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ORIGINAL ARTICLE

Urinary catheter indwelling clinical pathogen biofilm formation, exopolysaccharide characterization and their growth influencing parameters

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Abstract Self-reproducing microbial biofilm community mainly involved in the contamination of indwelling medical devices including catheters play a vital role in nosocomial infections. The catheter-associated urinary tract infection (CA-UTI) causative *Staphylococcus aureus*, *Enterobacter faecalis*, and *Pseudomonas aeruginosa* were selectively isolated, their phenotypic as well as genotypic biofilm formation, production and monomeric sugar composition of EPS as well as sugar, salt, pH and temperature influence on their *in vitro* biofilm formation were determined. From 50 culture positive urinary catheters *S. aureus* (24%), *P. aeruginosa* (18%), *E. faecalis* (14%) and others (44%) were isolated. The performed assays revealed their varying biofilm forming ability. The isolated *S. aureus ica*, *E. faecalis esp*, and *P. aeruginosa cup* A gene sequencing and phylogenetic analysis showed their close branching and genetic relationship. The analyzed sugar, salt, pH, and temperature showed that the degree of CA-UTI isolates biofilm formation is an environmentally sensitive process. EPS monosaccharide HPLC analysis showed the presence of neutral sugars (ng/μl) as follows: glucose (*P. aeruginosa*: 44.275; *E. faecalis*: 4.23), lactose (*P. aeruginosa*: 7.29), mannitol (*P. aeruginosa*: 2.53; *S. aureus*: 2.62; *E. faecalis*: 2.054) and maltose (*E. faecalis*: 7.0042)

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revealing species-specific presence and variation. This study may have potential clinical relevance for the easy diagnosis and management of CA-UTI.

1. Introduction

Healthcare associated infections have emerged as an important ground of morbidity and mortality among hospitalized patients, visitors, and staff. An estimate carried out during the year 2002 accounts that these nosocomial infections cost about $6.7 G (billion) in the United States and £1.06 G (around $1.7G) in the United Kingdom per year (Graves, 2004). The regular occurrence of nosocomial infections is often associated in contact with biomaterials like heart valves, artificial veins, joint prostheses and urinary tract catheters (Dohnt et al., 2011) which are implicated as some of the highly significant risk factors. Among the hospital acquired infections, catheter-associated urinary tract infection (CA-UTI) is the most common one. The United States Centers for Disease Control and Prevention alone records nearly 560,000 CA-UTIs. Biofilm formation is central to CA-UTI pathogenesis (Desai et al., 2010). This biofilm formation is a major facet in nosocomial infections in which soon after the attachment to surfaces pathogens colonize and form a sessile biofilm community, which can develop both on abiotic and biotic surfaces. These biofilms play an imperative role in pathogen physiology, persistence besides serving as a source of various infections. It also acts as an adhesive foundation, defense barrier that protects the embedded cells against detachment by flow shear (Jung et al., 2013). Hence, once became mature, this biofilm is recalcitrant to clearance by both the host immune response and antimicrobial therapies (Brady et al., 2011). Also, the biofilm and their counterpart planktonic cells contrast considerably in their physiology, gene expression pattern, and even morphology. Since they are less sensitive to antimicrobial agents, controlling their growth could be significantly challenging once they are formed (Landini et al., 2010). Furthermore, this biofilm lifestyle's associated exogenous stress high tolerance, ineffectiveness to antibiotics or other biocide treatments in their eradication (Rendueles et al., 2013) makes use of antibiotics or other antimicrobial agents against a biofilm infection unproductive.

Generally biofilm is made up of non-randomly distributed microcolonies of bacterial cells (15–20% by volume) in a shaped matrix or glycocalyx, the extracellular polymeric substances (EPS) 75–80% by volume (Saini et al., 2011). Hence, the EPS are made up mainly of polysaccharides besides proteins, nucleic acids, lipids, etc., production is the paramount event during biofilm development. The clinical significance of biofilms is their low antimicrobial sensitivity while displaying enhanced pathogenicity (pathogenic synergism). Bacteria living in biofilms exhibit 100- to 1000-fold increase in their antibiotic tolerance in comparison to their free-swimming counterparts (Moscoso et al., 2009; Dufour et al., 2012). The antibiotic resistant common mechanisms including efflux pumps, modifying enzymes, and target mutations could not be accounted for the bacterial protection in the biofilm. The possibility of slow or incomplete penetration of the antibiotics, alternation of chemical microenvironment within the biofilm and formation of a subpopulation having unique, and highly protected, phenotypic cell differentiation similar to spore formation are speculated or assumed as possible reasons (Stewart and Costerton, 2001). In addition, the matrix trapped and concentrated extracellular enzymes such as β-lactamases, formaldehyde lyase, and formaldehyde dehydrogenase inactivates susceptible, typically positively charged, hydrophilic antibiotics (Socransky and Haffajee, 2002). It is proposed that EPS can interact with antibiotics in a manner leading to a decline in their antibacterial activity (Tetz et al., 2009). Sometimes the biofilm matrix acts as an ion-exchange resin so that the strongly charged or highly chemically reactive agents are unable to reach the biofilm's deeper zones (Socransky and Haffajee, 2002). Hence, antimicrobials express restricted ability to eradicate bacteria deep into biofilms, in part owing to their binding with the biofilm outer layer components (ten Cate, 2012). The association of molecules like EPS and DNA within the biofilm constitutes a physical barrier to the diffusion of antimicrobial agents (Bordi and de Bentzmann, 2011). Consequently, the chemotherapeutic agents find difficulty in penetrating the polysaccharide matrix to reach and affect the microorganisms. So, the matrix helps to increase the chances of the colonies' survival by protecting bacteria deep inside the biofilm from antibiotics and antiseptics (Gurenlian, 2007). Hence manifestation of these characters that make sessile microorganisms more resistance to antimicrobial agents than their planktonic counterparts is the highly human being damaging property of biofilms (Villa and Cappitelli, 2013).

The principal component of EPS determines the physical properties of the biofilm while the bacterial cells determine its physiological properties (De Beer and Stoodley, 2006). They are accountable for most of their physical, chemical, and biological properties (Ruzicka et al., 2011). The occurrence of different types of polysaccharides and their production is the species and strain dependent one. The *Pseudomonas aeruginosa* alginate, staphylococcal polysaccharide intercellular adhesion, streptococcal and lactobacilli glucans and fructans are examples of best-known biofilm-associated EPS (Ruzicka et al., 2011). They act as a backbone of the biofilm by binding the biofilms bacteria together in a sticky web of tangled EPS fibers which connect cells as well as anchor them to a surface and to each other (Sihorkar and Vyas, 2001). They play an important role in attachment, detachment, mechanical strength, antibiotic resistance exo-enzymatic degradation activities (De Beer and Stoodley, 2006), cell-to-cell interconnection, interactions between subpopulations, tolerance, and exchange of genetic material (Harmsen et al., 2010). Therefore, EPS plays a vital role not only in their formation but also behavior (Ruzicka et al., 2011). Hence, they are deemed to be necessary for biofilm lifestyle existence and global expression of bacterial pathogen's virulence (Xiao et al., 2012).