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REVIEW ARTICLE

Escherichia coli biofilm: development and therapeutic strategies

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Summary

Escherichia coli biofilm consists of a bacterial colony embedded in a matrix of extracellular polymeric substances (EPS) which protects the microbes from adverse environmental conditions and results in infection. Besides being the major causative agent for recurrent urinary tract infections, *E. coli* biofilm is also responsible for indwelling medical device-related infectivity. The cell-to-cell communication within the biofilm occurs due to quorum sensors that can modulate the key biochemical players enabling the bacteria to proliferate and intensify the resultant infections. The diversity in structural components of biofilm gets compounded due to the development of antibiotic resistance, hampering its eradication. Conventionally used antimicrobial agents have a restricted range of cellular targets and limited efficacy on biofilms. This emphasizes the need to explore the alternate therapeuticals like anti-adhesion compounds, phytochemicals, nanomaterials for effective drug delivery to restrict the growth of biofilm. The current review focuses on various aspects of *E. coli* biofilm development and the possible therapeutic approaches for prevention and treatment of biofilm-related infections.

Introduction

Escherichia coli is a Gram-negative bacterium which is a facultative anaerobic in nature. It is a rod-shaped and nonsporulating bacterium that can be easily and inexpensively grown in laboratory conditions. It has been considered the model organism for different studies in biological engineering and industrial microbiology (Lee 1996). Most of the *E. coli* strains are found in the intestine of warm-blooded organisms where it benefits the host by preventing colonization of pathogenic bacteria (Singleton 1999). However, there are certain strains of *E. coli* that are majorly responsible for morbidity and mortality as in the case of various medical device associated infections such as urethral and intravascular catheters, prosthetic joints and shunts and prosthetic grafts (Reisner *et al.* 2014). *Escherichia coli* caused infections get aggregated and are difficult to eradicate due to formation of 'biofilms' (Danese *et al.* 2000). A biofilm is defined as an aggregate of micro-organisms that live together as a community and are often found attached to solid surfaces in moist environment. Microbes in a biofilm secrete a variety of protective substances called the EPS that enhance their survival efficiency. *Escherichia coli* biofilms are found to be the major causative agent of many intestinal infections. The dense bacterial cells in biofilm communicate with each other via the chemical signalling pathway known as quorum sensing (QS). During QS, bacterial cells secrete autoinducer substances (AI) to the extracellular milieu and once the required high density is attained, they upregulate biofilm formation and maturation (Sturbelle *et al.* 2015). AI help the bacterial cells in the biofilm to secrete virulence factors, modulate the host immune response and accrue genetic changes. Biofilm renders the penetration of conventional antibiotics hard and make the cells less susceptible to the antibiotics (Ito *et al.* 2009; Mittal *et al.* 2015). Hence there is an urgency to explore alternate therapeutic agents to combat the diseases originating especially due to *E. coli* biofilm formation.

Escherichia coli infections

The biofilm formation by *E. coli* contributes to the occurrence of various infections and makes their eradication

difficult. Factors like different extracellular appendages which contribute in *E. coli* surface colonization and their finely regulated expression and activity lead to formation of mature biofilms (Beloin *et al.* 2008). There are certain pathogenic strains of *E. coli* that are the major cause of morbidity and mortality. These enteropathogenic strains are further classified into two types: intestinal *E. coli* (InPEC) and extraintestinal *E. coli* (ExPEC). The InPEC group consists of various toxin-producing *E. coli* strains associated with Crohn's disease, enteric syndromes and haemorrhage. ExPEC group is a common leading cause of urinary tract infections (UTI), neonatal sepsis, meningitis in humans and various infectious diseases in animals including mastitis. (Vogeleer *et al.* 2014). *E. coli* strains causing UTI are termed uropathogenic *E. coli* (UPEC). *Escherichia coli* has also been the cause of various medical device associated infections in devices such as prosthetic grafts and joints, shunts as well as urethral and intravascular catheters. The formation of biofilm by *E. coli* on catheters makes catheter-associated urinary tract infections (CAUTI) one of the most frequent nosocomial infections (Reisner *et al.* 2014). UPEC isolates are a genetically heterogeneous group that possess different virulence factors necessary for persistence and colonization of the bacteria in the urinary tract, overcoming host defences and extra intestinal diseases. These virulence factors include fimbrial adhesins (F1C fimbriae S, P and type 1), afimbrial adhesin, toxins (haemolysin and cytotoxic necrotizing factor), siderophores (aerobactin system) and capsular polysaccharide (group II capsules). Genetic mobile elements are responsible for the virulence factors and genes for toxins and colonization factors, required for the pathogenesis, maybe found on plasmids.

Biofilm formation

There are four major steps involved in biofilm formation: (i) initial adhesion or attachment (reversible); (ii) early development of biofilm structure (irreversible); (iii) maturation of the developed biofilm and (iv) dispersion of cells from the biofilm to return to planktonic state.

Initial adhesion

Biofilm can form in any favourable environment that has proper nutrient conditions. The surfaces for attachment of cells can be abiotic such as metal, glass, plastic, medical implants, stainless steel or biotic such as epithelial cells, human skin and animal tissues. Apart from the environmental conditions like temperature, pH and ionic force of the medium, there are repulsive electrostatic and hydrodynamic forces in a liquid environment that inhibit the biofilm formation. Peritrichous flagella, that helps to overcome these forces acts as a mechanism for active motility of *E. coli* and increases the interaction between *E. coli* and the surface therefore providing the first cell-to-surface contact for adhesion. Motility is an important factor for adhesion but nonmotile bacteria can also attach to the surface with the expression of robust adhesion factors (Beloin *et al.* 2008).

