Original Article

Evaluation of Ventilator Associated Respiratory Tract Infections (VARTI) by Common Anaerobic and Atypical Bacteria among the Patients of ICU of a Tertiary Care Hospital in Bangladesh

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Abstract

Background and aim of the study: *Ventilator-associated respiratory tract infection (VARTI) is theleading cause of higher mortalityand morbidity in ICU compared to non-ICU patients. Thisarticle reveals etiology of VARTI by commonanaerobic and atypical bacteria in ICU of Dhaka Medical College.*

Materials and Methodology: *This is a cross-sectionalstudy where 200 endotracheal aspirate (ETA) samples were taken from clinically suspected patients of VARTI. After proper screening, bacteria were identified by PCR as because anaerobic and atypical bacteria cannot be easily grown in conventional culture media.*

Result: *Sixty three (31.5%) samples were found to have VARTI. Among the common atypicalbacteria causing, M. pneumoniae was found in 4 (6.35%) and L. pneumophila in 2 (3.17%) of the 63 samples. In search of the anaerobic bacteria causing VARTI,Peptostreptococcus was detected in 3 (4.76%) while Fusobacterium nucleatum andPrevotella melaninogenicawere detected in 2 (3.17%) each of the 63 samples.*

Conclusion: *Anaerobic and atypical bacteria are implicated in VARTI in ICU although at a relative low rate.*

Key word: *Intensive care unit (ICU), Ventilator associated respiratory tract infection (VARTI).*

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Introduction

Health-care-associated infections(HAIs) are major problems worldwide[1]and is particularly higher in intensive care units (ICUs) due to use of various external devices such as mechanical ventilator[2]. Study revealed that ICUs account for 11.98% of the total nosocomial infection, even though they occupy less than 10% of the total bed capacity in a tertiary hospital.[3] Majority of infections in ICUs are VARTI[4] which occurs in patients receiving mechanical ventilation for more than 48 hours.[5]It includes both ventilator associated pneumonia (VAP) and ventilator associated tracheobronchitis (VAT). In ICU, VARTI appear to double the number of deaths compared to those without VARTI.[6] It results from aspiration of oropharyngeal contents into the lungs followed by colonization of *atypical and anaerobic bacteria. Common a*naerobic bacteria include*Fusobacterium spp, Prevotella spp.* and *Actinomyces spp*. Atypical bacteria,such as *Mycoplasma pneumoniae* and *Legionella pneumophila*are also implicated in VARTI although these pathogens have not been studied systemically and their role is unclear.[7]

Classical microbiological techniques cannot provide a rapid and efficient way to diagnose anaerobic and atypical bacteria because most of them grow either slowly or not at all in conventional culture media, leading to delayed and missed diagnosis.[8]So, this studywasdesigned to determine etiology of VARTI by common anaerobic and atypical bacteria by PCR in ICU of DMCH.

Methodology

This cross-sectional study was conducted in ICU of Dhaka Medical College Hospital (DMCH), during 1st July 2015 to 30th June 2016. Research protocol was approved by the research review committee (RRC) and ethical review committee (ERC). Endotracheal aspirates (ETA) were collected from 200 patients of ICU who fulfilled the clinical definition of VARTI followed by screening through microbiological and radiological definitions.[6] So, samples that fulfilled the clinical,microbiological and radiological definitions of VARTI,[9] were included for the study.Finally, PCR was done to all these samples to detect anaerobic and atypical bacteria causing VARTI in ICU.

Clinical definition of VARTI

Patients using endotracheal tube (ETT) for e" 48 hours plus temperature (>38^0C) or leukocyte count (>12,000/mm^3, or <4000/mm^3) plus new onset of purulent endotracheal secretions or change in character of sputum or increased respiratory secretions.[9]

Sample collection

In each clinically suspected patient of VARTI, aseptically, a 50 cm 14 Fr sterile suction catheter was introduced through the Endotracheal tube (ETT) for 24-26 cm to obtain ETA by suction without giving saline. Cut tips of the catheters were collected inside the sterile test tubes with cotton plug and sent to the laboratory.[10]

Screening of samples

Microbiological criteria: Gram stained ETA samples, showing polymorphonuclear leukocyte (PMNL) with or without bacteria.[9]

*Radiological criteria:*Chest x ray showing either new or progressive and persistent infiltrate or consolidation or cavitation (VAP) or transient infiltrate or no radiographic change (VAT).[9]

Sample processing

In laboratory, 2 ml sterile normal saline and few glass beads were added in the test tube that contains the cut tips of catheter. The test tube was vortexed well to make a homogenous mixture of the sticky sample[11]After that, the cut tips and the glass beads were removed and the vortexed fluid was taken into an eppendorf tubeand centrifuged at 14000 g for 10 minutes.The supernatantwas discarded by sterile pipette and the deposit waspreserved for further study at -20^0C as pellet.[12]

