ORIGINAL ARTICLE

ASSESSMENT OF BIOFILM FORMATION BY THE CAUSATIVE ORGANISMS OF VENTILATOR ASSOCIATED PNEUMONIA AT INTENSIVE CARE UNIT OF A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction: The endotracheal tube participates in the pathogenesis of ventilator-associated pneumonia by the elimination of natural defense mechanisms, thereby allowing the entry of bacteria by the aspiration of subglottic secretions and ultimately these all will play role in the formation of biofilm on the endotracheal tube.
Aims and objectives: Present study was done to assess biofilm formation by bacterial clinical isolates from endotracheal tube of ventilator associated pneumonia patients and to assess drug resistance in association with biofilm.
Material and method: All isolates are identified by standard biochemical reaction and antibiotic susceptibility testing was done as per CLSI guidelines. Detection of biofilm is done by using tissue culture plate method.
Results: Total 56 isolates are recovered from 42 patients of ventilator associated pneumonia; from it 34 (65.4%) isolates are strongly positive by tissue culture plate method. Most common organisms isolated which producing strong biofilm are *Pseudomonas aeruginosa* and *Acinetobacter spp.*
Conclusion: The presence of an endotracheal tube in the airway, although critical for the management of the mechanically ventilated patient, also contributes to the development of ventilator associated pneumonia by disrupting normal protective mechanism which is associated with the intraluminal formation of biofilm by multidrug resistant organisms.

Keywords: Antibiotic resistance, Biofilm, Multidrug resistant organisms, Tissue culture plate method, Ventilator associated pneumonia.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is a major healthcare-associated complication with considerable attributable morbidity, mortality and cost. Inherent design flaws in the standard cuffed tracheal tubes form a major part of the pathogenic mechanism causing VAP. The formation of folds in the inflated cuff leads to micro aspiration of pooled oropharyngeal secretions into the trachea and biofilm formation on the inner surface of the tracheal tube helps to maintain bacterial colonization of the lower airways. By the elimination of natural defence mechanisms, thereby allowing the entry of bacteria by the aspiration of subglottic secretions or the formation of biofilm on the endotracheal tube. Common nosocomial pathogens like Pseudomonas aeruginosa are known to produce exopolysaccharide and generate the complex biofilm structure, which allows adhesion to abiotic surfaces and protection against antibiotic action. Multiple studies have identified bacterial biofilm on the inner lumen of endotracheal tubes, which represents a permanent source of infectious material.[1]

We aimed this study to assess biofilm formation by bacterial clinical isolates from endotracheal tube of Ventilator associated pneumonia patients and to assess drug resistance in association with biofilm.

MATERIAL AND METHOD

This was a prospective study done at Intensive care unit of a tertiary care hospital during May 2011 to October 2011. Diagnosis of VAP patients has done as per CDC criteria.[4] In this study we have included only those patients whose ET aspirate and ET tube culture results were grown phenotypically similar pathogens.

These samples were processed as per standard diagnostic practice. The medical devices were directly cultured by roll plate method on blood agar, MacConkey agar & chocolate agar. The observation was done after 24 hour of incubation for any isolation. The isolates were further identified to the species level using phenotypic tests as per standard protocols. All the organisms were subjected to antimicrobial susceptibility testing including detection of various resistance mechanisms like ESBL,Cabapenemase resistance, Inducible clindamycin & MRSA (in case of Gram positive organisms) by manual methods as per recent CLSI guidelines.[6] we screened all isolates for their ability to form biofilm by TCP/ microtitre plate method as described by Christensen *et al* [6] with a modification in duration of incubation. Incubation period of 20 hours was studied.

The following pathogens were considered as MDR: methicillinresistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase producing Gram-negative *Enterobacteriaceae* (ESBL), *Pseudomonas aeruginosa* and other non-fermenting organisms (*Acinetobacter baumannii*, *Stenotrophomonas maltophilia*) resistant for three or more of the following antibiotic classes: antipseudomonal cephalosporins or penicillins, carbapenems, fluoroquinolones and aminoglycosides (MDR NF).VAP episodes caused by MDR organism plus non-MDR organism were classified as 'MDR' episodes.

