ORIGINAL RESEARCH

Prevalence of microbial biofilms in the hospital environment in a Tertiary care hospital, Solapur

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ABSTRACT

Background: Biofilms are ubiquitous in healthcare settings and are associated with health care associated infections. Biofilms are composed of complex microbial community embedded in an extracellular polymeric matrix (EPS). Bacteria commonly involved in biofilm formation are *Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*. Formation of biofilm make the bacteria less susceptible for antimicrobial agents and disinfectants. Through cleaning practices with mechanical rubbing and use of appropriate disinfectant are needed to decrease the biofilm formation. **Material & methods:** 115 swabs were collected from ICU, Operation theater and wards. Swabs were cultured on Blood & MacConkey agar. Identification of organism were done as per standard microbiologic techniques. Antibiotic susceptibility done as per CLSI guidelines. Biofilm detection was done by Tube method described by Christensen *et al*.**Result:** 139 organisms were isolated of which Coagulase negative staphylococci were predominant (30.2%) followed by Klebsiella spp,(22.3%). The predominant biofilm producing organism was Klebsiella spp (51.6%) followed by CONS.(33.3%). Both the organism were strong biofilm producer. Most of the oeganisms were multidrug resistant **Conclusion:** Hospital environment is colonised with biofilm producing bacteria. CONS & Klebsiella spp are common organism producing biofilms. Good cleaning practices are required to prevent biofilm formation.

Key words: Biofilm, Bacteria, Tube method.

INTRODUCTION

Prevalence of Healthcare infections are increasing due to susceptible population and also emergence of multidrug resistant organisms. Among the many contributing factors for these infections, biofilms are emerging as an important predisposing factor. According to a recent public statement from the National Institutes of Health, more than 70 % of all microbial infections are caused by biofilms."[1,2]. A Biofilmis any group of microorganismsin which cells are irreversibly attached to each other on a surface. These adherent cells are frequently embedded within a self-produced matrix ofextracellular polymeric substance (EPS), which is also referred to as slime. It is a polymeric-conglomeration generally composed of extracellular DNA, proteins, and polysaccharides.[3,4]Biofilms are ubiquitous and are usually found on solid substrates submerged in or exposed to an aqueous solution. The structural and physiological complexity of biofilms indicate that they are co-ordinated and co-operative groups, analogous to multicellular organisms[4]. Biofilms may form on living or non-living surfaces and are prevalent in natural, industrial and hospital settings[5].

The life cycle of biofilms involves 3 phases[6]-

a) Attachment or Colonization- Biofilms form when bacteria adhere to surfaces in aqueous environments and begin to excrete a slimy, glue-like substance that can anchor them to a variety of materials including metals, plastics, soil particles, medical implant materials and most significantly, human or animal tissue.
b) Growth & Development- After the initial colonization, the biofilm grows through a combination of cell division and recruitment. Cells in different regions of a biofilm also exhibit different patterns of gene expression. Biofilm

bacteria can move in numerous ways that allow them to easily infect new tissues.[7].

c) Detachment or External Colonization- The periodic release of planktonic bacteria from some biofilms causes many chronic relapsing infections[8].

Bacteria commonly involved in biofilm production include *Staphylococcus epidermidis ,Enterococcus faecalis*, *Staphylococcus aureus*, , *Streptococcus viridans*, *Escherichia coli*, *Klebsiella pneumoniae*, etc.[7.8]*Mycobacterium avium* complex and fungi like yeast are also reported in biofilms[9].This is especially concerning where human health may be affected, for example on medical devices biofilms can decrease the effectiveness of sterilization procedures, resulting in increased medical infections[10]. In the hospital environment, biofilms can grow in showers, bathroom surfaces, inside water and sewage pipes, wash basin corners, floors, counters, trolleys[11].

Indwelling medical devices on which biofilms may develop on central venous catheters, contact lenses, endotracheal tubes, intra-uterine devices, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints & urinary catheters.[12]

Biofilms have been associated with infection in-Chronic sinusitis,Chronic wounds, Otitis media & other Inner Ear Infections, Cystic Fibrosis, Endocarditis, Osteomyelitis ,Periodontal Infections ,Urinary Tract Infections[13]

The formation of biofilms can make bacteria less susceptible to antimicrobial agents , the thick, slimy layer can protect bacteria down in the middle of the biofilm from the effects of an antimicrobial.[1]The survival power of bacteria in a biofilm is much higher than planktonic bacteria. Biofilms can be detected by Tissue culture Plate (TCP), Tube method (TM), Congo Red Agar method (CRA), Bioluminescent assay, Piezoelectric sensors, and Fluorescent microscopic examination etc.[14]These biofilms harbouring multi-antibiotic-resistant organisms found in hospital surfaces can contribute to the risk of infection transmission[14]. With this background, the present study is planned to study the prevalence of biofilms in different areas of the ARMCHRC hospital so that it will be helpful to take appropriate cleaning measures to prevent health care associated infections.

