

## Early detection of ventilator-associated pneumonia using quantitative endotracheal aspirate cultures

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**Abstract:** Ventilator associated pneumonia is commonly seen in patients in ICU, it results in increased costs of the treatment and increase in duration of hospitalization of patients. The objective of the present study was to determine the prevalence and antibiotic susceptibility profile of bacteria colonizing the endotracheal tubes in ICU. The study was carried out for a period of 5 years from Jan 2012 to Nov 2016. Data of 254 cultures were collected the sensitivity of cultures were recorded and analyzed. Endotracheal aspirates (ETA) and quantitative ETA tip cultures with a threshold of  $10^5$  to  $10^6$  bacteria per milliliter of exudates that is considered as optimal/ significant. Culture pairs were assessed for change in (1) species of bacteria isolated and (2) change in empiric antibiotic coverage. The results were analyzed using appropriate statistical methods. In 254 positive Cultures, the most frequently isolated organism was *Acinetobacter baumannii* (29.9%) followed by *Klebsiella pneumonia* (25.94%), *Pseudomonas aeruginosa* (18.11%), *E.coli* (8.25%). The total number of intubated cases complicated to Ventilator associated pneumonia ( VAP ) were 54 cases ( 21.65% ) VAP associated mortalities – 10 cases ( 18 .18 % ). Endotracheal aspiration is a simple, cost effective and it has proven to be highly effective as a diagnostic Technique. It is important to understand that Endotracheal tubes are susceptible to infections. Clinicians need to be aware of it and take prompt action if infection develops. Certain measures can reduce the risk of infections that includes early discontinuation of invasive devices, reduce intubation rates, recumbent patient positioning 30-45°, use of prophylactic probiotics.

**Keywords:** Ventilator associated pneumonia, endotracheal aspirates, culture

## INTRODUCTION

Ventilator-associated pneumonia (VAP) refers to bacterial pneumonia that developed in patients who have been mechanically ventilated for duration of more than 48 hours. It ranges from 6 to 52% and it can reach up to 76% in some specific settings [1]. It is the second most common nosocomial infection in the intensive care unit (ICU) and the most common in mechanically ventilated patients. Ventilator-associated pneumonia (VAP) is associated with increased mortality, morbidity, and medical costs. The costs of management are increased when infection involves resistant organisms, and due to unnecessary and prolonged therapy. Efforts at an accurate diagnosis, therapy and prevention can reduce the cost burden of this illness.

Nosocomial pneumonia is the most commonly acquired infection in hospitalized patients, particularly those on mechanical ventilators in the intensive care unit. The impact of this infection is dramatic, increasing mortality, length of stay, and utilization of resources such as mechanical ventilation and antibiotic therapy. Nosocomial pneumonia increases the hospital costs per episode and can increase hospital length of stay by as much as 14 days [2]. In spite of aggressive, accurate therapy, some patients die as a direct result of pneumonia and it is difficult to put a cost value on this endpoint. One of the most important types of this infection is pneumonia which commonly occurs in relation to the endotracheal intubation and mechanical ventilation named ventilation associated pneumonia (VAP)

The presence of an endotracheal tube is the most important risk factor, resulting in a violation of natural defense mechanisms against micro aspiration around the cuff of the tube. Infectious bacteria obtain direct access to the lower respiratory tract via: (1) micro aspiration, which can occur during intubation itself; (2) development of a biofilm laden with bacteria (typically Gram-negative bacteria and fungal species) within the endotracheal tube; (3) pooling and trickling of secretions around the cuff; and (4) impairment of mucociliary clearance of secretions with gravity dependence of mucus flow within the airways [3]. Pathogenic material can also collect in surrounding anatomic structures, such as the stomach, sinuses, nasopharynx, and oropharynx, with the replacement of normal flora by more virulent strains. This bacterium-enriched material is also constantly thrust forward by the positive pressure exerted by the ventilator. Whereas reintubation following extubation increases VAP rates, the use of non-invasive positive pressure ventilation has been associated with significantly lower VAP rates. Host factors such as the severity of underlying disease, previous surgery, and antibiotic exposure have all been implicated as risk factors for development of VAP [4].