Early development of biofilm

During early phase of biofilm development, the synthesis of the flagella is repressed as the attachment to the surface makes the adhered *E. coli* cells sessile. Several small molecules such as cyclic-diguanylic acid (c-di-GMP) are responsible for the shift from planktonic to sessile state. The concentration of c-di-GMP is low in motility state and it rises during biofilm formation. Adhesive organelles such as type 1 fimbriae and curli fimbriae play a major role in the irreversible attachment of *E. coli* to the surface (Wood 2009). Type 1 fimbriae or pili, found in *E. coli*, are important for the initial attachment to abiotic surfaces and they are encoded by the *fim* gene. Their expression is induced by adhesion and initial development of biofilm. Curli fimbriae, encoded by the *csg* gene, are the extracellular structures that attach to the proteins of the extracellular matrix. They also provide adhesion to abiotic surfaces by enhancing the cell-to-surface interaction and then facilitate the cell-to-cell communication (Beloin *et al.* 2008). Genes attributed in biofilm formation have been enlisted in Table 1.

Maturation

Once the cells are firmly adhered to the surface, they start aggregating through cell-to-cell interaction. The bacteria in the phase of maturation also produce the extracellular matrix which provides a three-dimensional structure to the biofilm. Autotransporters (for cell-to-cell interaction) and EPS (for matrix formation) are both crucial for biofilm maturation.

i Autotransporters: The proteins that do not require the help of accessory proteins for their translocation to the outer membrane are called autotransporter proteins (Beloin *et al.* 2008). Antigen 43 (Ag43) is the key autotransporter encoded by the *flu* gene. It promotes cell-to-cell adhesion, thus facilitating auto-aggregation and three-dimensional development. The transporter proteins (AidA and TibA) are associated with virulent strains of *E. coli* and cause aggregation and promote biofilm formation (Vogeleer *et al.* 2014).

ii The extracellular polymeric Substances-EPS: The EPS is the characteristic feature of biofilm that distinguishes it from planktonic bacteria. The EPS matrix is the medium through which bacterial cells are attached to the

Table 1 Genes involved in biofilm formation and development (http://www.uniprot.org; http://biocyc.org/ECOLI)

Biofilm Genes	Encoded proteins	Function	Location	Mass (in Daltons)	Web reference
csgD	CsgBAC operon transcription regulatory protein	Regulates curli fimbriae production and positively affects biofilm formation and stress regulation	Cell inner membrane, Peripheral membrane protein	24 935	[1]
hha	Haemolysin expression-modulating protein Hha	Repress the transcription of fimbrial genes thereby decreasing biofilm formation	Cytoplasm	8628	[2,3]
bcsA operon	Cellulose synthase catalytic subunit	Catalyses the formation of cellulose which is an extracellular component for mechanical and chemical protection of the cell	Cell inner membrane, Multipass membrane protein	99 785	[4]
*pga*C	Poly-beta-1, 6-N-acetyl-D-glucosamine synthase	Synthesis of PGA polymer that helps in biofilm adhesion	Cell inner membrane, Multipass membrane protein	50 766	[5]
fimB	Regulatory protein-FimB	FimB protein regulates type 1 fimbriae production	Cytoplasm	22 993	[6,7]

surface and facilitate cell-to-cell as well as cell-to-surface interactions. It provides support to biofilm cells and gives the biofilm a three-dimensional architecture, thus providing a protective as well as structural role. Water is one of the major components of the EPS, along with extrapolymeric polymers, proteins, nucleic acids, nutrients, lipids and other metabolites (Flemming and Wingender 2010). The polysaccharides secreted in the matrix are responsible for providing shape and structural support to the biofilm. The *E. coli* biofilm contain three major exopolysaccharides: β-1,6-N-acetyl-D-glucosamine polymer (PGA), cellulose and colanic acid, however, lipopolysaccharide and capsules are also important factors in the formation of *E. coli* biofilm. PGA helps biofilm formation by mediating cell-to-cell adhesion and attachment to surfaces. It also serves as an adhesin that stabilizes the *E. coli* biofilm. The *E. coli pgaABCD* operon encodes proteins including PgaC glycosyltransferase which is involved in the synthesis, export and localization of the PGA polymer. Cellulose synthesis is responsible for rigid biofilm formation. The genetic analysis of *E. coli* reveals *bcsABZC* operon which encodes the cellulose synthase protein BcsA. Colanic acid forms a capsule around bacterial cells and protects them from specific environmental conditions, however, it is also shown to have an inhibitory effect on biofilm formation as it masks Antigen 43 and AidA (Vogeleer *et al.* 2014). The other components such as lipopolysaccharides (LPS O antigen) and capsular polysaccharides (polysaccharide K antigen) in the matrix play an important indirect role in biofilm formation by facilitating interaction between the bacterial cells and the environment. LPS also plays significant role in adhesion by interacting with the cell-surface-exposed adhesion factors (Beloin *et al.* 2008).

iii Quorum sensing: QS is a cell-density-dependent chemical signalling system in which individual cells release small signal molecules called autoinducers or quormons, to the surroundings to promote the intraspecies communication. These autoinducers are specific for different species and can modulate gene expression (Daniels *et al.* 2004). QS is the process used by bacteria to communicate and coordinate their behaviour and function like a multicellular organism and it also controls gene expression during biofilm formation and maturation. Once the AI reaches a high concentration, it interacts with a regulatory protein that modulates gene expression of virulence factors and increase motility, fimbriae and heat-labile toxin expression (Sturbelle *et al.* 2015). Two types of AIs have been described in Gram-negative bacteria (AI-1 and AI-2). AI-1 molecules are N-acyl-homoserine lactones (AHL) and AI-2 is a unique furanosyl borate diester. AHL autoinducers share a common homoserine lactone moiety and differ only in their acyl group.

The AI-1 regulatory system consists of two structural genes – luxI that encodes the AI-1 synthase and luxR that encodes the AI-1 response regulator. LuxI and LuxR homologues are present in a wide variety of Gram-negative bacteria and control numerous processes ranging from virulence to biofilm formation. *Escherichia coli* is not able to synthesize AHL but its genome encodes *sdiA* which is an AI-1 sensor and a *luxR* homolog. AHL synthesized by other bacteria are sensed by LuxR encoded by *sdiA*. It has been shown that SdiA upregulates *uvrY* and *csrA* genes which enhance the biofilm formation, motility and virulence of *E. coli* (Beloin *et al.* 2008). AI-2 is responsible for both inter- and intraspecies bacterial QS. AI-2 has been shown to significantly increase biofilm biomass through

motility QS regulator (MqsR) and enhance flagellar motion and motility through MotA. MqsR regulates flageller movement through QseBC two-component system in which *qseB* encodes the response regulator and *qseC* results in synthesis of the sensor kinase. MqsR stimulates QseB, which controls the motility in *E. coli* through the master regulon *flhDC* which later stimulates MotA and FliA leading to biofilm formation. Also, MqsR induces curli expression through *crl* and stimulates motility through *csrA* (Barrios *et al.* 2006; Li *et al.* 2007).