RESULTS

Total 104 patients included in this study, 68 (65.4%) were males & 36 (34.6%) were females. The mean age was 43.8 years. The incidence of VAP in our study was 49.03%, with 51 of 104 patients developing VAP. From these 51 patients of VAP 42 patients whose ET aspirate and ET tube culture results were grown phenotypically similar pathogens. Total 56 isolates were recovered from 42 patients. Distribution of causative organisms of VAP is demonstrated in Figure-1.

Figure 1: Distribution of causative organisms of VAP

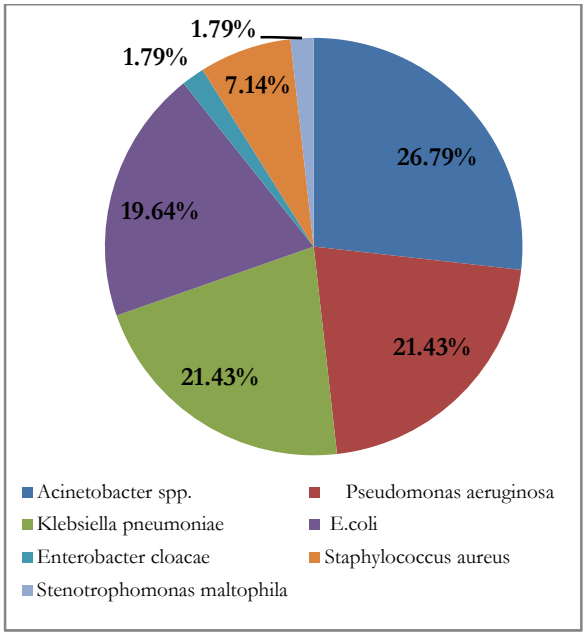

Table 1: Antibiotic susceptibility pattern in gram negative organisms (% sensitive)

Organism	CPZ	CPM	PIT	IPM	MRP	GEN	AK	NT	LE	PB	CL
Acinetobacter spp	0	0	3	13	17	13	13	30	17	100	100
Pseudomonas aeruginosa	41	41	35	76	76	59	47	47	24	100	100
E-coli	22	22	33	100	100	67	89	61	17	100	100
Klebsiella pneumoniae	0	0	33	57	57	57	71	48	19	100	100

CPZ=Ceftazidime, **CPM**=Cefepime, **PIT**=Piperacillin/Tazobactam, **IPM**=Imipenem, **MRP**=Meropenem, **GEN**=Gentamicin, **AK**=Amikacin, **NT**=Netilmycin, **LE**=Levofloxacin, **PB**=Polymyxin B and **CL**=colistin.

In this study 4 isolates of *Staphylococcus aureus* were isolated and all strains were methicillin resistant (MRSA) and only sensitive to vancomycin, linezolid and teicoplanin.

In our study we found that from 56 isolates 37 (66.1%) isolates were MDR and from them 27 (48.2%) isolates were associated with strong biofilm formation. *Acinetobacter spp.* was the most common organism isolated (26.8%) and also associated with strong biofilm formation (33.3%). It was also the most common multidrug resistant organism (35.1%) followed by *Pseudomonas aeruginosa* (18.9%), *Klebsiella pneumoniae* (18.9%), *E-coli* (13.5%) and *Staphylococcus aureus* (10.8%) in our study.

DISCUSSION

VAP pathogenesis is a dynamic process, involving a wide spectrum of pathogens and complex interactions with host defenses. Bacteria causing VAP usually originate in the oropharynx. The endotracheal tube increases the risk of VAP 6- to 20-fold, facilitating bacterial access to the lung and providing a nidus for the growth of biofilm-encased bacteria . Poor patient outcomes are associated with late-onset disease (> 5 d) and infections caused by multidrug-resistant bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter* species, or methicillin-resistant *Staphylococcus aureus*.

Table-2: Results of biofilm detection and their association with drug resistance

Organism	No. of organism	Assessment of Biofilm formation results				Drug resistance	
		Strong biofilm production	Moderate biofilm production	Weak biofilm production	Negative for Biofilm production	MDR organism	Non-MDR organisms
Acinetobacter spp.	15	9	2	4	--	13	2
Pseudomonas aeruginosa	12	5	1	3	3	7	5
Klebsiella pneumoniae	12	7	--	3	2	7	5
E.coli	11	4	1	5	1	5	6
Enterobacter cloacae	1	--	1	--	--	--	1
Staphylococcus aureus	4	2	1	1	--	4	--
Stenotrophomonas maltophila	1	--	--	1	--	1	--
Total	**56**	**27**	**6**	**17**	**6**	**37**	**19**