AIMS & OBJECTIVES

1. Detection of biofilms in the hospital environment.
2. Identify the organisms forming these biofilms.
3. Study their antibiotic profile.
4. To reanalyse the biofilms after thorough cleaning and disinfection.

MATERIAL AND METHODS

The present study was a prospective study carried out for a duration of six months in a tertiary care hospital. Material for the study include swabs collected from these locations

Sites	Location	Number	Total
Wash Basin corners	ICU	04	22
	Operation theaters	06	
	Wards	12	
Bathroom junction between wall & floor	ICU	06	57
	Wards	35	
	Special rooms	16	
Dressing trolleys surfaces.	Operation theaters	7	14
	Wards	7	
Bedside table surfaces.	ICU	10	22
	Wards	12	
Total			115

SAMPLE COLLECTION PROCEDURE

Sterile Swabs moistened with sterile BHI were rubbed on the surfaces mentioned above and inserted into sterile culture tubes and sent to Microbiology laboratory without delay (within half an hour).These swabs were used to inoculated 5% Sheep blood agar and MacConkey agar. Simultaneously smears were prepared on clean grease free slide, stained with gram stain and observed under microscope. Findings were documented. Inoculated media incubated at 37^0C overnight. Next day colony characteristics were studied. The isolated organisms identified by colony characteristics, gram reaction, morphology, various biochemical tests, motility etc as per the standard protocol. All the isolates were subjected to antibiotic susceptibility testing as per CLSI guidelines by Kirby-Bauer's disk diffusion method. The isolated organisms were subjected to Biofilm detection by Tube method described by Christensen *et al.*[15]. This is a qualitative method for biofilm detection.

BIOFILM DETECTION

A loopful of test organism was inoculated in 10 mL of Trypticase soy broth with 1% glucose in test tubes. The inoculated tubes incubated at 37ºC for 24 hrs. Then tubes decanted and washed with phosphate buffer saline (pH 7.3) and dried. The tubes were then stained with crystal violet (0.1%). Excess stain washed with deionized water. Tubes were dried in inverted position. In every batch one test tube of trypticase broth with 1% glucose was also inoculated with positive control and negative control.

The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible film lining the wall and the bottom of the tube was seen.

- Scoring of the Biofilm- The amount of biofilm formed was scored as-

a) Weak/None - 1
b) Moderate - 2
c) High/Strong -3

The experiment was performed in duplicate and repeated three times.

- Controls used for the Study- Positive biofilm producer *Staphylococcus epidermidis* ATCC 35984, *Staphylococcus aureus* ATCC 35556, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 and *Staphylococcus epidermidis* ATCC 12228 (non-slime producer) .

Sites, which showed the organisms producing films were subjected to thorough cleaning with freshly prepared 1% Sodium Hypochlorite solution with contact period of 30 minutes with mechanical rubbing for three consecutive days. Then again swabs were collected, and cultured as above and also biofilm production was tested.

OBSERVATIONS AND RESULTS

In the present study from 115 swabs collected showed 139 isolations, of which Coagulase negative staphylococci were predominant (30.2%) followed by Klebsiella spp,(22.3%) as shown in table no 1.

Table No 1: Showing the various organisms isolated.

Sr. No.	Name of the organism	Number
1	Coagulase Negative Staphylococci (CONS)	42 (30.2%)
2	*Klebsiella spp*	31(22.3%)
3	Coagulase positive Staphylococcus (COPS)	29 (20.8%)
4	*E.coli*	20 (14.4%)
5	*Pseudomonas aeruginosa*	17 (12.2%)
	Total	139

These organisms when subjected to biofilm production Klebsiella spp (51.6%) was the predominant biofilm producer followed by CONS.(33.3%).