LuxS-like synthase plays a major role in the production of AI-2 and after synthesis is transported outside the cell. During the stationary phase of cell-cycle growth, the extracellular AI-2 can be taken up by the cell with the help of luxS controlled transporter proteins LsrABCD, an ATP-binding cassette (ABC) transporter. AI-2, once inside the cell, is phosphorylated by LsrK kinase and promotes its own uptake by repressing the activity of LsrR repressor. Once the repression by LsrR on the *lsr* transporter genes is off, it further increases the synthesis of Lsr transport proteins and intake of AI-2 (Table 2).

iv Stress resistance genes: Stress tolerance is the property of pathogenic bacteria to survive in hostile environments. During the formation of the *E. coli* biofilm, various stress resistance genes are induced that protect the biofilm in harsh environments (Table 3). Hfq protein helps in the formation of *E. coli* biofilms in the harsh environment of the urinary tract. YcfR/BhsA induces indole production that forms biofilm resistant to acid, heat, peroxide and cadmium. The *ymg*B gene produces the protein AriR (Regulator of acid resistance influenced by indole), which imparts acidic resistance for *E. coli* to survive the low pH of the

Table 2 Genes involved in quorum sensing in *Escherichia coli* (http://www.uniprot.org/; http://biocyc.org/ECOLI; Brito *et al.* 2013 and Barrios *et al.* 2006)

Gene name	Protein synthesized	Function	Location	Molecular weight (in Daltons)	Web reference
luxS	S-ribosylhomocysteine lyase	Synthesizes AI-2. Stimulates biofilm formation and controls the biofilm architecture	Cytoplasm	19 416	[8]
mqsR	mRNA interferase MqsR	Regulates motility quorum sensing and positively regulates qseBC	Cytoplasm*	11 232	[9]
qseB	Transcriptional regulatory protein QseB	Response regulator in flagella synthesis as it activates transcription of FlhDC and regulates motility	Cytoplasm	24 678	[10]
qseC	Sensor protein QseC	Plays the role of sensor kinase, which is the receptor for autoinducers. Might activate QseB by phosphorylation	Cell inner membrane; Multi-pass membrane protein	50 282	[11]
pfs	5′-methylthioadenosine/ S-adenosylhomocysteine nucleosidase	Precursor for AI-2. Converts S-adenosyl homocysteine (SAH) to S-adenosyl homocysteine by removing adenine. Prevents the toxic consequences due to SAH accumulation. Regulates bacterial-type flagellum assembly	Cytoplasm	24 354	[12,13]
flhD	Flagellar transcriptional regulator FlhD	Sigma factor that activates the class 3 flagellum operons. Component of Flagellar Motor Complex	Cytolpasm	13 316	[14]
fliA	RNApolymerase sigma factor FliA	Generates force to rotate the flagellar motor	Cytoplasm	27 521	[15]
motA	Motility Protein A	Phosphorylates AI-2 into a molecule that binds and de-represses the LuxS regulated repressor (*lsrR)*	Cell inner membrane; Multipass membrane protein	32 011	[16]
lsrK	Autoinducer 2 kinase LsrK	Represses the LsrABCD transporter complex in the absence of AI-2	Cytoplasm	57 545	[17]
lsrR	Transcriptional regulator LsrR	*LsrR* is derepressed and inactivated in the presence of AI-2 as it binds to the phosphorylated AI-2	Cytoplasm	33 797	[18]
csrA	Carbon storage regulator	Motility and flagellum biosynthesis through the post-transcriptional activation of *flhDC* expression	Cytosol	6856	[19]

*Indicates those proteins for which the location is verified from the PSORT database. PSORT is a predictory tool that is used to predict the location of protein in the bacterial cells.

stomach (Wood 2009). C-di-GMP is linked to curli production and hence, overproduction of c-di-GMP increases *E. coli* biofilm production. The gene *rpo*S encodes the sigma S factor that regulates the stress response. The expression of the regulatory and structural genes, including genes for curli, cellulose, other matrix components and enzymes involved in the synthesis and degradation of biofilm (e.g. C-di-GMP), depends upon the sigma S factor (Serra and Hengge 2014). Certain prophages are also known to play a role in biofilm formation. DLP12 prophage is recently identified to induce stress resistance in *E. coli* biofilms. Deletion of the lysis genes of this prophage reduces curli production, thereby reducing biofilm formation (Rueggeberg *et al.* 2013).

Dispersion

This final step includes detachment of bacteria from the mature biofilm and their dispersal, that is, the transmission of the bacteria to a planktonic state, which can lodge at distant site and form biofilm. Detachment can occur as a result of enzymatic degradation of the biofilm matrix and QS in response to environmental changes related to nutrition levels and oxygen depletion by some external force (Soto *et al.* 2011). According to a study by Vogeleer *et al.*, modulation of type IV bundle-forming pili which is a crucial surface structure in enteropathogenic *E. coli* ExPEC and aggregative adherence fimbriae in enteroaggregative *E. coli* result in the detachment of bacteria from the biofilm and surface (Vogeleer *et al.* 2014).

Antibiotic resistance

The most frequent causative agent for UTI has been recognized as *E. coli* and most of these isolates were recognized as resistant to antibiotics ampicillin, amoxicillin-clavulanic acid, norfloxacin, cefuroxime, ceftriaxone and co-trimoxazole. Diabetes, renal disease and use of intra uterine device are some of the risk factors associated with UTI which complicate the infection and increases the cost of treatment, morbidity and mortality (Niranjan and Malini 2014). Formation of biofilm further complicates the infection and increases the dwelling of significant number of cells within a biofilm which are more tolerant to antibiotics than those cells that grow planktonically. Biofilm formation has been associated with medical devices including catheters, ventilators, contact lenses and their treatment is difficult. It has also been shown that cells from a disrupted biofilm typically become susceptible to antibiotics when grown planktonically (Zuroff *et al.* 2010).

