

Original Research Article

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A Study of Microbial Colonization and their Antibiotic Resistance Pattern in Endotracheal Aspirate Cultures in Intensive Care Unit Patients at a Tertiary Care Hospital of Eastern UP, India

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ABSTRACT

Nosocomial infections are a critical issue among intubated patients. Respiratory tract infection in relation to intubation and/ or mechanical ventilation has been reported to vary from 4% to 28% and this rate has been thought to be 21 times higher than in patients without endotracheal tube. The aim of our study was to identify the pathogens isolated from culture of endotracheal tube aspirates and determine their antibiogram. Patients on mechanical ventilation (MV) for >48 h in the intensive care units (ICU), critical care unit (CCU), PICU and NICU were included in this study. All the endotracheal aspirate samples were transported to the Microbiology laboratory for culture and sensitivity testing. In our study we observed a total of 101 ETAs culture positive isolates. The most common micro-organism was *Klebsiella pneumoniae* 28 (27%) and in Gram positive it was *CONS* 12 (12%). The highest resistance was found in 3rd generation cephalosporins and Penicillin group antibiotics, the least in carbapenems and polymyxin B. The key is early identification of the causative microbial agents, antibiotic sensitivity and administration of selected antibiotics. Our observations provide useful information regarding such respiratory infection. Thus ET tip cultures could be useful adjunct to the ATS strategy for ensuring appropriate treatment of intubated patients and in context of a bundled approach to the prevention of ET tip infections.

Keywords

Endotracheal tube, Ventilator-associated pneumonia, Nosocomial infection

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Introduction

Nosocomial infections are the leading cause of mortality and morbidity among the patients admitted in ICU in the present time. One of the most important types of this infection is pneumonia which commonly occurs in relation to the endotracheal tube and mechanical ventilation named ventilator associated pneumonia. Ventilator-associated pneumonia (VAP) is the most frequent

intensive care unit (ICU)-acquired infection, occurring in 9–24% of patients intubated for longer than 48 hours (Tandia *et al.*, 2015). Nosocomial pneumonia represents approximately one quarter of all nosocomial infections and tracheal intubation increases the risk of infection from 6 to 20 times (American Thoracic Society, 2005; Ferreira *et al.*, 2016). In the literature, the incidence of respiratory tract infection in relation to intubation and/ or mechanical ventilation has been reported to

vary from 4% to 28% and this rate has been thought to be 21 times higher than in patients without endotracheal tube (Diaz *et al.*, 2008; Chastre and Fagon, 2002; Fortaleza *et al.*, 2009; Chevret *et al.*, 1993; Haley *et al.*, 1981).

Patients with mechanical ventilation have an increased risk for respiratory tract infection because the tube which has been inserted in trachea reduces the clearance of bacteria and increases the leakage of secretion around the cuff of tube and disable the ciliary tract by damaging it (Tandia *et al.*, 2015; Ferreira *et al.*, 2016; Diaz *et al.*, 2008). Microbial adhesion on the tube itself results in biofilm formation is one of the major cause of infection. In long-term ventilated patients subglottic secretions can accumulate above the cuff of the ETT and hence present as an ideal growth medium for bacteria (Haley *et al.*, 1981; <https://www.bactiguard.se/en/our-products/endotracheal-tube>).

By micro-aspiration along the cuff, these contaminated secretions might pass into the lower respiratory tract and become a potential cause of lower airway-infection including VAP (<https://www.bactiguard.se/en/our-products/endotracheal-tube>). Artificial airways generally are colonized with potentially pathogenic microbes. Colonizing bacteria may arise from the upper airway, ventilator tubing or reservoirs (Depuydt *et al.*, 2006). Lower respiratory tract infections in intubated patients include ventilator-associated tracheobronchitis (VAT) and ventilator-associated pneumonia (VAP), these infections are increasingly caused by multidrug-resistant bacteria, which colonize the patient's oropharynx and enter the lower respiratory tract around the endotracheal tube cuff or through the lumen. Progression of colonization to VAT and, in some patients, to VAP is related to pathogenesis and virulence of invading bacteria (Donald *et al.*, 2010).