**MATERIALS AND METHODS**

This study was undertaken in Medicity Institute of Medical Sciences Hyderabad. Institutional Ethical committee permission was obtained for the study. Data was collected from Jan 2012 and Nov 2016. Media -Blood agar, MacConkey agar, Chocolate agar, Chromagar, soya casein digest broth. ET tip culture: Using a sterile forceps, remove ET tip from transport tube, Rolled the tip back and forth across the entire surface of as BAP, CHROM in addition SCDA using sterile forceps and exerting slight downward pressure. If the tip is too long, using sterile scissors, cut the end

closest to the top of the tube (proximal end) prior to rolling on the plates. The proximal end may be rolled on a second plate if desired. Clinical sample was diluted in 1 in 100 or 1 in 1000 and Gram stain examination was performed using 100 µl of the fluid, subsequently 10 µl of diluted sample was uniformly inoculated on to blood agar, chocolate agar, and McConkey agar. Samples with large mucus plugs were liquefied and homogenized by vortexing for one minute with glass beads followed by centrifuging at 3000 rotations per minute for 10 minutes. The plates were incubated at 35°C - 37°C later number of colonies were counted on each plate and multiplied by the appropriate dilution factor to express the colony count as CFU/ml. The quantitative plates were read at 24 and 48hrs and if after 24 hours no growth was observed, further incubated for 24 hours and observed for growth. If growth was seen, performed gram's staining The organisms isolated from ET culture were identified by standard conventional methods and negative breakpoint combo 42 & identification by walkaway 96 s fully automated for identification & sensitivity by MIC values. Comparatively, the antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines [5] An isolate was considered as MDR if found resistant to three or more antimicrobials belonging to different classes/groups of antimicrobials.

**RESULTS**

The most commonly isolated organism was Acinetobacter Baumanii in 76 cases (29.9%) followed by Klebsiella Pneumoniae in 66 patients (25.94%) of the patients and Pseudomonas Aeruginosa 46 patients (18.11%) other isolated organisms are shown along with their percentage in table 1.

**Table-1: Showing the number of Organisms isolated and their percentage.**

Sl. No	Organism	Number	Percentage
1.	Acinetobacter Baumanii	76	29.9
2.	Klebsiella Pneumoniae	66	25.94
3.	Pseudomonas Aeruginosa	46	18.11
4.	Escherichia Coli	21	8.25
5.	Burkholderia Cepacia	5	1.96
6.	Stenotrophomonas Maltophilia	1	0.39
7.	MRSA	3	1.17
8.	Staphylococcus Hemolyticus	2	0.78
9.	OTHERS	34	13.36
10.	Total	254	100