Table 3 Genes involved in stress and antibiotic resistance (http://www.uniprot.org; http://biocyc.org/ECOLI)

Genes	Proteins	Function	Location	Mass (in Daltons)	Web reference
ycfR	YcfR or BhsA	Mediate stress by inducing indole synthesis; regulates biofilm formation	Cell outer membrane	8815	[20]
flu	Ag43	Adhesin protein that helps in auto-aggregation	Ag43 alpha chain: Secreted onto cell surface; Ag43 beta chain: Cell outer membrane	106 825	[21]
*ymg*B or *ariR*	AriR	Regulates the expression of genes involved in acid resistance and biofilm formation	Cytoplasm	9694	[22,23]
rpoS	RNA polymerase Sigma factor RpoS	Regulates genes induced under stress conditions	Cytoplasm	37 972	[24]
hfq	RNA-binding protein Hfq	Stabilizes to small regulatory RNA in response to stress and stress responses mediated by the sigma factors RpoS	Cytoplasm	11 166	[25,26]
yafQ	mRNA interferase YafQ	Enhances tolerance of *Escherichia coli* biofilms to specific antibiotics	Cytoplasm*	10 847	[27]
rapA	RNA polymerase-associated protein RapA	Alters gene regulation in biofilm including that of *yhcQ*. It enhances the biofilm antibiotic resistance	Cell inner membrane*	109 769	[28]
yhcQ	p-hydroxybenzoic acid efflux pump subunit AaeA	Encodes a multidrug resistance pump	Cell inner membrane	34 775	[29]
motA	Motility Protein A	Component of Flagellar Motor Complex. It generates force to rotate the flagellar motor	Cell inner membrane	32 011	[16]

*Indicates those proteins for which the location is verified from the PSORT database. PSORT is a predictory tool that is used to predict the location of protein in the bacterial cells.

The correlation between biofilm and antibiotic resistance was studied in CAUTI and it was found that most frequently isolated pathogen was *E. coli*. It was observed that biofilm strains were more resistant to ampicillin, cephaotaxime, norfloxacin and nalidixic acid than non-biofilm strains. In another study, ampicillin was found to affect biofilm formation at different stages viz. attachment, early development and maturation, and it was observed that cells in the attachment and early development phase did not form biofilm after 24-h of discontinuing ampicillin treatment but cells in the mature stage formed biofilm within 72-h of discontinuing ampicillin treatment (Ito *et al.* 2009). To understand the molecular players that regulate antibiotic resistance in *E. coli* biofilm, its mutant strain was isolated. It was discovered that the *rapA* gene altered the gene regulation of the biofilm that ensued lower expression of the 22 genes (Table 3). Deletion of *yhcQ* gene which encodes the putative MDR (multidrug resistance) pump decreased the biofilm penicillin G resistance in *E. coli*. Moreover the *rapA* mutation also reduced the extent of matrix coverage facilitating the penetration of penicillin. Thus, the development of penicillin resistance was not only attributed to MDR pump but also to matrix (Lynch *et al.* 2007).

Therapeutics

The increased expression of resistance markers within the biofilm as well as the diffusion limitations of the extracellular matrix have made biofilm bacteria recalcitrant to treatment with antibiotics. In UPECs, antibiotic resistance mechanism has evolved as it has been observed that there is a decrease in susceptibility to first-line agents such as nitrofurantoin, ampicillin, fluoroquinolones and sulphamethoxazole/trimethoprim. Therefore, there is an urgent need for the development of new therapeutic strategies to eradicate biofilm infections by *E. coli*. Some of the recent advances in strategies designed to treat biofilms by killing the bacteria or targeting different developmental stages of biofilm formation have been discussed (Chibeu *et al.* 2012).

Antiadhesion agents

Curli are adhesive amyloid fibres present on the cell surface of *E. coli* that help to maintain cell–cell and cell-surface interactions and lead to biofilm formation. Pili are extracellular adhesive fibres, which mediate biofilm formation, binding and invasion into host cell. Type 1 pili contain the FimH adhesin at their tip and play a major role in UPEC pathogenesis. FimH adhesin leads to the binding of the bacteria to mannosylated receptors on the luminal surfaces of mammalian bladder epithelial cells which lead to pathogenesis of UTI. Type 1 pili are therefore essential virulence factors that are an excellent target for therapeutic intervention. The inhibition of curli and pili formation can help in treatment of *E. coli* biofilm. Structural knowledge of the target protein has led to design and screening of small molecule-based inhibitors. These molecules can traverse through cell membrane and thus target various components of cellular machinery. Type 1 pilus subunit polymerization in UPEC *E. coli* was inhibited by *N*-(4-chloro-phenyl)-2-{5-[4-(pyrrolidine-1-sulfonyl)-phenyl]-[1,3,4]oxadiazol-2-yl sulfanyl}-acetamide (AL1). The *in vivo* and *in vitro* data showed that the synthesis of type I pili was disrupted resulting in suppressed biofilm formation and also adherence to human bladder cells (Lo *et al.* 2014).

The type 1 pili of *E. coli* binds to mannosylated receptor present on urinary bladder to trigger infection. The knowledge of the receptor-binding site for FimH adhesin has led to the design of mannosides which fit the binding pocket of FimH mannose and inhibit its binding to the host receptor. The FimH antagonists were designed and the optimized monomeric biphenyl mannosides displayed enhanced potency, relative to FimH inhibitors (Han *et al.* 2010; Kostakioti *et al.* 2013).