DNA extraction

Three hundred microliter distilled water was added with pellet in the eppendorf tube and vortexed. Then it was heated at 100^0C for 10 minutes in a heat block and was placed in an ice pack for 5 minutes.After that, eppendorf tube was centrifuged at 13000 rpm for 6 minutes at 4^0C and thesupernatant waspreserved at -20^0C for PCR.[13]

Diagnostic criteria for VAP and VAT [9]

Variables	VAP	VAT
Clinical signs and symptoms	Temperature (> 30^0C) or leukocyte count (> 12000/mm^3 or < 4000/mm^3) plus new onset of purulent endotracheal secretion or change of sputum or increased respiratory secretion.	
Radiology: CXR or CT scan	New or progressive and persistent infiltrate or consolidation or cavitation	Transient infiltrate or noradiographic change
Microbiological criteria	Gram stained ETA sample showing PMNLs with or without bacteria	

Primers of this study

For detection of anaerobic bacteria

Bacteria		Primer Sequence (5'-3')	Size (bp)
Prevotella melaninogenica	F	TCATCTTACCGAAAAAAT	141
	R	TGGGACGTTCGTTTGTTT	
Fusobacterium nucleatum	F	*AGAGTT TGATCC TGGCT*	360
	R	*GTCATCGTGCACACAGAT*	
Peptostreptococci	F	AGAGTTTGATCTGGCTCG	553
	R	ACGGGCGGTGTGTC	

For detection of atypical bacteria

Bacteria		Primer Sequence (5'-3')	Size (bp)
Chlamydia pneumoniae	F	GTTGTTCATGAAGGCCTACT	437
	R	TGCATAACCTACGGTGTGTT	
Legionella pneumophila	F	AGGGTTGATAGGTTAAGAG'	386
	R	CCAACAGCTAGTTGACATCG	
Mycoplasma pneumoniae	F	TCAATCTGGCGTGGATCTCT	180
	R	GTCACTGGTTAAACGGACTAC	

Results

The study started with 200 clinically suspected VARTI patients of ICU of DMCH and after proper screening 63 (31.5%)samples were finally selected for this study. This study searched for atypical bacteria such as *Mycoplasma pneumoniae, Legionella pneumophila* and *Chlamydia pneumoniae* and anaerobic bacteria such as *Peptostreptococcus*,*Fusobacterium nucleatum* and *Prevotella melaninogenica*.As because these bacteria cannot be cultured in routine media,they were detected by PCR. PCR was done to all of the 63 samples for two times, once for atypical bacteria and then for anaerobic bacteria where few samples revealed mixed bacteria.

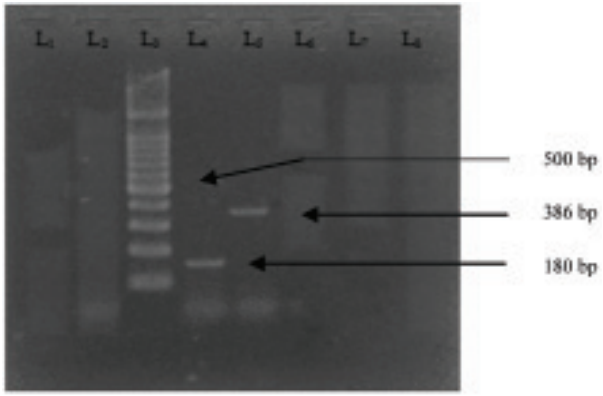

Figure: *Amplified DNA of 180 bp for Mycoplasma pneumoniae gene (lane 4), DNA of 386 bp for Legionella pneumophila (lane 5), 100 base pair DNA ladder (lane 3), negative sample (Lane 6 and 7), negative control E. coli ATCC 25922 (lane 8)*

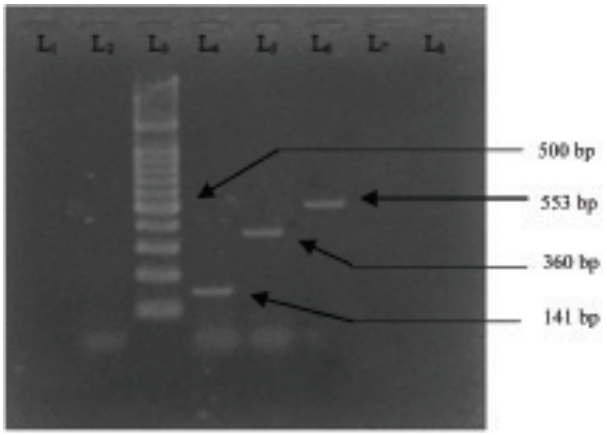

Figure: Amplified DNA of 141 bp for *Prevotella melaninogenica*, gene (Lane 4), DNA of 360 bp for *Fusobacterium nucleatum* gene (Lane 5), DNA of 553 bp for *Peptostreptococci* gene (Lane 6), 100 bp DNA ladder (Lane 3) negative sample (Lane 7) and negative control *E. coli* ATCC 25922(Lane 8).