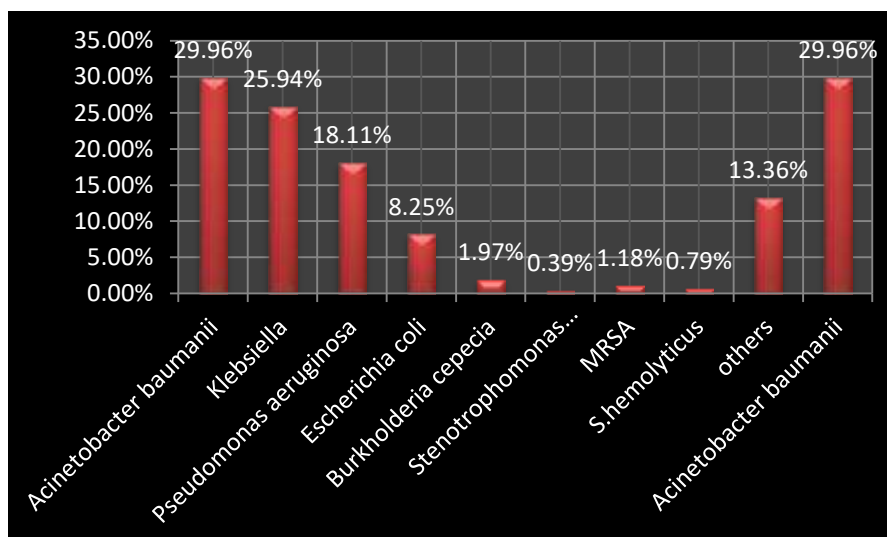


Fig-1: Percentage prevalence of each isolate

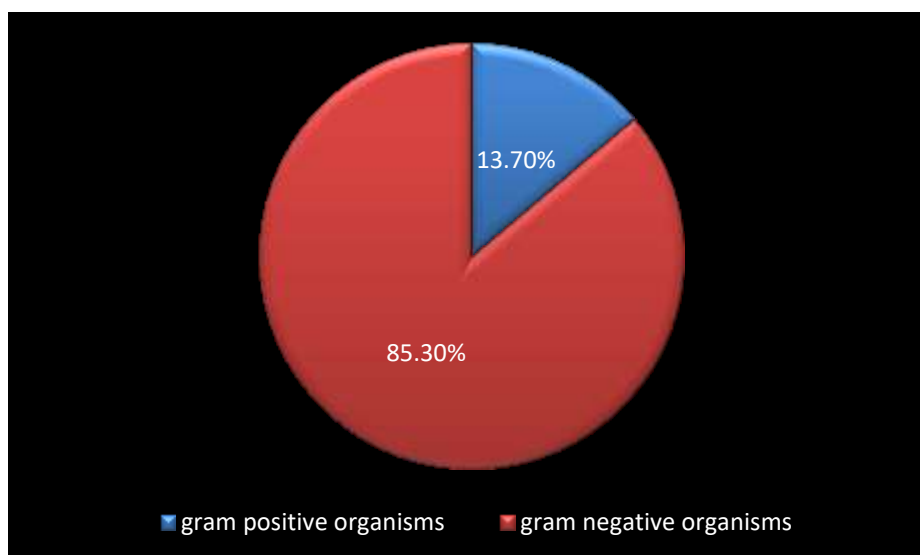


Fig-2: percentage prevalence of gram positive & gram negative organisms (excluding candida spp)

The gram positive organisms involved in various cases were 13.07% and gram negative organisms isolated were 85.3% given in figure 2.

Comparatively the antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines. The isolates were tested for ampicillin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), amoxicillin-clavulanic acid (10/20 µg), co-trimoxazole (1.25/23.75 µg), cefepime (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), piperacillin-tazobactam (100/10 µg) and imipenem (10 µg) (Hi-mediA). After adding

inoculums of 0.5 McFarland turbidity standards, specified antibiotic discs placed 2 cm apart from each other with sterile forceps and were incubated for 16-18 hours at 37°C aerobically. The degree of sensitivity was determined by measuring zone of growth inhibition around the disc. The growth of bacterium would be inhibited around the discs containing antibiotics to which the bacterium is susceptible, while no inhibitory zone around resistant ones. The results were interpreted as sensitive, intermediately sensitive and resistant to the different drugs. The zone of inhibition was interpreted according to the Kirby-Bauer antibiotic sensitivity chart [6]. The susceptibility of common micro-organisms are given in table 3, 4, 5 and 6 below.

**Table-3: showing susceptibility percentage of Acinetobacter baumannii in ET culture**

Antibiotics	Number (N = 76)	Percentage susceptibility
Ampi/sulbacatam	4	5.26%
Amikacin	9	11.84%
Ceftriaxone	2	2.63%
Levofloxacin	15	19.73%
Cotrimoxazole	3	3.98
Tobramycin	4	5.26%
Pip / tazo	6	7.89%
Tetracycline	3	3.9%
Imipenem	17	22.35%
Meropenem	17	22.35%
Colistin	76	100%

**Table-4: showing the susceptibility profile of Klebsiella in ET culture**

Antibiotics	Number N = 66	Percentage susceptibility
Ampi/sulbacatam	2	3.03%
Amikacin	36	54.5%
Augmentin	13	19.7 %
Ceftriaxone	9	13.63%
levofloxacin	20	30.3%
Ciprofloxacin	5	7.5 %
Cotrimoxazole	11	16.7%
Tobramycin	16	24.2%
Gentamycin	22	33.3%
Pip / tazo	28	42.4%
Tetracycline	19	28.8%
Imipenem	55	83.3%
Meropenem	55	83.3%
Colistin	76	100%

**Table-5: Susceptibility profile of Pseudomonas in ET culture**

Antibiotics	Number N = 46	Percentage susceptibility
Ceftazidime	13	28.2%
Amikacin	25	54.3%
Cefepime	16	34.7%
levofloxacin	16	34.7%
Ciprofloxacin	7	15.2%
Gentamycin	15	3.98
Tobramycin	16	34.7%
Pip / tazo	20	43.3%
Imipenem	32	68.4%
Meropenem	30	65.1%
Colistin	46	100%

**Table-6: Susceptibility profile of Escherichia coli in ET culture**

ANTIBIOTICS	Number N = 21	Percentage susceptibility
Augmentin	1	4.76%
Amikacin	14	66.6%
Ceftriaxone	1	4.7%
levofloxacin	2	9.5%
Cotrimoxazole	4	19%
Tobramycin	5	23.8%
Pip / tazo	4	19.04 %
Tetracycline	2	9.52%
Imipenem	18	85.7%
Meropenem	18	85.7%
Colistin	76	100%

The numbers of patients showing colonization in 254 cases were 189 (74.4%) and the number of cases progressing to Ventilator acquired pneumonia were 55

(21.65%) a number of patients with mortality were 10 (18.18%) given in table 7.