Another inhibitor, mannoside, against type 1 pilus adhesin, FimH, controlled bacterial invasion and thus reduced CAUTI caused by UPEC. Mannosides were shown to act synergistically with trimethoprim–sulfamethoxazole in treatment of infection due to UPEC (Guiton *et al.* 2012). In another study, curli subunit CsgA was targeted by rationally designed 2-pyridone compounds which prevented *E. coli* biofilm formation. Curli is abundant in matrix of bacterial biofilm and its inhibition prevents colonization, invasion and reduce the biofilm biomass (Cegelski *et al.* 2009; Andersson *et al.* 2013).

The curlicides BibC6 and FN075 have a common chemical lineage to ring-fused 2-pyridones known as pilicides. They inhibit major curli subunit protein CsgA and hence curtail the formation of curli in UPEC. The curlicides also retain pilicide activities and inhibit both curli-dependent and type 1-dependent biofilms (Cegelski *et al.* 2009). Fluorescent pilicides and curlicides have been synthesized using coumarin and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) fluorophores and it was found to have improved antibiofilm activity (Chorell *et al.* 2012).

Phage therapy

Phages are found in abundance and can be isolated from a wide range of environments. They are usually specific to narrow host ranges and due to their self-replication,

low dosage is sufficient. Their high mutation rate helps them to adapt as the host bacteria undergoes genetic alterations to survive in a given environment. Phages have been effective in eradicating biofilm of single or mixed bacterial species and could lyse biofilm grown on medical devices and filtration membranes.

Certain phages possess virion-associated polysaccharide depolymerases which help them to degrade capsules. It was found that mature biofilms are more effectively eradicated in depolymerase-producing phages than the nonsecreting ones, therefore, they have been used along with antimicrobials to facilitate deeper penetration by degrading the EPS. The phage depolymerases have an important part in the degradation of the EPS matrix of the biofilm, which facilitate the permeation of the phages inside the biofilm layers resulting in bacterial cell lysis. However, phages that are not capable of producing EPS depolymerases are also used in biofilm degradation. These include phages such as T7 engineered to express recombinant dispersin B (DsbB) and naturally occurring phages such as T4 (Chibeu *et al.* 2012).

There have been studies in which a combination of bacteriophages has been applied for the erradication of biofilms. A cocktail of bacteriophages has a greater impact on the biofilm bacteria as their combination causes lysis of the bacterial cells of the biofilm much more than that caused by a single bacteriophage. One phage could facilitate the infection by the other phage by degrading the polysaccharides in bacterial biofilm matrix with depolymerase. These enzymes are highly species-specific and hence to target different bacteria especially for mixed biofilm, T7 phage has been engineered to express lactonase which can degrade AHLs from many bacteria. They were found to be effective against mixed biofilm of *Pseudomonas aeruginosa* and *E. coli* (Pei and Lamas-Samanamud 2014). The combination of phage with antibiotic has resulted in remarkable decrease in antibiotic-resistant *E. coli* (Coulter *et al.* 2014). Recently it has been shown that phage resistant bacteria overproduce colanic acid. Second infection with phage carrying colanic acid-degrading enzyme can restrain the development of phage-resistant bacteria (Kim *et al.* 2015).

Phytochemicals

Plants are being increasingly explored as the possible antitherapeutic agent as they can kill the micro-organism with diverse mechanisms of action with minimal chance for bacteria to develop resistance to it. The phytochemicals such as 7-hydroxycoumarin (7-HC), indole-3-carbinol (I3C), salicylic acid and saponin have shown inhibitory activity against the planktonic culture of *E. coli* and *Staphylococcus aureus* and were also able to restrict the growth of biofilm partially. The phytochemicals I3C and 7-HC had a more pronounced effect on QS inhibition and bacterial motility for both *E. coli* and *Staph. aureus*. I3C exhibited synergistic activity with antibiotics against resistant strains of *Staph. aureus* (Monte *et al.* 2014).

Ginkgolic acid and Ginkgo biloba extract have shown significant inhibition of enterohaemorrhagic *E. coli* O157:H7 biofilm formation by downregulating curli and prophage genes (Lee *et al.* 2014). The β-sitosterol glucoside isolated from citrus fruit inhibited *E. coli* O157:H7 biofilm formation and motility by suppressing the levels of RssAB and HNS of flagellar master operon *flhDC* (Vikram *et al.* 2013). In a recent study, it was found that the phenolic acids (gallic acid and ferulic acid) inhibited bacterial motility of *E. coli*. Both gallic acid and ferulic acid caused total inhibition of swarming in *E. coli* and thus reduced the biofilm mass considerably (Borges *et al.* 2012). In another study, phenolic-rich maple syrup extract (PRMSE) was tested for its antibiofilm activity on pathogenic bacteria including *E. coli*. The transcriptome analysis revealed that PMRSE effectively repressed multiple drug resistance genes and genes associated with motility, adhesion and biofilm formation (Maisuria *et al.* 2015).

Antimicrobial peptides

Antimicrobial peptides (AMPs) are the integral part of innate immunity and are produced by varied living organisms to fight against infection. It has been seen that a chance for the development of bacterial resistance is very limited when the bacterial growth is restricted with AMP. Based on the ability of amino acid residues to form a helix, adhere to the surface and possess antimicrobial activity, KABT-AMP was designed which showed antimicrobial activity against *E. coli* (Thankappan *et al.* 2013).

Two antibacterial peptides containing tryptophan (KT2 and RT2) were designed and found to be highly effective against multidrug-resistant, enterohaemorrhagic *E. coli* O157:H7 biofilm at 1 μmol l^{-1} concentration. It was proposed that these peptides could traverse inside the cell and eventually bind the DNA for its antimicrobial action (Anunthawan *et al.* 2015). Bacteriocin isolated from *Citrobacter freundii* showed antimicrobial activity against a wide range of bacteria including *E. coli* in both planktonic as well as in biofilm form (Shanks *et al.* 2012).

Nanoparticles

Nanoparticles, being more stable and having high bioavailability, can be delivered efficiently as antimicrobial agents. Silver nanoparticles are known to be flexible,

stable and can restrict the infection and biofilm formation of *E. coli*. The silver nanoparticles due to its small size and enhanced surface to volume ratio can be incorporated in medical devices and wound dressings. The mechanism of silver toxicity has been attributed to thiol group that renders many enzymes inactive inhibiting DNA replication, and translation of crucial proteins. Silver nanoparticles have been synthesized from the aqueous extract of *Calotropis procera* flower and were found to be effective against enterotoxic *E. coli* biofilm and significantly decreased the colonization in small intestine of infant mouse model (Salem *et al.* 2015).