Over the past decade, the incidence of lower respiratory tract infection due to multidrug resistant (MDR) pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative bacilli (eg, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) has been increasing (Chevret *et al.*, 1993). Understanding the pathogenesis of VAT (ventilator-associated tracheobronchitis) and VAP (ventilator associated pneumonia) is crucial for establishing principles for therapy and prevention. Hence intubation with mechanical ventilation increases the risk of bacterial pneumonia (Chevret *et al.*, 1993; Nseir *et al.*, 2008).

Widespread use of antibiotics in intensive care units is a potential cause of the emergence of nosocomial infections caused by antibiotic-resistant bacteria (Kollef and Fraser, 2001; Chandra Mouli *et al.*, 2016). Current guidelines for the management of VAP strongly recommend the use of early, appropriate empirical antibiotic therapy based on patient risk factors for multidrug-resistant pathogens (Chevret *et al.*, 1993; Kollef and Fraser, 2001; Chandra Mouli *et al.*, 2016).

Routine endotracheal aspirate cultures of critically ill patients in ICUs may be predictive of patients who are at high risk of invasive disease, and may guide the selection of appropriate empirical therapy based on the predominant pathogens identified in microbial cultures in the event of the development of VAP (Joseph *et al.*, 2010).

The decision regarding choice of antibiotic has to be based on microbiological culture and sensitivity pattern. Therefore the aim of our study was to identify the pathogens from culture of endotracheal tube and determine the risk factors of disease and their antibiogram in patients admitted in tertiary care hospital of Eastern UP.

Materials and Methods

This was an Observational Cross– Sectional study of 1 year period from June 2016 to May 2017 conducted at Department of Microbiology, BRD Medical College, Gorakhpur.

All the patients on mechanical ventilation (MV) for >48 h in the different intensive care units (ICU), critical care unit (CCU) and PICU, NICU of BRD Medical college and hospital were included in this study. Patients with pneumonia prior to MV or within 48 h of MV were excluded.

The following data was collected from the patients enrolled in the study including age, gender, and underlying illness with all clinical information, duration of hospitalization, duration of mechanical ventilation and details of prior antibiotic therapy.

All the endotracheal aspirate samples were transported to the Microbiology laboratory and immediately inoculated on Blood agar, MacConkey agar and chocolate agar. The culture plates were incubated at 37°C for 48 hours before issuing a negative result. Any growth of microorganism on the culture plates was identified by colony characteristics, Gram staining and standard biochemical tests (Meligy *et al.*, 2015; Collee *et al.*, 1996). Bacterial concentrations (cfu/mL) were calculated. Microorganisms with counts > 10⁴cfu/mL were submitted for identification and antimicrobial susceptibility testing (Collee *et al.*, 1996; Clinical Laboratory Standard Institute, 2006).

Antibiotic susceptibility of microorganisms isolated from quantitative endotracheal aspirate (ETA) cultures was carried out using the Kirby–Bauer disk diffusion method, on Mueller–Hinton (Hi-media) agar plates by using the commercially available antibiotic

disks from Hi-Media (Mumbai, India) as per the Clinical and Laboratory Standard Institute (CLSI) guidelines (Clinical Laboratory Standard Institute, 2006; Clinical Laboratory Standard Institute, 2010).

The following antibiotics disk were used for Gram positive organisms - Ampicillin Sulbactam (10 units), Vancomycin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Penicillin G (10µg), Linezolid (10µg), Piperacillin/Tazobactam (100/10 µg), Imipenem (10 µg), Cefotaxime (30µg), Co-trimoxazole (5 µg), Clindamycin (10µg), Teicoplanin (30 µg), Chloramphenicol (30 µg), Erythromycin (15µg) and Cefoxitin (30µg) for MRSA.