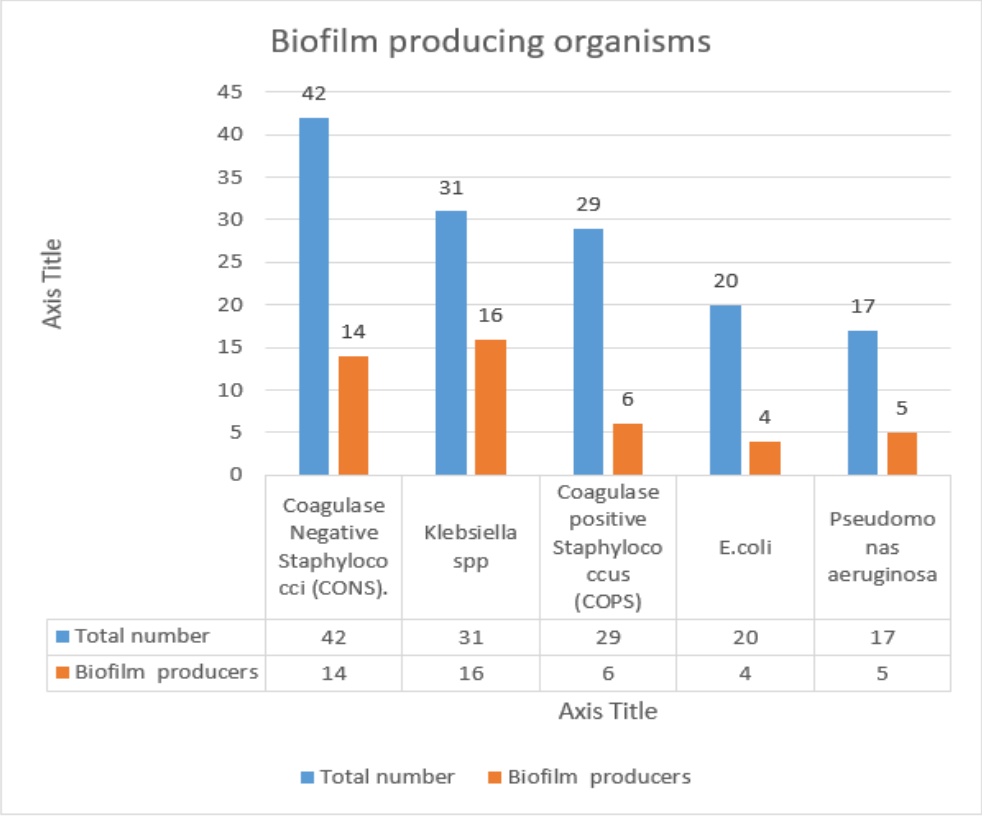

Figure 1

Grading of biofilm production showed that CONS (57.1%) and klebsiella aerogenes (56.3%) were strong biofilm producers as shown in table no 2

Table 2: Showing the distribution of grades biofilms by different organisms.

Organism	Biofilm grade			Total
	Weak	Moderate	Strong	Total
CONS (42)	02 (14.3%)	04 (28.6%)	08 (57.1%)	14
COPS (29)	03 (50%)	02(33.3%)	01(16.6%)	06
Klebsiella (31)	04 (25%)	03(18.7%)	09 (56.3%)	16
E.coli (20)	02 (50%)	02 (50%)	00	04
Pseudomonas (17)	01(20%)	03 (60%)	01(20%)	05
Total	12	14	19	45

Biofilm producing organisms are responsible for many infections and are notoriously difficult to eradicate Antibiotic susceptibility pattern of the isolates showed all the organisms were resistant to 2-4 antibiotics. So they were multidrug resistant.

Through cleaning with appropriate disinfectant and mechanical rubbing resulted in decrease colonisation of biofilm producing organism which is shown in the following table.

Table 3: Showing the number of organisms and biofilm production after thorough cleaning.

Organism	Isolations	Biofilm demonstrated
CONS (42)	13 (30.1%)	02 (15.4%)
COPS (29)	08 (27.6%)	00
Klebsiella (31)	14 (45.2%)	03 (21.4%)
E.coli (20)	01 (5%)	00
Pseudomonas (17)	09 (52.9%)	02 (22.2%)
Total	45	07 (15.6%)

DISCUSSION

Contamination of inanimate environment around the patients constitute an important reservoir of multidrug resistant organisms for health care associate infections. Biofilms are found in moist environments such as instruments, devices used in hospitals. Consistent with our study Hassan A *et al.*[14] has reported CONS 37.!%, *E.coli* (27.1%), *Klebsiella pneumonia* (15.7%), *Staphylococcua aureus* (11.4%), *Pseudomonas aeruginosa* (4.2%) as biofilm producers. Wojtyczka RD *et al.*[16] has also shown CONS as predominant pathogen showing biofilm producers in Surgical operation theatre (35.8%) and surgical wards (22.5%).

Ahamed SM *et al.*[17] has shown that tube method of biofilm detection in Pseudomonas aeruginosa detected totally 59% of the strains.

After cleaning thoroughly we could show that 69% of CONS, 61% of *Klebsiella aerogenes* and 47% of *Pseudomonas aeruginosa* were inhibited.

Vickery K *et al.*[18] has evaluated efficiency of disinfectants and demonstrated sodium [hypochlorite 1/10 dilution -1/50 was effective with contact time of 15 min in removing organisms. Smith K *et al.*[18]9 showed that following biocide treatment 0-11% of cells in MRSA biofilm survived and upto 80% of cells in *Pseudomonas aeruginosa* biofilm survived.

CONCLUSION

1. Hospital environments are colonised with biofilm producing bacteria.
2. These bacteria were CONS, *Klebsiella aerogenes, pseudomonas aeruginosa* etc.
3. Predominant biofilm producers were, *Klebsiella aerogenes* and CONS
4. With thorough cleaning with appropriate disinfectant (1% sodium hypochlorite) with mechanical rubbing can reduce the biofilms.

SUMMARY

In the present study 139 isolates were obtained from 115 environmental swabs. CONS, klebsiella aerogenes, Pseudomonas aeruginosa were predominant. Biofilm production was demonstrated by all organisms. Biofilms can be inhibited by cleaning 1% sodium hypochlorite along with mechanical rubbing.

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