In a study, [11] it was documented that the interior of the ETT of patients undergoing mechanical ventilation rapidly became colonized with Gram-negative micro-organisms which commonly appeared to survive within a biofilm. While it appears that colonization of the ETT may begin from as early as 12 h, it is most abundant at 96 h. This investigation further suggests that the common sequence of bacterial colonization of patients undergoing mechanical ventilation is firstly the oropharynx/ upper gastrointestinal tract, followed by the lower respiratory tract, leading on to ETT colonization. Colonization of the ETT with microorganisms commonly causing nosocomial pneumonia appears to persist in many cases despite apparently successful treatment of the previous pneumonia. The organisms isolated (sometimes multiple) in secretions obtained by suctioning of the lower respiratory tract of these cases and deemed to be the likely cause of the pneumonia were *Pseudomonas aeruginosa* (6 cases), *Acinetobacter* (5 cases) *Klebsiella pneumonia* (3 cases), *Proteus mirabilis* (3 cases) and *Enterobacter spp.* (1 case). Propotional bacterial isolates from endotracheal tubes in study by Feldman et al is as our study. With regard to the management of ETT biofilm formation and colonization, a number of options have been considered. While regular ETT changes may seem appropriate, recent studies have suggested that this may be associated with a higher incidence of nosocomial pneumonia.[7, 10] The reasons for this are not entirely clear, but may relate to the passing of the airway access tube through areas (e.g. the naso- or oropharynx) that are already colonized in critically ill cases with common nosocomial pathogens. Others have suggested that the interior of the ETT could be "brushed" using a specially developed wire mesh instrument or that specific materials could be developed for airway access tubes that impede biofilm formation, these being steps which may prevent nosocomial pneumonia. [8, 9] In conclusion, it has been noted that endotracheal tube colonization and biofilm formation occurs in many patients undergoing mechanical ventilation, from a very early stage. Biofilm formation may in many cases precede the development of nosocomial pneumonia, and perhaps more importantly, represent a persistent source of organisms causing recurrent infections. Further studies are needed to clarify the exact role of endotracheal tube colonization in the pathogenesis of nosocomial pneumonia. Similar findings are from the study of Timothy et al being *Pseudomonas aeruginosa* and members of the family Enterobacteriaceae (including *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis*, and *Providencia stuartii*) were isolated from 13 (29%) of 45 inner surfaces of tracheal tubes. We have shown that a layer of biofilm accumulates on the inner surfaces of tracheal tubes and that contaminated particles can be detached from this layer during mechanical ventilation.

Acinetobacter as strong biofilm producer was described by R Shrinivasa Rao et al [12] in 62% cases that correlating with present study. In study by Marta M. Wroblewska et al [13] demonstrated 12% of A. baumannii strains as strong producers, 41% – medium producers and 47% low producers of biofilm.

Strains of Acinetobacter spp., mainly Acinetobacter baumannii, are very important nosocomial pathogens, contributing significantly to morbidity and mortality of patients, particularly hospitalized in intensive care unit. Moreover, recent emergence of carbapenem resistance among these isolates further stresses their importance in etiology of hospital-acquired infections. Infections of hospitalized patients with Acinetobacter spp., often preceded by colonization, are frequently associated with invasive procedures and implantable medical devices. The ability of a strain to form a biofilm may be a significant factor facilitating this process. However, there are only scarse reports on biofilm formation by clinical strains of A. baumannii isolated from hospitalized patients and the numbers of tested isolates were 20.[14]

In our study we have correlated the ability of biofilm formation of an organism with multidrug resistance.

CONCLUSION

The presence of an endotracheal tube in the airway, although critical for the management of the mechanically ventilated patient, also contributes to the development of VAP by disrupting normal protective mechanism which is associated with the intraluminal formation of biofilm by multidrug resistant organisms.

Acronyms	
CDC	Centers for Disease Control and Prevention
CLSI	Clinical & Laboratory Standards Institute
ESBL	Extended spectrum of β-lactamase
ET/ETT	Endotracheal tube
MDR	Multidrug resistant
MDR NF	Multidrug Resistant Non-Fermenter
MRSA	Methicillin resisrtant *Staphylococcus aureus*
Non-MDR	Non Multidrug Resistant
TCP	Tissue Culture Plate method

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