In another study, the silver nanoparticles were embedded in orthorhombic nanotubes of lithium vanadium oxide (Li_2O_5/Ag) and they could restrict the growth of *E. coli* biofilm at the concentration of 60–120 μg ml^{-1}. The images obtained by scanning electron microscopy revealed the action of the nanocomposites on the surface of the bacteria in the form of surface perturbation thereby facilitating its use as a candidate for biofabrication of medical devices to prevent infectious diseases (Diggikar *et al.* 2013).

Wei He *et al.* tested a nanotechnology antimicrobial spray, JUC, against the *E. coli* biofilm formed in CAUTI. It formed an invisible, protective positively charged film on the surface after it was sprayed, and prevented the bacterial growth. In the clinical study, catheter sprayed with JUC therapy group, 4·52% of patients were diagnosed with CAUTI as compared to 13·04% in the control group (catheter sprayed with distilled water) (He *et al.* 2012). In a recent study, selenium and tellurium nanoparticles obtained from the strains *Stenotrophomonas maltophilia* and *Ochrobactrum* sp. MPV1, respectively, were found to be effective against both planktonic and biofilm form of *E. coli* JM109, *Ps. aeruginosa* PAO1, and *Staph. aureus* ATCC 25923 (Zonaro *et al.* 2015).

AMP LL-37 coated on magnetic nickel nanoparticles with the aid of polyacrylic acid, created as adhesion layer on nanoparticles, was found to be effective in killing *E. coli* (Chen *et al.* 2009).

Conclusion

Complications in *E. coli*-related infection have been mainly attributed to biofilm formation. Biofilm is considerably recalcitrant to antibiotics as compared to its planktonic culture. *Escherichia coli* biofilm formation is an intricate process which involves a number of steps such as initial adhesion, early development, maturation and dispersion. These steps are governed by a number of genes that serve specific functions in the formation of the biofilm. Type I fimbriae of *E. coli* plays a crucial role in its attachment to the surface and maturation is further facilitated by autotransporters and EPS. Recent discoveries have also identified stress resistance genes in the biofilm-formation process that help the biofilm to survive in hostile environments. The *rpoS* gene is majorly responsible for regulating genes and structural proteins involved in the synthesis and degradation of biofilm, under stress conditions.

Escherichia coli biofilm has been found to be resistant to a number of antibiotics, mostly accredited to putative multidrug resistance pump. The development of the extracellular matrix and the observed increased resistance to common antibiotics create a challenge to control the infections caused by *E. coli* biofilms. Recently there have been advances in exploring and developing new approaches and therapeutic methods to cure *E. coli* biofilm-related infections.

Molecular and structural understanding of *E. coli* biofilm has led to the advances in targeting specific agents to curtail infections. Type I pili of *E. coli* are crucial for the adhesion and initiation of *E. coli* biofilm formation. The curlicides and pilicides have been designed against curli subunit protein CsgA and type I pili, respectively, and have been shown to inihibit bacterial biofilm. The combination of phages have shown to complement lysis of bacteria through different mechanism of action and yielded promising results. Bacterial biofilm has been notorious in mounting resistance and phytochemicals and AMPs are able to address this issue through their diverse mechanism to target organisms.

One of the major problems faced by these remedial agents is stability and low bioavailability. Natural compound efficacy can be improved by incorporating them in nanoparticles or coating on a particular surface. Silver nanoparticles have shown great promise especially in coating the medical devices and wound dressings and were found to be effective against *E. coli* biofilm both *in vitro* and *in vivo* mouse model. An antimicrobial nanospray JUC, which was sprayed on a catheter was found to be efficacious in restricting *E. coli* biofilm formation. These new methods hold a great promise but the issues concerning *in vivo* efficacy, toxicity and large-scale production need to be addressed before these potential therapeuticals can reach the clinical stage.

Conflict of Interest

No conflict of interest declared.

References

Andersson, E.K., Bengtsson, C., Evans, M.L., Chorell, E., Sellstedt, M., Lindgren, A.E., Hufnagel, D.A., Bhattacharya,

M. *et al.* (2013) Modulation of curli assembly and pellicle biofilm formation by chemical and protein chaperones. *Chem Biol* **20**, 1245–1254.

Anunthawan, T., de la Fuente-Núñez, C., Hancock, R.E. and Klaynongsruang, S. (2015) Cationic amphipathic peptides KT2 and RT2 are taken up into bacterial cells and kill planktonic and biofilm bacteria. *Biochim Biophys Acta* **1848**, 1352–1358.

Barrios, G.A.F., Zuo, R., Hashimoto, Y., Yang, L., Bentley, W.E. and Wood, T.K. (2006) Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (mqsr, b3022). *J Bacteriol* **188**, 305–316.

Beloin, C., Roux, A. and Ghigo, J.M. (2008) *Escherichia coli* biofilms. *Curr Top Microbiol Immunol* **322**, 249–289.

Borges, A., Saavedra, M.J. and Simões, M. (2012) The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. *Biofouling* **28**, 755–767.

Brito, P.H., Rocha, E.P., Xavier, K.B. and Gordo, I. (2013) Natural genome diversity of AI-2 quorum sensing in *Escherichia coli*: conserved signal production but labile signal reception. *Genome Biol Evol* **5**, 16–30.

Cegelski, L., Pinkner, J.S., Hammer, N.D., Cusumano, C.K., Hung, C.S., Chorell, E., Aberg, V., Walker, J.N. *et al.* (2009) Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation. *Nat Chem Biol* **5**, 913–919.

Chen, G., Zhou, M., Chen, S., Lv, G. and Yao, J. (2009) Nanolayer biofilm coated on magnetic nanoparticles by using a dielectric barrier discharge glow plasma fluidized bed for immobilizing an antimicrobial peptide. *Nanotechnology* **20**, 465706.

