BIOFILMS- SURVEILLANCE IN A HEALTHCARE FACILITY AND EFFECT OF A BIOCIDE
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ABSTRACT

BACKGROUND
Biofilms form in environment, indwelling medical devices and industries. They are responsible for nosocomial infections. They can be detected in-vitro. Various biocides are being evaluated to remove them. We isolated 148 bacteria from 82 swabs collected from the hospital environment of which 37.2% were biofilm producers.

MATERIALS AND METHODS
Bacterial profile showed CONS (31.1%) and K. pneumoniae (25.7%) to be predominate with 41.3% and 55.3% biofilm producers respectively. Strong biofilm producers were K. pneumoniae (57.1%), CONS (52.6%), Pseudomonas aeruginosa (50%) and Acinetobacter baumannii (50%). After thorough cleaning with 1% sodium hypochlorite, the number of bacteria isolated was reduced to 54 from 82 samples with 42.6% biofilm producers.

RESULTS
Data analysis showed that biofilm producing organisms persisted as K. pneumoniae (57.1%), CONS (54.5%), Pseudomonas aeruginosa (40%). Even though 1% sodium hypochlorite showed reduction in bacterial load in the hospital environment, it did not exterminate the biofilm forming organisms.

CONCLUSION
We observed that with 1% sodium hypochlorite, along with mechanical rubbing, we could not eliminate the biofilm forming organisms. Thus, we need to study spectrum of biocides and their contact time against the biofilms in the hospital environment to effectively control them.

KEYWORDS
Biofilms, Sodium Hypochlorite.

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BACKGROUND
A biofilm is any group of micro-organisms such as bacteria, fungi, algae etc., in which cells stick to each other on a surface, which are embedded within a self-produced matrix of extracellular polymeric substance (EPS). This polymeric conglomerate is generally composed of extracellular DNA, proteins and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings.1,2

Microbes form biofilm in response to factors like cellular recognition of specific and non-specific attachment sites on a surface, nutritional signal, sub-inhibitory concentration of antibiotics. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells and large suites of genes are differentially regulated. Biofilm formation takes place in five stages –

1) Initial attachment.
2) Irreversible attachment.
3) Maturation –I.
4) Maturation –II.
5) Dispersion.

Biofilm formation begins with the attachment of free – floating planktonic micro-organisms to a surface. These attach initially with weak reversible adhesion via Van-der-Waal forces. If the colonies are not immediately separated from the surface, they can anchor more permanently using cell adhesion structures such as pili. Bacteria with increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacterium thus facilitating biofilm formation. Maturation starts once colonisation has established as it grows through a combination of cell division and recruitment. Polysaccharide matrices containing mainly glucose, galactose, mannose, fructose, fructose and N-acetylgulosamine etc., typically enclose bacteria and particulate matter. Further maturation progresses with development of water channels, that allows transport of essential nutrients, oxygen to the cells growing within. EPS traps extracellular enzymes and keeps them establishing external digestive system.3

Dispersal of cells from biofilm colony is an essential stage of biofilm life cycle. Dispersal enables the biofilms to spread and colonise new surfaces. Enzymes that degrade
the biofilm extracellular matrix, such as Dispersin B, DNase, may play a role in biofilm dispersal. Some species are not able to attach on their own but are instead able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonisation that the cells are able to communicate via quorum sensing.4

Biofilms are useful and also hazardous. Some uses of biofilms are-

1) Biofilms help in eliminating petroleum oil from contaminated oceans or marine systems by hydrocarbon degrading microbial communities-hydrocarbonoclastic bacteria (HCB).
2) Stromatolites - layers of earth are formed by biofilms. – Cyanobacteria.
3) They can live symbiotically with plants –nitrogen fixing rhizobium.
4) Used in microbial fuel cells (MFCs) to generate electricity from a variety of starting materials- complex organic waste and renewable biomass.
5) Biofilms are important in maintaining our gut with good flora.

Common Harmful Effects of Biofilms -

1) Cosmetic degradation.
2) Reduces the efficiency - cooling or heating pipes.
3) Affects the product quality in manufacturing.
4) Degradation of metals – reduces the life.
5) Corrosion of shower heads, water pipes, etc.
6) Bio-deterioration of sewage pipes.
7) Harmful health effects.