For Gram negative organisms, the following antibiotics were used - Amoxicillin (30 mcg), Ofloxacin (5µg), Imipenem (10 µg), Meropenem (10 µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Tigecycline (10µg), Tobramycin (10µg), Gentamicin (10µg), Colistin (10µg), Piperacillin/Tazobactam (100/10µg), Chloramphenicol (30 µg) and Co-trimoxazole.

Quality control: Reference strains *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27953) and *S. aureus* (ATCC 25923) were used as control reference strains for identification and drug susceptibility testing (Clinical Laboratory Standard Institute, 2010; Jasuja *et al.*, 2017).

Detection of methicillin resistance in Staphylococcus: Staphylococcus species were further screened for methicillin resistance by using 30 µg cefoxitin disc by modified Kirby Bauer disc diffusion method as per the standard guidelines (Jasuja *et al.*, 2017). If the zone diameter < 21mm, then it was considered methicillin resistant *S. aureus* (MRSA) and methicillin resistant CoNS (MR-CoNS) if < 24mm.

Detection of Extended Spectrum Beta-Lactamase production (ESBL) in Gram negative bacilli: Disk-diffusion method for ESBL screening was performed by using cefpodoxime, ceftazidime, cefotaxime and ceftriaxone, and the isolates showing zone of inhibition to Ceftriaxone <25mm, Cefotaxime <27mm, Cefpodoxime <17mm and Ceftazidime <22mm in Mueller Hinton Agar (Hi-Media Laboratories, Mumbai, India) for ESBL detection. Phenotypic confirmatory test for ESBL producers was done by both combined disc test (CDT) and double-disc synergy test (DDST) for all isolates that were screened positive, following CLSI guidelines. The confirmatory test was done by potentiated disc diffusion method using ceftazidime alone on one end and ceftazidime with clavulanic acid kept 20 mm apart. An increase in the zone of inhibition of equal or more than 5mm around the ceftazidime-clavulanic acid combination was taken as positive.

Determination of multidrug resistance in bacterial isolates

The isolates were defined as multidrug resistant if they were resistant to two or more than two class of antibiotics tested or microorganisms which showed resistance to at least one antibiotic among three or more antimicrobial categories (Meligy *et al.*, 2015; Clinical Laboratory Standard Institute, 2010; Jasuja *et al.*, 2017).

Statistical analysis

Data so collected was analyzed by using Software Statistical Package for Social Sciences (SPSS) 16. The statistical testing was carried out by employing chi square test.

Ethical issue

Ethical approval was obtained from Institutional Ethical Committee before the beginning of the study.

Results and Discussion

In our study we observed a total of 101 ETAs culture positive patients in which 56 were males and 45 were females. Out of 101 positive cases, 44 (43.56%) belonged to 0 to 5 years of age followed by 31 (30.69%) in 6 to 15 yrs, 14 (13.86%) in 16 to 25 yrs, 8 (7.92%) in 26-50yrs and 04 (3.96%) in <50yrs age group. The age and sex demographical distribution pattern is shown in table 1.

In 101 culture positive isolates, the frequency of isolation of Gram-negative bacteria ($n=79$, 78.2%) was found to be higher than ($n=22$, 21.7%) Gram-positive bacteria. The most common micro-organism isolated was *Klebsiella pneumoniae* 28 (27%) followed by *Pseudomonas aeruginosa* 22 (22%), *Acinetobacter spp.* 11 (11%), *E. coli* 9 (9%), *Citrobacter* 5 (5%) and *Proteus* 4 (4%). The most frequently identified micro-organism in Gram positive bacteria was *CONS* 12 (12%), subsequently followed by 8 (8%) *Staphylococcus aureus* and 2 (2%) *Streptococcus spp* (Figure 1).

Antibiotic resistance pattern among Gram positive bacteria: Gram positive bacteria were highly resistant to penicillin G (86%) which included *S. aureus* and CoNS which showed 87% and 91% resistance respectively.