Chibeu, A., Lingohr, E.J., Masson, L., Manges, A., Harel, J., Ackermann, H.W., Kropinski, A.M. and Boerlin, P. (2012) Bacteriophages with the ability to degrade uropathogenic *Escherichia coli* biofilms. *Viruses* **4**, 471–487.

Chorell, E., Pinkner, J.S., Bengtsson, C., Edvinsson, S., Cusumano, C.K., Rosenbaum, E., Johansson, L.B., Hultgren, S.J. *et al.* (2012) Design and synthesis of fluorescent pilicides and curlicides: bioactive tools to study bacterial virulence mechanisms. *Chemistry* **18**, 4522–4532.

Coulter, L.B., McLean, R.J., Rohde, R.E. and Aron, G.M. (2014) Effect of bacteriophage infection in combination with tobramycin on the emergence of resistance in *Escherichia coli* and *Pseudomonas aeruginosa* biofilms. *Viruses* **10**, 3778–3786.

Danese, P.N., Pratt, L.A. and Kolter, R. (2000) Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilms. *J Bacteriol* **182**, 3593–3596.

Daniels, R., Vanderleyden, J. and Michiels, J. (2004) Quorum sensing and swarming migration in bacteria. *FEMS Microbiol Rev* **28**, 261–289.

Diggikar, R.S., Patil, R.H., Kale, S.B., Thombre, D.K., Gade, W.N., Kulkarni, M.V. and Kale, B.B. (2013) Silver-decorated orthorhombic nanotubes of lithium vanadium oxide: an impeder of bacterial growth and biofilm. *Appl Microbiol Biotechnol* **97**, 8283–8290.

Flemming, H.C. and Wingender, J. (2010) The biofilm matrix. *Nat Rev Microbiol* **8**, 623–633.

Guiton, P.S., Cusumano, C.K., Kline, K.A., Dodson, K.W., Han, Z., Janetka, J.W., Henderson, J.P., Caparon, M.G. *et al.* (2012) Combinatorial small-molecule therapy prevents uropathogenic *Escherichia coli* catheter-associated urinary tract infections in mice. *Antimicrob Agents Chemother* **56**, 4738–4745.

Han, Z., Pinkner, J.S., Ford, B., Obermann, R., Nolan, W., Wildman, S.A., Hobbs, D., Ellenberger, T. *et al.* (2010) Structure-based drug design and optimization of mannoside bacterial fimh antagonists. *J Med Chem* **53**, 4779–4792.

He, W., Wang, D., Ye, Z., Qian, W., Tao, Y., Shi, X., Liu, L., Chen, J. *et al.* (2012) Application of a nanotechnology antimicrobial spray to prevent lower urinary tract infection: a multicenter urology trial. *J Transl Med* **10**, S1–S14.

Ito, A., Taniuchi, A., May, T., Kawata, K. and Okabe, S. (2009) Increased antibiotic resistance of *Escherichia coli* in mature biofilms. *Appl Environ Microbiol* **75**, 4093–4100.

Kim, M.S., Kim, Y.D., Hong, S.S., Park, K., Ko, K.S. and Myung, H. (2015) Phage-encoded colanic acid-degrading enzyme permits lytic phage infection of a capsule-forming resistant mutant *Escherichia coli* strain. *Appl Environ Microbiol* **81**, 900–909.

Kostakioti, M., Hadjifrangiskou, M. and Hultgren, S.J. (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med* **3**, a010306.

Lee, S.Y. (1996) High cell-density culture of *Escherichia coli*. *Trends Biotechnol* **14**, 98–105.

Lee, J.H., Kim, Y.G., Ryu, S.Y., Cho, M.H. and Lee, J. (2014) Ginkgolic acids and Ginkgo biloba extract inhibit *Escherichia coli* O157:H7 and *Staphylococcus aureus* biofilm formation. *Int J Food Microbiol* **174**, 47–55.

Li, J., Attila, C., Wang, L., Wood, T.K., Valdes, J.J. and Bentley, E.W. (2007) Quorum sensing in *Escherichia coli* is signaled by AI-2/LsrR: effects on small RNA and biofilm architecture. *J Bacteriol* **189**, 6011–6020.

Lo, A.W., Van de Water, K., Gane, P.J., Clan, A.W., Steadman, D., Stevens, K., Selwood, D.L., Waksman, G. *et al.* (2014) Suppression of type 1 pilus assembly in uropathogenic *Escherichia coli* by chemical inhibition of subunit polymerization. *J Antimicrob Chemother* **69**, 1017–1026.

Lynch, S.V., Dixon, L., Benoit, M.R., Brodie, E.L., Keyhan, M., Hu, P., Ackerley, D.F., Andersen, G.L. *et al.* (2007) Role of the rapA gene in controlling antibiotic resistance of *Escherichia coli* biofilms". *Antimicrob Agents Chemother* **51**, 3650–3658.

Maisuria, V.B., Hosseinidoust, Z. and Tufenkji, N. (2015) Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. *Appl Environ Microbiol* **81**, 3782–3792.

Mittal, S., Sharma, M. and Chaudhary, U. (2015) Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. *Pathog Glob Health* **109**, 26–29.

Monte, J., Abreu, A.C., Borges, A., Simões, L.C. and Simões, M. (2014) Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. *Pathogens* **3**, 473–498.

Niranjan, V. and Malini, A. (2014) Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. *Indian J Med Res* **139**, 945–948.

Pei, R. and Lamas-Samanamud, G.R. (2014) Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes. *Appl Environ Microbiol* **80**, 5340–5348.

Reisner, A., Maierl, M., Jörger, M., Krause, R., Berger, D., Haid, A., Tesic, D. and Zechnerc, E.L. (2014) Type 1 fimbriae contribute to catheter-associated urinary tract infections caused by *Escherichia coli*. *J Bacteriol* **196**, 931–939.

Rueggeberg, K.G., Toba, F.A., Thompson, M.G., Campbell, B.R. and Hay, A.G. (2013) A Q-like transcription factor regulates biofilm development in *Escherichia coli* by controlling expression of the DLP12 lysis cassette. *Microbiology* **159**, 691–700.