Table I: *Screening and selection of ETA samples of ICU.*

ETA samples from clinically suspected	Screening of samples				Confirmedcases of VARTI n (%)
	Microbiological criteria N = 200		Radiological criteria N = 120		
VARTI patients	Rejected samples	Selected samples	Excluded samples	Including samples	
200	80	120	57	63	63 (31.5%)

Table II

Detection of atypical bacteria among ETA samples by PCR (N = 63).

Bacteria	Detection by PCR n (%)
Mycoplasma pneumoniae	1+2**+1* (6.35)
Legionella pneumophila	1+1* (3.17)
Chlamydia pneumoniae	0 (00.00)
Total	6 (9.52)

*Indicates mixed infection of *Mycoplasma pneumoniae* and *Legionella pneumophila.*

** Indicates mixed infection of *Mycoplasma pneumoniae* and *Prevotella melaninogenica.*

Table III Detection of anaerobic bacteria among ETA samples by PCR (N = 63).

Bacteria	Detection by PCR n (%)
Fusobacterium nucleatum	2 (3.17)
Peptostreptococcus	3 (4.76)
Prevotella melaninogenica	2** (3.17)
Total	7 (11.11)

*Indicates mixed infection of *Mycoplasma pneumoniae* and *Legionella pneumophila.*

** Indicates mixed infection of *Mycoplasma pneumoniae* and *Prevotella melaninogenica.*

In this study,31.5% VARTI were detected in ICU of DMCH.In search of atypical bacteria causing VAP or VAT, PCR detected *Mycoplasma pneumoniae* in 6.35% and *Legionella pneumophila* in 3.17% of the 63 ETA samples. None of the ETA samples were positive for *Chlamydia pneumoniae*.Among the common anaerobic bacteria *Peptostreptococcus* were detected in 4.76% while *Fusobacterium nucleatum* and *Prevotella melaninogenica* were found in 3.17% each of the 63 samples.

Discussion

Regular surveillance of DAI in any healthcare setting is highly informative not only to clinicians but also to the hospital administration to decide strategies for prevention and control of nosocomial infections. However, due to difficulties in conducting such studies only few information is available on institutional DAI rate. [14]

In the present study, 31.5% VARTI were detected in ICU of DMCH which was similar to El-Din-Hamdy on 2014[15] who reported 34.20% VARTI in his study.In this study,*Mycoplasma pneumoniae* was detected in 6.35% and *Legionella pneumophila* in 3.17% of the 63 ETA samples by PCR.These findings were in accordance with the data reported by Akter on 2014 in DMCH,[12] where *Mycoplasma pneumoniae* was detected in 7.69% and *Legionella pneumophila* in 6.15%. Moreover, another study by Mokhless on 2010[16] reported 8.33% *Mycoplasma pneumonia* and 5% *Legionella pneumophila* in their study which also coincides with the present study.In contrast, Abukhabar on 2017[17] reported 3.33% *Legionella pneumophila* but no*Mycoplasma pneumoniae* in their study. This difference might be due to the fact that most of the patients of this study was of older age and smoker. *Mycoplasma pneumoniae* produces infection mostly in elderly people and shows special predilection to those with preexisting lung diseases.[18]

Data regarding the role of anaerobic bacteria in VARTI are conflicting. A prospective surveillance study reported that 57.70% of VAP patients becamecolonized by anaerobic bacteria.[19]On the other hand, Marik and Careau on 1999[20]reported no anaerobic infection even among 185 VAP patients. This deflection might be due to the fact that ICU patients of the second study were treated with antibiotics which might have prevented anaerobic infections.[21]This study revealed 11.11% anaerobic infections among 63 VARTI patients.

During our study period, we observed that, in ICU of DMCH, there is a discrepancy between the number of patients and the health care providers such as doctors and nurses. So, meticulous cleaning and time to time replacement of ETT was not possible which might be the reason for VARTI by anaerobic bacteria in ICU of DMCH.

Conclusion

VARTI remains a frequent health care associated infection, occurring in 10% to 20% of ICU patients (Jean-Louis). In reality, very few data are available about ICU, particularly VARTI by atypical and anaerobic bacteria. On the other hand, control of nosocomial infections highly depends on proper identification of the bacteria followed by rational use of antibiotics. So, this study will definitely be helpful about detection of atypical and anaerobic bacteria. Further study is urgently required to evaluate the drug sensitivity patterns of these bacteria.

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