**Table-7: Number of cases complicating to ventilator associated pneumonia**

Total no of cases showing colonization	189	74.4%
Total no of cases progressing to VAP	55	21.65%
Total no of cases with mortality	10	18.18 %

## DISCUSSION

In our study males (77.5%) were significantly affected when compared to females (22.47 %), incidence of gram negative organisms (85.3 %) were more than gram positive organisms (13.7 %) Foglia *et al.* [7] found in their study, in children most common etiologies of HABP and VABP were *P. aeruginosa* (25.2%- 38.4%), other gram-negative bacilli (26.6%-51.4%), and *S. aureus* (14.6%-28.4%). In our study we found the predominant isolate was *Acinetobacter baumannii* spp (29.9%) and greater than 78% of the strains were XDR *Acinetobacter* spp showing resistance to all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones, and aminoglycosides.

VAP cases due to *Acinetobacter* spp were 36.4 % (20 cases) with rest of them as colonizers, numbers of VAP cases with *Acinetobacter* spp causing mortality were 10 (18.18 %), most of the mortalities were due to combined pathogenesis of the predominant species *Acinetobacter* spp and *Klebsiella pneumoniae* spp. It was observed that a coinfection of *Acinetobacter* spp with *Klebsiella pneumoniae* showed more complications and mortalities. More than 72% strains of *Klebsiella pneumoniae* spp were ESBL'S and 40% of the cases were multidrug resistant, showing resistance to all betalactam /lactamase inhibitor combinations, Aminoglycosides, quinolones 65% of *Pseudomonas aeruginosa* spp isolated were MBL producers showing resistance to Carbapenems tested, these isolates showed 100% susceptible to colistin and polymyxin B [8]. The accurate diagnosis of newly developed pneumonia is

difficult in patients with an endotracheal tube or tracheostomy [9] since many other conditions, such as, tracheobronchitis pulmonary edema, and atelectasis, can mimic pneumonia [10]. Therefore bacteriologic tests are necessary to confirm pneumonia; Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) guideline is that samples of lower respiratory tract secretions should be obtained from all patients with suspected hospital acquired pneumonia (HAP) which should be collected before antibiotic changes [11]. Samples can include an endotracheal aspirate, bronchoalveolar lavage sample, or protected specimen brush sample. But qualitative endotracheal aspirate (EA) culture cannot differentiate colonization from infection. Although quantitative culture of Broncho alveolar lavage (BAL) fluid obtained by bronchoscopy can accurately diagnose pneumonia, this procedure is invasive and cannot be utilized in some patients, especially in those who are critical quantitative EA culture is non-invasive, easily learnt, and cheaper than quantitative BAL fluid culture, and previous results have suggested that EA can be used as a substitute for BAL in quantitative cultures. However, this issue is controversial and result thresholds have not been determined [12]. Furthermore, little data is available on quantitative EA cultures in India, or on the clinical applications of this methodology. Accordingly, we evaluated the clinical usefulness of quantitative EA cultures in intensive care unit (ICU) patients with pneumonia and sought to determine the result threshold level for positivity.

The impact of pneumonia can be even more dramatic if it involves a multi-resistant Gram-positive organism (such as methicillin-resistant *Staphylococcus aureus*) or a Gram-negative organism such as *Pseudomonas aeruginosa* or *Acinetobacter* spp. Resistant organisms can add to costs in a number of ways. First, since patients who acquire these organisms are already very ill and the availability of effective therapy is limited, pneumonia due to resistant organisms can lead to a higher mortality and length of stay than pneumonia due to antibiotic-sensitive organisms & in addition also can help spread these infections to adjacent patients [2]. Various studies have proposed different causative microorganism as the most common etiology for intubation related respiratory infections including *A. baumannii*, *Pseudomonas aeruginosa*, other *pseudomonas* spp, *Klebsiella pneumoniae* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in patients Which in return adds to cost of diagnosis, cost of treatment, cost of corrective and preventive actions.

## CONCLUSION

Endotracheal aspiration is a simple, cost effective and it has proven to be highly effective as a diagnostic Technique. It is important to understand that endotracheal tubes are susceptible to infections. Clinicians need to be aware of it and take prompt action if infection develops. Certain measures can reduce the risk of infections that includes early discontinuation of invasive devices; reduce intubation rates, recumbent patient positioning 30-45°, use of prophylactic probiotics.

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