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ORIGINAL ARTICLE

Bacterial Biofilms on Tracheostomy Tubes

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Abstract Tracheostomy is a commonly performed airway surgery for critically ill patients. Tracheostomy tube is an indwelling prosthesis, providing potential surface for growth of bacteria. Biofilm formation by bacteria as a self-protective mechanism, has led to worrisome antibacterial resistance and thus higher rate of nosocomial infections. A prospective observational study was conducted with a purpose of knowing most common organisms capable of forming biofilm on tracheostomy tube and their antibiotic sensitivity in our setting. Fifty seven percent of the isolates were found to be capable of biofilm production. *Acinetobacter baumannii (45%)* was the commonest biofilm producer isolated and the commonest multidrug resistant organism (35.7%), followed by *Klebsiella pneumoniae (28.5%)*. Both biofilm producers and non-biofilm producers were found most susceptible to Amikacin (43%), followed by Gentamicin (30%) and Ciprofloxacin (18.5%). No significant association was found between biofilms and ventilators (p value = 0.558) or pre-existing infection (p value = 0.66) using Chi square test. Potentially biofilm producing bacteria were isolated from tracheostomy tube inner surfaces just after a week of their insertion, in majority of patients. *Acinetobacter baumannii* and *Klebsiella pneumoniae* were the commonest biofilm forming organisms and Amikacin, Gentamicin and Ciprofloxacin were most sensitive drugs. Multi drug resistant organisms were also commonly found, stressing the need for sensitivity-based antibiotics. Ventilator usage had no strong association with biofilm formation. Patients with non-infectious conditions also harboured bacteria capable of biofilms in tracheostomy tubes demanding the need for stringent tube hygiene measures and prophylactic antibiotics.

Keywords Biofilm · Tracheostomy tubes · Antibiotic sensitivity · Multidrug resistance

Background

Biofilm formation is a unique self-protective mechanism of bacteria, as it protects them from host immune response and antimicrobial agents [1]. Biofilms are complex three-dimensional structures, which are composed of bacteria, living in an extracellular matrix made of polysaccharides, nucleic acids and proteins [2]. Studies showed that more than 60% hospital acquired infections are caused by biofilm forming bacteria on medical devices [3, 4].

Tracheostomy is a life-saving procedure performed among critically ill patients either because of airway obstructions or requiring prolonged mechanical ventilation for respiratory failure. Due to the presence of indwelling prosthesis for longer duration among tracheostomized patients, biofilm formation is inevitable on tracheostomy tubes; transmission of these biofilms to the lower airway tracts leads to severe complications like pneumonia or sepsis [5]. Different materials of tracheostomy tubes showed no change in susceptibility to biofilm formations [6]. Prolonged duration of tracheostomy had no significant correlation to biofilms, and they were formed as early as 7 days [2, 6, 7].

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Recent studies showed *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter species* as the common organisms forming biofilms on tracheal tubes [3, 4, 7, 8]. *Acinetobacter* was the most common multidrug resistant organism and was sensitive to Carbapenem and Colistin, while *Staphylococcus aureus* was sensitive to Linezolid and Carbapenem and *Pseudomonas* to Imipenem and Amikacin [9, 10].

An observational study aimed at knowing the most common organisms capable of forming biofilm, isolated from tracheostomy tubes and their antibiotic sensitivity in our setting was conducted.

Methods

A prospective observational study was conducted in south west coast of India, at a tertiary care hospital after obtaining Institutional ethics committee approval (YEC-1/2019/121) between June 2019 and June 2020. It included tracheostomized patients with tube in situ for more than 7 days, who presented to the department of Otorhinolaryngology and those who were admitted at our hospital in other departments.

A detailed patient information sheet was given to all participants, written informed consent obtained and history and reason for tracheostomy noted. Infection status of the patient and history of being on the ventilator was also noted. Using a sterile cotton swab stick, sample from the inner aspect of the portex tracheostomy tube and inner tube of the metal tracheostomy tube, was taken and sent for the standard protocol biofilm detection by tissue culture plate method.

Culture and sensitivity: The samples were inoculated on routine media like Blood agar and MacConkey agar. Colony characteristics were studied, and further identification and antibiotic sensitivity was done using BD Phoenix 100 automated system.

Tissue Culture Plate Method (Microtiter Plate):

Strains from fresh agar plates were inoculated in 3 ml of brain heart infusion (BHI) with 1% glucose and incubated for 24 h at 37^0C in stationary conditions and diluted 1 in 20 with fresh medium. Individual wells of sterile, polypropylene, 96 well microtiter plate were filled with 200µL of the diluted cultures and 200µL aliquots of only BHI + 1% glucose were dispensed into each of eight wells of the column 12 of microtiter plate to serve as control (to check nonspecific binding and sterility of media). After incubation (24 h at 37^0C), the microtiter plates content of each were removed by tapping the bottom plates. They were washed with 200µL of phosphate buffer saline (1 × PBS pH 7.2) to remove planktonic bacteria. The plates were then inverted and blotted on paper towels and allowed to dry for 15 min. Adherent organisms forming biofilms in plate were fixed with 2% Sodium acetate and stained with 0.1% w/v crystal violet and incubated at room temperature for 15 min. After removing the crystal violet solution, wells were washed with 1 × PBS to remove unbound dye. Finally, all wells were filled by 200µL ethanol (95%) to release the dye from the cells. Optical density (OD) of stained adherent bacteria was determined with an absorbance microreader at wavelength of 630 nm [11].