Staphylococcus aureus showed high degree of resistance to gentamicin (75%) followed by clindamycin and amoxicillin clavulanic acid (62% each), co-trimoxazole (50%), ampicillin sulbactam and azithromycin (37% each) and least resistance was found to teicoplanin and linezolid (12.5%) followed by vancomycin, ciprofloxacin and tetracyclin (25% each). CoNS were highly resistant to clindamycin (83%) followed by gentamicin (75%), amoxicillin clavulanic acid (66%), tetracycline (58%) ciprofloxacin (50%), co-trimoxazole and azithromycin (41%) and least resistance

was found to teicoplanin (8%) followed by vancomycin (16%), ampicillin-sulbactam (16%). However no resistance was detected to linezolid among *CoNS*. (Table 2) shows the antibiotic resistance pattern of isolates in Gram positive bacteria.

Methicillin resistance was observed in 61% of *Staphylococcus aureus* isolates and 53% of *CoNS* isolates by the Cefoxitin disc diffusion method.

Antibiotic resistance pattern among Gram negative bacteria: The highest resistance were found in two major beta-lactam antibiotic groups including 3rd generation cephalosporins and Penicillin group antibiotics, the least resistance was found to carbapenems and polymyxin B.

Klebsiella pneumoniae showed maximum resistance to third generation cephalosporins including cefotaxime (85%), cefoperazone (82%), ceftazidime (75%). Subsequently followed by amoxicillin clavulanic acid (68%), gentamicin (57%), ampicillin and tetracycline (50% each), ciprofloxacin (46%), tobramycin (42%) and least resistance to imipenem (7%) and polymyxin B (39%). *E. coli* was highly resistant to cefotaxime (77%), cefoperazone and amoxicillin clavulanic acid (66% each). ceftazidime, piperacillin/tazobactam and gentamycin (55%) showed similar resistance pattern and lowest resistance were found in imipenem and polymyxin B (33%). In *Citrobacter spp.* high resistance was found in cefoperazone (80%) and similar resistance to cefoperazone, cefotaxime and gentamicin (60%) and least to imipenem, ampicillin and tobramycin (20% each). *Proteus spp* also showed high resistance to cefoperazone and ceftazidime (75%) equally and least to imipenem (20%). In non-lactose fermenters *Acinetobacter spp* showed high resistance to cefoperazone (90%), ceftazidime (81%), cefotaxime (72%) and amoxicillin

clavulanic acid (81%) and least to imipenem (18%) and polymyxin B (27%). Similarly *Pseudomonas aeruginosa* also showed high resistance to cefoperazone and amoxicillin clavulanic acid (68%), and least to imipenem (4%) and polymyxin B (18%). Most of the Gram negative organisms were highly resistant to amoxicillin and cephalosporins and none of the isolates were found resistant to colistin (Table 3).

The Gram negative isolates (79) were tested for ESBL production, among which 47 (59%) were ESBL producers.

The percentage of beta lactamase producing organisms is as follows: *Klebsiella pneumoniae* 17/28 (60.71%), *E. coli* 5/9(55.55%), *Citrobacter spp.* 3/5(60%), *Proteus spp.* 2/4 (50%) and in non-lactose fermenters (NLFs), *Acinetobacter baumannii* 7/11 (63.63%), *Pseudomonas aeruginosa* 12/22 (54.54%).

Respiratory tract infections due to the mechanical ventilation or tracheal intubation are the most important and leading cause of morbidity in ICU, NICU and emergency. The key treatment is early identification of the causative microbial agents, antibiotic sensitivity profile and administration of selected antibiotics. Endotracheal tubes are susceptible to impaction and attachment of micro-organisms therefore it is important to be aware of the relevant factors and responsible organisms to take prompt action.

In this study, 101 positive microbial cultures obtained from the ETAs or tracheostomy cases were considered for the identification of pathogens and their antibiotic resistance pattern. In our study, of all the culture positive patients 56 were males and 45 were females. Joseph *et al.*, also reported quite similar gender ratio in his prospective observational study (Joseph *et al.*, 2010).

