defined before they can be considered as possible therapeutic strategies (Kharidia et al., 2011). A recent work, focused on reduced biofilm formation by multidrug-resistant *P. aeruginosa* strains isolated from patients with cystic fibrosis, revealed that the bacterium was killed within preformed biofilms. Lytic peptides are another group of antimicrobial peptides assessed for their inhibitory effects on biofilm formation. Lytic peptides bind the lipopolysaccharide moieties of the bacterial cell membrane, disrupting membrane stability (Kharidia et al., 2011). Studies on *S. aureus* have shown that a lytic peptide prevented *in vitro* biofilm formation and was also capable of diffusing into the deep layer of preformed biofilm, killing 99.9% of biofilm bacteria. This peptide retained activity under highly acidic environments and in the presence of excess of metals, conditions that mimic the *S. aureus* biofilm environment.

### BACTERIAL ANTIBIOFILM POLYSACCHARIDES

Polysaccharides, as sugar polymers, have the capacity to act as lectin inhibitors. Lectins are proteins that specifically recognize and bind sugars without modifying these molecules. In bacteria, the primary function of lectins is to facilitate attachment or adherence of bacteria to host cells. These proteins play an important role in biofilm formation, and are essential for bacterial colonization and infection. Lectins are mainly located on the surface of bacteria cells where they can access and bind to the glycan substrates present on the surface of host cell. By competing for the sugar binding domain of lectins, polysaccharides can inhibit lectin-dependent adhesion of pathogens and biofilm formation. In fact, several plant, microbial and milk polysaccharides have been shown to block various lectins from human pathogenic bacteria by competitive inhibition (Qin et al., 2009). Polysaccharides mediate cell-to-surface and cell-to-cell interactions that are critical for biofilm formation and stabilization. Recent evidence indicates that some bacterial exopolysaccharides inhibit or destabilize biofilm formation by other species (Qin et al., 2009). Antibiofilm properties of polysaccharides are believed to depend on their ability to: a) alter the physical characteristics of bacterial cells or abiotic surfaces; b) act as signaling molecules that impact the gene expression patterns of susceptible bacteria; or c) competitively inhibit multivalent carbohydrate-protein interactions, thereby interfering with adhesion. Many studies are reported on the ability of some bacterial polysaccharides to inhibit biofilm formation by several bacteria, including *E. coli* strains, *P. aeruginosa*, *K. pneumoniae*, *Staphylococcus* and *Enterococcus* (Rendueles et al., 2013.). Most of these antibiofilm agents are able to inhibit the biofilm formation

of a broad range of bacteria, suggesting that they may play an essential role in microbial competition and niche exclusion. Mutants unable to synthesize or export such polysaccharides are typically deficient in adherence and biofilm formation and thus are highly sensitive to killing by antibiotics and host immune defense (Maria et al., 2014).

### Anti-biofilm enzymes

Enzymes that degrade biofilm extracellular matrix may play a role in biofilm dispersal and may be useful as anti-biofilm agents. N-acetyl-D-glucosamine-1-phosphate acetyl transferase is an essential peptidoglycan and lipopolysaccharide precursor in Gram-positive and negative pathogens, respectively, is among the enzymes targeted for matrix disruption (Maria et al., 2014). Treatment with such enzymes prevented *Staphylococcus* and *Enterococcus* biofilm formation and disperse preformed biofilms *in vitro* (Guiton et al., 2009). For example, Dispersin-B is a glycoside hydrolase that cleaves  $\beta$  1–6 N-acetylglucosamine polymers in the bacterial peptidoglycan layer. Dispersin-B treatment has been shown to be effective against *S. aureus* and *S. epidermidis* biofilms and bacteria (Kaplan, 2010).

### Catheters coated with hydrogels or antibiotics

A high number of antimicrobial agents and other chemical compounds have been used to coat catheters. Silver alloy has been used in hydrogel coated urinary catheter observing a decrease of up to 45% of CAUTI (Devenport and Keeley, 2005; Raad et al., 2012). Minocycline rifampicin coated catheters have been shown to inhibit the biofilm formation of Gram-positive and negative pathogens, except *P. aeruginosa* and *Candida* spp. (Lellouche et al., 2012; Fisher et al., 2015).

Nanoparticles of MgF have been used for coating glass surfaces observing an inhibition of biofilm formation by both *E. coli* and *S. aureus* (Lellouche et al., 2009). Catheters have also been coated with these nanoparticles and a significant reduction of bacterial colonization was observed over a period of 1 week in comparison with the catheter uncoated catheter control. This group also demonstrated the antibacterial and antibiofilm activity of yttrium fluoride (YF3) nanoparticles which showed low solubility and provided extended protection (Roy et al., 2013).

Microwave irradiated CaO nanoparticles (CaO-NPs) have also shown the potential to inhibit biofilm formation against Gram-negative and positive bacteria (Fey, 2010). Silver nanoparticles have also been used for impregnating medical devices due to the silver antimicrobial properties (Costerton et al., 1994). Several studies have demonstrated the "*in vivo*" and "*in vitro*" inhibition of biofilm formation by numerous bacterial species and

using determined nanoparticle concentrations.

### Iontophoresis

Iontophoresis is a physical process in which ions flow diffusively in a medium driven by an applied electric field. This method enhances the efficacy of antibiofilm agents "*in vitro*" (Lu et al., 2007). Thus, it has been observed that low electrical currents enhance the activity of tobramycin and biocides against *P. aeruginosa* biofilm.