At the end samples were disposed according to the standard biomedical waste management protocols. Results obtained were charted on an excel sheet and patient information was destroyed and data was used only as group information under the study categories.

Results

Thirty-five patients in the age range of 20–75 years were enrolled in the study, of which 10 were females and 25 were males. It was observed that 57% (n = 20) of the isolates were biofilm producers and 43% (n = 15) were non biofilm producers.

Among the biofilm forming bacteria isolated in our study (Table 1), *Acinetobacter baumannii* (45%) was the commonest, followed by *Klebsiella pneumonia* (20%) and *Staphylococcus aureus* (10%). *Klebsiella pneumonia* (40%) was the commonest non biofilm forming organism, followed by *Pseudomonas aeruginosa* (33%).

Most organisms were sensitive to multiple antibiotics and few were multidrug resistant (MDR). As seen in Table 2, both groups were found to be most susceptible to Amikacin (43%), followed by Gentamicin (30%) and Ciprofloxacin (18.5%). Multidrug resistant strains were found in 14 patients (Table 3); *Acinetobacter baumannii* being the commonest organism (35.7%) followed by *Klebsiella pneumoniae* (28.5%) and *Pseudomonas aeruginosa* (21.4%). It was also reported that *E. Coli* and *S. Aureus* were multidrug resistant in one patient each: *Staph aureus* was resistant to, Daptomycin, Linezolid, Tetracycline, Vancomycin, Erythromycin, Clindamycin, Gentamicin, Penicillin and *E.coli* was resistant to Amikacin, Gentamicin, Tetracycline, Cefepime, Ceftazidime, Imipenem and Meropenem.

In biofilm forming group ten (50%) patients were on ventilator: *Acinetobacter baumannii* and *Klebsiella pneumonia* were found in four patients each and *Staphylococcus aureus* in two patients. There were seven (46%) patients in non-biofilm forming group on ventilator, out of which *Klebsiella pneumoniae* was found in four patients and

Table 1 Biofilm producers and non-biofilm producers among isolated organisms

Organisms	Biofilm producers (n = 20)	Non-Biofilm producers (n = 15)
K. Pneumoniae	4(20%)	6(40%)
A. Baumannii	9(45%)	0(0%)
S. Aureus	3(15%)	2(13.3%)
P. Aeruginosa	2(10%)	5(33.3%)
E. Coli	2(10%)	2(13.3%)

Pseudomonas aeruginosa in two and *Staphylococcus aureus* in one patient.

Among biofilm forming group four (20%) patients were admitted with pre-existing infectious aetiology and three (20%) patients in non-biofilm forming group. *Klebsiella pneumoniae* was found to be the commonest organism in both the groups.

Discussion

Observations of the present study add on to the evidence available in literature that biofilms are rampant on implanted medical devices. While our study showed 57% of isolates having biofilm positivity, other studies had similar rates of more than 60% bacterial biofilm formation [3]. There are some studies which have found as high as 73, 90 and 95% biofilm formation on medical prosthesis [1, 2, 12, 13].

Acinetobacter baumannii (45%) was the commonest biofilm forming organism followed by *Klebsiella pneumonia* (20%), which is similar to another study conducted by Mahendra et al.[9] Gil-Perotin S et al. in their study, noticed that most isolated bacteria were *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [13]. In other studies by Radji et al. and Inglis TJ et al., *Pseudomonas aeruginosa* and members of the family *Enterobacteriaceae (E.coli* and *Klebsiella pneumoniae*) were commonly isolated from tracheostomy tubes and ventilator filters [10, 12].

Observations of this study revealed that Aminoglycosides (Amikacin and Gentamicin) were most suitable antibiotics for the isolates. Since both are injectable drugs, they can conveniently be used for in-patients thus helping in preventing nosocomial infections. However, Ciprofloxacin and Tetracycline were the next best drugs for patients who did not require hospitalization.