Figure - 1: Isolated microorganisms from intubated patients of ICU, CCU, NICU, PICU at BRD Medical College Gorakhpur.

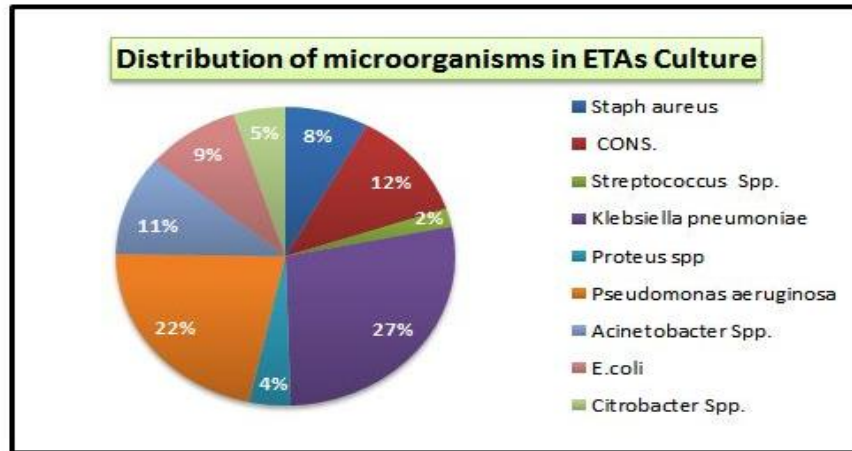


Table.1 Demographic distribution of ETA positive culture patients

AGE GROUP	MALE	FEMALE	TOTAL
0-5 YEARS	28	16	44
6-15 YEARS	13	18	31
16 - 25 YEARS	08	06	14
26 - 50 YEARS	05	03	08
<50 YEARS	02	02	04
TOTAL	56	45	101

Table.2 Antibiotic resistance pattern among Gram positive bacteria

MICRO.	AMC	AZM	CI	PG	TE	COT	LZ	TP	VM	AM	CM	GEN
<i>Staphylococcus aureus</i> (n=8)	5	3	2	7	2	4	1	1	2	3	5	6
<i>CONS.</i> (n=12)	8	5	6	11	7	5	0	1	2	4	10	9
<i>Streptococcus spp.</i> (n=2)	1	0	1	1	0	1	0	1	1	0	0	1
Total (n=22)	14	8	9	19	9	10	1	3	5	7	15	16

Table.3 Antibiotic resistance pattern among Gram negative bacteria

Micro.	AMC	AK	CFS	CE	CAZ	PT	TE	IPM	CI	TB	GEN	PB
<i>Klebsiella pneumoniae.</i> (n=28)	20	14	23	24	21	15	14	2	13	12	16	11
<i>E.coli</i> (n=9)	6	3	6	7	5	5	4	1	4	2	5	3
<i>Proteus spp.</i> (n=4)	2	1	3	2	3	2	1	1	3	2		2
<i>Citrobacter spp.</i> (n=5)	2	1	3	3	4	3	2	1	2	1	3	2
<i>Acinetobacter spp.</i> (n=11)	9	5	10	8	9	6	6	2	6	5	7	3
<i>Pseudomonas aeruginosa</i> (n=22)	15	8	16	15	13	12	12	1	14	6	8	4
Total (n=79)	54	31	61	59	55	43	39	8	42	28	42	25

We found the frequency of isolation of Gram-negative bacteria to be higher than Gram-positive bacteria, similarly Abdollahi *et al.*, and Ferreira *et al.*, had also shown high frequency of Gram-negative microorganisms isolated in ETAs culture (Ferreira *et al.*, 2016; Abdollahi *et al.*, 2013). In our study, the most common micro-organism was *Klebsiella pneumoniae* among all isolates followed by *Pseudomonas aeruginosa*, *Acinetobacter spp.*, *E. coli*, *Citrobacter spp.* and *Proteus*. The most frequently identified micro-organism in Gram positive bacteria was *CONS* subsequently followed by *Staphylococcus aureus*. This finding is in accordance with the results of Bassant *et al.*, who found that the most prevalent isolates in quantitative ETA cultures were GNBs (77.3%) such as *Klebsiella*, *Acinetobacter*, *Pseudomonas* and *Proteus spp.*, whereas Gram-positive organisms accounted for 22.7% represented by *CONS* (Meligy *et al.*, 2015). Amini *et al.*, also conducted a descriptive study showing similar distribution of isolated microorganisms (Amini *et al.*, 2009).

