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Isolation and identification of microbes from biofilm of Urinary catheters and antimicrobial Susceptibility evaluation

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ABSTRACT

Objective: Bacterial species colonize indwelling catheters as biofilm induce complications in patients care. **Methods:** From the biofilm matrix seven species of microbes were isolated. The predominant bacteria seen in catheters were *E.coli*, (27 percent) *P.mirabilis* (20 percent) and *S.epidermis* (18 percent). **Results:** The biomass of microbes associated with the biofilm was estimated. The mean dry weight of biomass of bacteria associated with a catheter that was used for over a month time was in the range 2.5±0.04g – 3.1 ± 0.6g. **Conclusion:** But it was found to colonize the microtitre plate to attain a peak growth at 84h. P.mirabilis isolated from the biofilm was able to tolerate the antibiotics tetracycline, Penicillin, Kanamycin and Gentamycin at a dose level of 20 μg/ml. The study indicated that the catheter has to be replaced if biofilm formation was noticed.

1. Introduction

Bacteria such as *Staphylococcus aureus* have shown adaptability to changing environments in the healthcare setting, but also more recently in the wider community [13, 12]. Opportunistic pathogens such as S. aureus and Pseudomonas aeruginosa, along with other bacteria, can develop resistance through an elaborate array of mechanisms. These include modification of the target protein or binding site, enzymatic destruction of antibacterial agents, active efflux of drugs from the bacterial cell or by acquisition of genetic material from other resistant strains [14].

Biofilms have been defined in the literature as "microorganism attached to a surface and covered with an exo-polysaccharide of microbial origin" [2]. Biofilm formation is a developmental process, which initially involves the adhesion of bacterial cells to a surface via various surface proteins and/or conditioning films. Once adhered to the surface, the bacteria undergo a variety of changes involving the activation and down regulation of many genes and production of an exopolysaccharide that covers and protects the cells [8].

[7] Reported that Bacterial biofilms are complex, mono or polymicrobial communities adhering to biotic or abiotic surfaces. The formation of biofilm is mediated by mechanical, biochemical and genetical factors. Biofilm enhance the virulence of the pathogen and have their potential role in various infections like dental caries, systic fibrosis, osteonecrosis, urinary tract infection and eye infections. In total 30 *P. aeruginosa* isolates from urinary tract and catheter associated infections were identified and characterized by [19]. A comparable system has been used by [20] to observe the biomass in a planar micro fluidic reactor. Both systems are limited because they are only applicable for planar systems. The optical biomass sensor developed for the in vitro urinary tract catheter system has the advantage that it monitors the biomass even in the non-planar catheter.

It is possible that phytochemicals with well known antibiotic properties could also potentially possess antipathogenic activities too. Such antipathogenic compounds, in contrast to antibacterial compounds neither kill bacteria nor stop their growth and are assumed to

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not result in the development of resistant strains [15]. QSI compounds have been identified from awide range of natural resources, particularly medicinal plants, edible vegetables and fruits, marine sponges and seaweeds [15, 16].

A worrying feature of biofilm-based infections is represented by the higher resistance of bacterial and fungal cells growing as biofilms to antibiotics and disinfecting chemicals as well as resisting phagocytosis and other components of the body's defense system, when compared to planktonic cells [17]. Biofilm forming bacteria isolated from urinary tract infection, relation to catheterization and susceptibility to antibiotics [18].

2. Materials and methods

2.1. Isolation of bacteria from catheters

Catheters from patients being cared for in hospitals in Chidambaram, India were collected. Sections 1-2 cm and 3-4 cm from the catheter tip were cut and suspended in quarter-strength Ringer's solution in (10ml) in sterile universal containers, as explained by Sonification for 5 min at 35 kHz in a transonic water bath and by vortex mixing for 2 minutes was used to remove and disrupt the colonizing biofilms. The resulting cell suspensions were cultured onto Cystine lactose electrolyte deficient media (CLED) Chromogenic UTI and tryptone soya agars (Hi-media). After 24 h incubation the resulting colonies were identified using standard procedures.

2.2. Determination of biofilm dry weight

The microbes that were detached from a one cm size of infected catheter tip were centrifuged at 10,000 rpm for five minutes at room temperature. The pellet so obtained was transferred to preweighed cellulose nitrate paper (0.45 μm pore size, 25 mm diameter), dried at 800c overnight and weighed. This weight was taken as a measure of the dry biomass.

2.3. Monitoring biofilm formation by Proteus mirabilis

Proteus mirabilis catheter film was grown in Lueria Bertani medium and samples were drawn at 12 hour intervals. One hundred microlitres (1:100 diluted LB broths) of these samples were inoculated in Microtitre plate (MTP) and incubated at 300c for ten hours. MTP wells were then rinsed thoroughly thrice with 0.15M PBS to remove free floating organisms. Crystal violet stain was then added to each well and the plates were incubated at room temperature for 15 minutes. Excess of stain was removed by rinsing with distilled water. The stain that was taken up by biofilm forming organisms was extracted twice in 200 μl aliquots of 95% ethanol, 100 μl of which was transferred to a new MTP and the absorbance was determined in a plated reader at 600 nm.