Acinetobacter baumannii (35.7%) was found to be highly resistant to multiple drugs followed by *Klebsiella pneumoniae* (28.5%) and *Pseudomonas aeruginosa* (21.4%) which makes major concern and complexity in

Table 3 Antibiotics resistance pattern of multidrug resistant organisms (%)

Antibiotic	*A. Baumannii* (n = 5)	*K. Pneumonia* (n = 4)	*P. Aeruginosa* (n = 3)
Amikacin	66.6	40	42.85
Gentamicin	77.7	80	57.1
Ciprofloxacin	77.7	80	71.4
Cefepime	88.8	80	57.1
Ceftazidime	88.8	90	57.1
Levofloxacin	88.8	90	100
Tetracycline	88.8	100	100
Imipenem	100	80	100
Meropenem	100	100	100
Chloramphenicol	100	70	100

Table 2 Antibiotic sensitivity pattern of both biofilm producing and non-biofilm producing organisms (%)

Antibiotics	*P. Aeruginosa* (n = 7)	*K. Pneumonia* (n = 10)	*A. Baumannii* (n = 9)	*S. Aureus* (n = 5)	*E. Coli* (n = 4)
Amikacin	57.15	60	33.3	0	50
Gentamicin	42.9	20	22.3	40	25
Ciprofloxacin	28.6	20	22.3	0	25
Cefepime	42.9	20	11.2	0	0
Ceftazidime	42.9	10	11.2	0	0
Levofloxacin	0	10	11.2	0	25
Tetracycline	0	0	11.2	40	0
Imipenem	0	20	0	0	50
Meropenem	0	20	0	0	25
Chloramphenicol	0	70	0	0	0

antibiotics use. This is comparable with the study conducted by Mahendra et al., where they found *Acinetobacter species* was highly resistant to most of the antibiotics except Colistin [9]. In a study conducted by Radji M et al., *Staphylococci*, *Acinetobacter, Pseudomonas species*, were highly multidrug resistant [10]. These findings are comparable with our study.

Acinetobacter baumannii (40%), *Klebsiella pneumonia* (40%) and *Staphylococcus aureus* (20%) were isolated from samples from patients who were on ventilator. This is similar to observations of study conducted by Gil-Perotin S et al. where *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the common organisms to be isolated from endotracheal aspirate of patients on ventilator [13]. For the association between ventilators and biofilm formation, p value (0.558) was not found to be statistically significant using Chi square test. Hence there was no significant association between ventilator assisted respiration and biofilm forming bacteria in our study.

Similarly, in both biofilm forming and non-biofilm forming groups, only 20% patients were admitted with infectious aetiology. The p value (0.660) was not statistically significant using Chi square test for association between pre-existing infection and biofilm formation. It signifies that infections need not always be present at the time of admission for forming biofilms during hospital stay. Hence there is a need for various cautious measures by clinicians like tracheal tube swabs for biofilms, appropriate choice of sensitivity-based antibiotics and tracheostomy tube hygiene by autoclaving at hospital and regular washing of tubes including the inner aspect using Hydrogen peroxide, at home.

Klebsiella pneumonia was reported to be most common in non-biofilm forming and second most common among biofilm forming groups. It was also the second most common multidrug resistant organism. Interestingly, *Klebsiella pneumonia* species of multidrug resistant strains were found more in patients who were on ventilator (77%). Radji et al. also found *K. Pneumoniae* to be multidrug resistant strains in Intensive care unit patients probably because of extensive and inappropriate usage of broad-spectrum antibiotics [10]. So, clinicians maybe cautious of this feature of *Klebsiella*, when isolated from tracheostomy tube.

One of the limitations of our study was the lack of availability of Scanning Electron Microscope (SEM) which could give us direct evidence of the biofilms on tracheostomy tubes. Hence, we relied on the indirect evidence provided by tissue culture plate method. Gu et al. in their study of middle ear biofilms gave a data of 82% concordance of biofilm presence, seen on SEM, with the presence of biofilm producing organisms in culture [14]. In another study by Ferreira et al. on endotracheal tube (ET) biofilms, they compared different techniques of biofilm identification- tracheal aspirate, sonication fluid, and centrifuged sonication fluid from ET tubes. They found 83.3% concordance in these methods of diagnosis [15]. Hence, we can deduce that detection of biofilm by tissue culture plate method to be an evidence for high chance of the clinical presence of biofilms.

Conclusions

With 57% of patients having tracheostomy tube in situ for more than just a week, harbouring biofilm producing organisms, clinicians cannot ignore their presence, while choosing antibiotics. *Acinetobacter baumannii* and *Klebsiella pneumoniae* were the commonest biofilm forming organisms and Amikacin, Gentamicin and Ciprofloxacin were most sensitive drugs. Multi drug resistant organisms were also commonly found, stressing the need for sensitivity-based antibiotic. Ventilator usage had no strong association with biofilm forming bacteria. Patients with non-infectious conditions also developed bacterial colonies capable of biofilms in tracheostomy tubes, hence demanding evaluation, appropriate prophylactic antibiotics and stringent tube hygiene measures at hospital and home. However further studies with large sample size with respect to tracheostomy exclusively and SEM evidence, are needed to know geographical variations and to standardize the treatment protocols.

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