Salem, W., Leitner, D.R., Zingl, F.G., Schratter, G., Prassl, R., Goessler, W., Reidl, J. and Schild, S. (2015) Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli*. *Int J Med Microbiol* **305**, 85–95.

Serra, D.O. and Hengge, R. (2014) Stress responses go three dimensional – the spatial order of physiological differentiation in bacterial macrocolony biofilms. *Environ Microbiol* **16**, 1455–1471.

Shanks, R.M., Dashiff, A., Alster, J.S. and Kadouri, D.E. (2012) Isolation and identification of a bacteriocin with antibacterial and antibiofilm activity from *Citrobacter freundii*. *Arch Microbiol* **194**, 575–587.

Singleton, P. (1999) *Bacteria in Biology, Biotechnology and Medicine*. pp 444–454. Chichester: Wiley.

Soto, S.M., Marco, F., Guiral, E. and Vila, J. (2011) Biofilm formation in uropathogenic *Escherichia coli* strains: relationship with urovirulence factors and antimicrobial resistance. In *Clinical Management of Complicated Urinary Tract Infection* ed. Nikibakhsh, A., pp.159–170. Croatia: Intech.

Sturbelle, R.T., Avila, L.F., Roos, T.B., Borchardt, J.L., de Cássia, D.S., da Conceição, R., Dellagostin, O.A. and Leite, F.P. (2015) The role of quorum sensing in *Escherichia coli* (ETEC) virulence factors. *Vet Microbiol* **18**, 245–252.

Thankappan, B., Jeyarajan, S., Hiroaki, S., Anbarasu, K., Natarajaseenivasan, K. and Fujii, N. (2013) Antimicrobial and antibiofilm activity of designed and synthesized antimicrobial peptide, KABT-AMP. *Appl Biochem Biotechnol* **170**, 1184–1193.

Vikram, A., Jayaprakasha, G.K., Uckoo, R.M. and Patil, B.S. (2013) Inhibition of *Escherichia coli* O157:H7 motility and biofilm by β-sitosterol glucoside. *Biochim Biophys Acta* **11**, 5219–5228.

Vogeleer, P., Tremblay, Y.D.N., Mafu, A.A., Jacques, M. and Harel, J. (2014) Life on the outside: role of biofilms in environmental persistence of Shiga-toxin producing *Escherichia coli*. *Front Microbiol* **5**, 317.

Wood, T.K. (2009) Insights on *Escherichia coli* biofilm formation and inhibition from whole-transcriptome profiling. *Environ Microbiol* **11**, 1–15.

Zonaro, E., Lampis, S., Turner, R.J., Qazi, S.J. and Vallini, G. (2015) Biogenic selenium and tellurium nanoparticles synthesized by environmental microbial isolates efficaciously inhibit bacterial planktonic cultures and biofilms. *Front Microbiol* **6**, 584.

Zuroff, T.R., Bernstein, H., Randolfi, J.L., Taracido, L.J., Stewart, P.S. and Carlson, R.P. (2010) Robustness analysis of culturing perturbations on *Escherichia coli* colony biofilm beta-lactam and aminoglycoside antibiotic tolerance. *BMC Microbiol* **10**, 185.

Web references

[1] http://www.uniprot.org/uniprot/P52106, (accessed 18 January 2016).

[2] http://www.uniprot.org/uniprot/P0ACE3, (accessed 18 January 2016.

[3] http://biocyc.org/gene?orgid=ECOLI&id=EG10439-MONOMER, (accessed 18 January 2016.

[4] http://www.uniprot.org/uniprot/P37653, (accessed 18 January 2016).

[5] http://www.uniprot.org/uniprot/P75905, (accessed 18 January 2016).

[6] http://www.uniprot.org/uniprot/P0ADH5, (accessed 18 January 2016).

[7] http://biocyc.org/gene?orgid=ECOLI&id=EG10312, (accessed 18 January 2016).

[8] http://www.uniprot.org/uniprot/P45578, (accessed 18 January 2016).

[9] http://www.uniprot.org/uniprot/Q46865, (accessed 18 January 2016).

[10] http://www.uniprot.org/uniprot/P52076, (accessed 18 January 2016).

[11] http://www.uniprot.org/uniprot/P40719, (accessed 18 January 2016).

[12] http://www.uniprot.org/uniprot/P0AF12, (accessed 18 January 2016).

[13] http://biocyc.org/gene?orgid=ECOLI&id=EG11090-MONOMER, (accessed 18 January 2016).

[14] http://www.uniprot.org/uniprot/P0A8S9, (accessed: 18 January 2016).
[15] http://www.uniprot.org/uniprot/P0AEM6, (accessed 18 January 2016).
[16] http://www.uniprot.org/uniprot/P09348, (accessed 18 January 2016).
[17] http://www.uniprot.org/uniprot/P77432, (accessed 18 January 2016).
[18] http://www.uniprot.org/uniprot/P76141, (accessed 18 January 2016).
[19] http://www.uniprot.org/uniprot/P69913, (accessed 18 January 2016).
[20] http://www.uniprot.org/uniprot/P0AB40, (accessed 18 January 2016).
[21] http://www.uniprot.org/uniprot/P39180, (accessed 18 January 2016).
[22] http://www.uniprot.org/uniprot/P75993, (accessed 18 January 2016).
[23] http://biocyc.org/gene?orgid=ECOLI&id=G6606-MONOMER, (accessed 18 January 2016).
[24] http://www.uniprot.org/uniprot/P13445, (accessed 18 January 2016).
[25] http://www.uniprot.org/uniprot/P0A6X3, (accessed 18 January 2016).
[26] http://biocyc.org/gene?orgid=ECOLI&id=EG10438-MONOMER, (accessed 18 January 2016).
[27] http://www.uniprot.org/uniprot/Q47149, (accessed 18 January 2016).
[28] http://www.uniprot.org/uniprot/P60240, (accessed 18 January 2016).
[29] http://www.uniprot.org/uniprot/J7R6D3, (accessed 18 January 2016).