2.4. Susceptibility of Proteus mirabilis to antimicrobial agents

Proteus mirabilis isolated from catheter was tested for antimicrobial susceptibity. The antimicrobial agents used were penicillin, tetracycline, gentamysin and kanamycin. Antimicrobial agents at varying concentration (0.2 μg/ml to 1.28 μg/ml depending upon the MIC) were added to these wells, incubated for 5 hours and then stained with crystal violet.

2.5. Statical analysis

Two general linear models were fitted to estimate the amount of biofilm produced by six microorganisms to assess the statistical significance of (P-0.005) the differences between different strains.

3. Results

Out of the 45 catheters screened, 37 were found with a biofilm formation. The biofilm formation was heavy on the catheters, which were used for a long time. Catheters that were used for over 25 months had a dense biofilm matrix. An examination of biofilm that was detached from the catheter was found to harbour consortia of microbial communities. Seven species of microbial strains were isolated from the biofilm matrix. An assessment of the different bacteria that constituted the biofilm revealed that the Escherichia coli incidence was high (27%) followed by, Proteus mirabilis (20 %), Staphylococcus epidermis (18 %) and S.aureus (16%) incidence. The incidence of Candida albicans (12 %), Pseudomonas aeruginosa (5 %) and Neisseria gonnorhaea (02 %) was found less. (Table 1).

Table 1.
Microorganisms screened and identified from infected catheters

Microorganisms	Number of catheters colonized each species (%)
Proteus mirabilis	27
Staphylococcus aureus	20
Staphylococcus epidermidis	18
Pseudomonas aeruginosa	16
Escherichia coli	12
Candida albicans	05
Neisseria gonnorrhaea	02

Proteus mirabilis is the most commonly recovered bacteria from patient's encrusted catheters [9]. Hence it was chosen for further study. The nature of biofilm formation by P.mirabilis was evaluated using in vitro experiment. The growths of P.mirabilis increased gradually and formed a peak at 84 h (Table 2).

The growths of microbes on the catheters were estimated in relation to the duration of usage. The catheters that had been used for over 2 month harbours a high growth of microbial colonies. The mean dry weight biomass of bacteria associated with a unit length (1 cm) of catheters was in the range 2.5±0.4g to 3.1±0.6 g after thirty days of usage (Table 3).

Table 2.
Biofilm forming characteristics of Proteus mirabilis isolated from catheter

S.no	Adhesion time (h)	Growth of P.mirabilis (OD Value)
1	12	0.19
2	24	0.32
3	36	0.41
4	48	0.61
5	60	0.72
6	72	0.81
7	84	0.85
8	96	0.68
9	108	0.52

Table 3.
Dry biomass of biofilm matrix catheters in relation to the time of indwelling periods

Catheters dwelling periods (days)	Dry Biomass g/infected 1 cm piece of catheter
0	2.5±0.4
30	2.7±0.3
60	2.6±0.7
90	3.0±0.3
120	2.9±0.6
150	3.1±0.6

4. Discussion

Escherichia coli and Proteus mirabilis were predominant in patient undergoing long term Catheterization. This indicates their high colonization in urinary tract. [4] Had isolated eleven species of bacteria from urinary tract catheters. The catheter associated bacteria had been reported to develop encrustation and blockage in catheters [3]. Blockage in catheters led to urine retention and caused pyelonephritis, septicemia and endotoxic shock. Encrustations and blockage in the lumen of the catheter was reported mainly due to the colonization of urease-producing bacteria, particularly Proteus mirabilis [1]. A comparison of the bacteria isolated from the catheters examined in the present study with the database of the composition of biofilms recovered from patient's catheter in the catheter research laboratory of Cardiff school of Biosciences, UK [4] confirmed the predominant occurrence of Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa in the catheter biofilms. [5] also reported that the bacteria Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa and Enterococcus faecalis are common in catheter biofilms.

The results indicated that P.mirabilis started its adhesion from 12th h and reached the maximum growth at 84 h. After 84 h the decline phase had started. [6] Had isolated P.mirabilis from around 40% of urine samples and reported that the long-term catheterized patients who suffer from catheter encrustation probably acquire P.mirabilis from their own fecal flora.

Bacteria in biofilms are notoriously difficult to eradicate with bactericidal drugs. In the present study P.mirabilis isolated from the catheters was resistant to Penicillin, tetracycline, gentamycin and kanamycin at a concentration, 20 μg/ml. Concentrations of Tetracycline, Penicillin, Kanamycin above 25 μg/ml could show little anti P.mirabilis activity. The resistance of biofilm cells to antibacterial agents is a clinically important, feature. [11] Reported that the replacement of old, biofilm -ladder catheters before antibiotic treatment is a sensible option.

Conflict of interest statement

We declare that we have no conflict of interest.

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