The development of a biofilm makes the aggregate cell colonies increasingly resistant to detergents, disinfectant and antibiotics. Microbes tend to readily form biofilms in healthcare settings. Traditionally biofilms are associated with wet or damp surfaces such as indwelling medical devices, tubing’s on medical devices Healthcare associated infections increase the morbidity, mortality and prolong the hospitalisation. They also increase the cost. According to a recent public statement from the National Institutes of Health, more than 65% of all microbial infections are caused by biofilms. These infections are difficult to treat and tend to relapse. Hospital environment cleaning and disinfection plays a major role in reducing the healthcare associated infections. Biofilms are associated with infections like – Gingivitis, dental plaque, dental caries. Contact lens, Joint prosthesis, Heart valves, Catheter associated infections etc. In all these conditions hospital environment may act as a source of infection.5

Various organisms associated with biofilms are staphylococcus epidermidis, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa etc.

Biofilms can be detected in the laboratory by various methods like Tissue culture plate (TCP), Tube method (TM), Congo Red agar method (CRA), Bioluminescent assay, Piezolectric sensors, Fluorescent microscope, Confocal microscope, Magnetic Atomic Force (MAF) microscope etc.6

Removal of biofilms can prevent their harmful effects especially in healthcare settings. Among the various disinfectants evaluated sodium hypochlorite and peroxygens like hydrogen peroxide, sodium percarbonate were found to be more effective.7

With this background we planned to do point surveillance for biofilms in a surgical Nursing Home and also test the efficiency of sodium hypochlorite in removing them.

MATERIALS AND METHODS

Prospective point surveillance was undertaken in a 30 bedded surgical nursing home, Sterile swabs were rubbed on the surfaces of the OT table, Boyles trolley, dressing trolleys, patients side tables. Bed rails wash basins in operation theatre, special rooms and general room. All the swabs were sent to laboratory immediately. The areas from where samples were collected were thoroughly washed first with detergent followed by 1% sodium hypochlorite along with mechanical rubbing. Then allowed to dry. Again, swabs were rubbed, and samples collected and sent to laboratory. So totally 164 swabs were collected, 82 before cleaning and 82 after cleaning.

Each swab collected was inoculated on 5% sheep blood agar and MacConkey agar plates and Gram stain was also performed. Inoculated plates were incubated at 37°C overnight. Colonies grown were identified using standard protocol of biochemical tests.8

All the isolated organisms were tested for their biofilm producing capacity using tube method described by Christensen et al.9 Accordingly one loop full of test isolate was inoculated into 10 ml of Trypticsoya broth with 1% glucose medium in a test tube and incubated at 37°C for 24 hours. Then the tubes were decanted and washed with phosphate buffer saline (PH 7.3). Tubes were dried and stained with 1% crystal violet for five minutes. Excess stain was washed with distilled water. Tubes dried in inverted position. Positive control strain was staphylococcus epidermidis ATCC 35984. Biofilm is present when a visible film was present on the walls and bottom of the test tube. Then grading of biofilm was done. 1- Weak, 2- moderate, 3-strong. Results were documented and analysed and compared before and after cleaning.

RESULTS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number</th>
<th>Biofilm Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONS</td>
<td>46</td>
<td>19</td>
</tr>
<tr>
<td>COPS</td>
<td>24</td>
<td>03</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>38</td>
<td>21</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>22</td>
<td>08</td>
</tr>
<tr>
<td>E.coli</td>
<td>14</td>
<td>02</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>04</td>
<td>02</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 1. Bacterial Profile and Biofilm Production Before Cleaning
From 82 samples collected before cleaning a total of 148 organisms were isolated of which 57 (37.2%) were biofilm producers. Coagulase negative Staphylococci (CONS) was the predominant organism 46 (31.1%) with 19 (41.3%) biofilm producers, of which 52.6% were strong biofilm producers. Similar observations were made in various studies, e.g., Elhabibi T et al have shown that 100% of *Pseudomonas aeruginosa* from various clinical specimens to be biofilm producers. Whereas Elhabibi T et al have shown that 100% of *Pseudomonas aeruginosa* from various clinical specimens were biofilm producers of which 90% were strong biofilm producers.

**CONCLUSION**

The types of organisms that develop biofilms are quite broad and include a number of known pathogenic bacteria, fungi and algae. A biofilm enhances the virulence, so involved in chronic bacterial infections. In the hospital environment, it is imperative that suitable biocides and infection control procedures are enforced to limit this risk of infection. We observed that with 1% sodium hypochlorite along with mechanical rubbing we could not reduce the biofilm forming organisms. Thus, we need to study spectrum of biocides and their contact time against the biofilms in the hospital environment to effectively control them.

**REFERENCES**