Our studies as well as the other ones in similar settings confirm that *Klebsiella pneumoniae*, *P.aeruginosa* and *CoNS* are among the most 3 prevalent isolated organism from endotracheal tube aspirate (Jasuja *et al.*, 2017, Abdollahi *et al.*, 2013; Amini *et al.*, 2009).

It is important to understand that although there are minor variations in relative frequency of obtained organisms, different studies have reported varying sources of isolations upon different geographical area and applied methodology.

In the current study, the frequency of polymicrobial infection was present in 7 (6.93%) patients. Several researchers estimated that polymicrobial infections accounted for 20–60% of patients (Wilson *et*

al., 2012).

Gram positive bacteria were highly resistance to penicillin G, gentamycin and clindamycin least resistance were found to teicoplanin, linezolid and vancomycin. Methicillin resistance was observed in 61% of *Staphylococcus aureus* and 53% in *CoNS*. In this respect, our study was quite similar to the other studies, Bassant *et al.*, reported methicillin resistance was detected in 60% of GPC isolates. All isolates were 80% resistant to penicillin G and 100% resistant to clindamycin. Another study Abdollahi *et al.*, claimed that 84% Gram positive organism including *Staphylococcus aureus* and *CoNS* were resistant to cefoxitin and showed increasing frequency of multidrug resistance in GPC which represent the emergence of MDR in MV patients (Meligy *et al.*, 2015, Abdollahi *et al.*, 2013).

Antibiotic resistance pattern among Gram negative bacteria: the highest resistance were found in two major beta-lactam antibiotics including 3rd generation cephalosporins and Penicillin group of antibiotics. All the lactose fermenting Enterobacteriaceae were highly resistant to cephalosporins including cefoperazone, cefotaxime and ceftazidime similarly non lactose fermenters *Pseudomonas aeruginosa* and *Acinetobacter spp* also showed high resistance to cefoperazone and amoxicillin clavulanic acid and least to imipenem and polymyxinB.

Among Gram negative isolates tested for ESBL production; 50 out of 79 (63%) were ESBL producers. Our findings were similar when compared to the Kiran Tandia *et al.*, and Abdollahi *et al.*, who reported high degree of resistance in cephalosporins and Penicillin group of antibiotics in their studies (Tandia *et al.*, 2015, Abdollahi *et al.*, 2013).

The micro-organisms that have been documented to colonize ETTs and grow in the

form of a biofilm are numerous, including the multidrug-resistant bacteria MRSA and Gram-negative bacilli such as *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *Acinetobacter spp.* Therefore, making effective antibiotic policy directed at reducing such colonization could help to reduce the rates of antibiotic-resistant infections.

Our observations provide useful information regarding the microbiology of respiratory infection occurring in intubated patients. In the present study, antimicrobial resistance rate was alarming in the culture of ETA isolates obtained from MV patients. This study also highlighted the importance of using empirical antimicrobial therapies to target multidrug-resistant pathogens at early-onset VAP. Therefore, ETA culture provides accurate and reliable information in respect to early treatment strategy, swift administration of antibiotics as per susceptibility results and should be routinely included as a part of management protocol.

Thus ET tip cultures could be a useful adjunct to the ATS (American Thoracic Society) strategy for ensuring appropriate treatment of intubated patients and in the context of a bundled approach to the prevention of ET tip infections.

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Source of Support

Nil

Conflict of Interest

None declared.

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