### Enzyme inhibitors

Urease, the enzyme that allows *P. mirabilis* to hydrolyze urea to ammonium ions, has been an important target in the study of new antibiofilm compounds. In this sense, fluorofamide has been a candidate molecule because it is able to prevent the increase in pH by *P. mirabilis* "*in vitro*", thereby inhibiting the formation of urea crystal and the subsequent encrustation and catheter obstruction. Other natural compounds, such as vanillic acid, natural plum juice and germa-lactones among others, presented the ability to strongly inhibit bacterial growth as well as the formation of crystals in catheters by the inhibition of the urease enzyme.

In one study, Giwerzman et al. (1991) generated a bacteriophage which expressed a biofilm-degrading enzyme during infection. The enzyme associated with the bacteriophage was DspB and it is produced by one species of Actino bacillus. DspB hydrolyses a crucial adhesion needed for biofilm formation and integrity in both *E. coli* and *Staphylococcus* (Raad et al., 1992) and attacks the bacterial cells in the biofilm and the biofilm matrix simultaneously. The percentage of eradication using this bacteriophage-enzyme combination was about 99.9% (Freeman et al., 1989). In recent years, the second messenger, c-di-GMP, has been studied in depth because it is highly conserved among bacterial species, being an important candidate for studies on biofilm inhibition. C-di-GMP is synthesized via diguanylate cyclases (DGC). Inhibition of DGC activity leads to a reduction in biofilm formation by a decrease in the intracellular levels of c-di-GMP.

Liposomes can be applied in the eradication of formed biofilm because when the antibiotic is encapsulated in a liposome carrier it does not interact with the EPS, improving its antibiofilm effect, and it is protected from degradation by antibiotic-inactivating enzymes (such as  $\beta$  lactamases) which can appear in the biofilm matrix (Siddiq and Darouiche, 2012).

In bacterial interference, the colonization of a surface by nonpathogenic bacteria could prevent the adherence of pathogenic bacteria thereby avoiding infection (Traunter et al., 2003). Several avirulent strains of *E. coli* have been used as a method to reduce urinary catheter

colonization by a wide variety of pathogens (Traunter et al., 2002; Anderson et al., 1991). Thus, the *E. coli* HU2117 strain, derived from *E. coli* 83972, that causes persistent colonization without symptomatic infection (Otto et al., 2001; Hull et al., 2002; Curtin and Donlan, 2006) has been used for coating urinary catheters, observing a reduction of biofilm formation by other pathogens (Trautner et al., 2002).

### Bacteriophages

These phages have been incorporated into hydrogel-coating catheters, and a reduction has been observed in biofilm formation by *S. epidermidis* and *P. aeruginosa* (Carson et al., 2010; Schmidt et al., 2012). In addition, the use of lytic bacteriophages against established biofilm of *P. mirabilis* and *E. coli* caused a reduction of three to four log cycles (Hensel and Xiao, 2009). These lytic phages also prevented biofilm formation on catheters coated with hydrogel containing bacteriophages. The reduction of formation observed was about 90% (Hensel and Xiao, 2009).

### Low-energy surface acoustic waves

It has been demonstrated that surface acoustic waves (SAW) interfere with adhesion of planktonic micro-organisms to cellular surfaces (Mack et al., 2004). SAW reduces biofilm bio burden on catheter segments in suspensions with several Gram-negative and positive bacteria as well as fungi, indicating its efficacy against a broad spectrum of micro-organisms.

### Anti-adhesion agents

The main characteristic of an anti-adhesive compound is that it specifically interact with the adhesins of the pathogen, inhibiting the union between pathogen and eukaryotic cell (Lohr et al., 2011; Jepson et al., 2001). These anti-adhesive compounds cause a decrease in invasion or infection of host epithelial cells, also avoiding recurrence. One of the compounds most frequently studied is cranberry extract (Foo et al., 2000). The anti adherence effect of cranberry against uropathogenic *E. coli* (UPEC) is due to the presence of A-type proanthocyanidin trimers in the cranberry extract (Foo et al., 2000; Hamblin et al., 2004) that acts as an anti-adhesion agent. Salicylate is a member of a large group of pharmaceuticals referred to as non-steroidal anti-inflammatory and it is the active component of the analgesic aspirin. Salicylate has been shown to decrease biofilm formation of UPEC, inhibiting type 1 fimbriae expression (Advances in Biology vol. 2014).

Antimicrobial photodynamic therapy (Grinholc et al., 2008) in recent studies has shown that the antimicrobial

effect can be obtained with the use of photosensitizers belonging to different chemical groups. Most studied PSs are phenol thiazine dyes methylene blue and toluidine blue O, porphyrin and its derivatives, fullerenes and cyanines and its derivatives (Grinholc et al., 2010, 2011; Nakonieczna et al., 2010).

### Need for future research

To better understand and control biofilms on indwelling medical devices, research must progress in several key areas. More reliable techniques for collecting and sampling biofilms should be developed. Model systems should be developed and used to study biofilm processes on various indwelling medical devices. These systems should closely simulate the *in vivo* or *in situ* conditions for each device, while at the same time providing reproducible and accurate results.

### Conflict of interests

The authors have not declared any conflict of interests.

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