



### Original Research Article

## A Study on Microbiological and Antibiotic Susceptibility Profile of Endotracheal Aspirate in Patients Suspected of Ventilator-Associated Pneumonia in a Tertiary Care Hospital

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### Abstract

**Background:** Respiratory infections in critically ill patients are associated with high morbidity and mortality. Patients who are mechanically ventilated are at high risk of acquiring respiratory tract infections due to a complex interplay between the endotracheal tube, host immunity, and virulence of invading bacteria. To initiate empiric antimicrobial therapy knowledge of local antimicrobial resistance patterns are essential.

**Aim:** The aim of our study was to identify the bacterial pathogens and their antibiotic susceptibility of endotracheal aspirate samples collected from the patients on mechanical ventilation for more than 48 hours.

**Materials and Methods:** This prospective study was done at Saveetha Medical College and Hospital, Chennai. One hundred and seven samples were collected, bacteriological identification and antibiotic susceptibility were tested for pathogen grown in endotracheal cultures received.

**Results:** Culture was positive in sixty-one out of one hundred and seven samples (i.e., 57% of all the samples) out of which gram-negative bacteria accounted for (59 out of 61) 96.72% cases and rest 2 cases were *Candida* spp. *Pseudomonas* spp 37% (22 out of 61) was the commonest organism isolated followed by *Klebsiella* spp 33% (20 out of 61), *Acinetobacter* spp 20% (12 out of 61), *Escherichia coli* 3.3% (2 out of 61), *Enterobacter* spp 3.3% (2 out of 61) and *Proteus* spp 1.6% (1 out of 61). ESBL rate was highest among *Acinetobacter* spp (100%) followed by *Klebsiella* spp (40%) and *Pseudomonas* spp (27%). Most of the *Acinetobacter* spp 83% (10 out of 12) were Multidrug-resistant whereas five isolates of each of *Klebsiella* spp (25%) and *Pseudomonas* spp (23%) were found to be MDR and sensitive to only colistin and tigecycline. Fungal isolates consisted of one case of *Candida albicans* and *Candida non-albicans* each.

**Conclusion:** Gram-negative bacteria especially *Pseudomonas* spp, *Klebsiella* spp, *Acinetobacter* spp are most commonly encountered pathogens isolated in endotracheal samples of ventilator-associated pneumonia patients. Among these pathogens, *Acinetobacter* spp. shows very high ESBL positivity, while these characteristics are moderately expressed in *Klebsiella* spp and less commonly in *Pseudomonas* spp. Multidrug resistance also followed the same trail among these common pathogens.

**Keyword:** Bronchoalveolar lavage, Endotracheal aspirates, Extended Spectrum Beta-Lactamase (ESBL), Multidrug-resistant.

## Introduction

Endotracheal intubation is an important procedure for life-threatening conditions. The uses of invasive therapeutic procedures have saved many lives but it can also cause life-threatening consequences due to severe persistent resistant infections.<sup>[1]</sup> The invasive therapeutic and diagnostic methods have increased the incidences of nosocomial infections, particularly in ICU's.<sup>[1,2]</sup>

The endotracheal tube (ETT) appears to be an independent risk factor for VAP by impairing mucociliary clearance, disrupting the cough reflex, and promoting the accumulation of tracheobronchial secretions in the lung.<sup>[3-5]</sup>

Ventilator-associated pneumonia (VAP) is the second most common hospital-acquired infection (HAI), accounting for 15% of HAIs and has the highest morbidity and mortality.<sup>[6]</sup> A large number of bacteria are found to be causative factors for colonization that may later proceed to infection.<sup>[7]</sup> Gram-negative bacteria are predominant ones. Most common gram-negative bacteria are *Pseudomonas aeruginosa*, *Acinetobacter spp.* and *Klebsiella pneumoniae*.<sup>[8,9]</sup>

Prolonged hospital stay and colonization with *Pseudomonas* species itself is an independent risk factor for gram-negative bacterial infection. The longer the hospital stay is as in case of head injury, the more are the chances of a change of bacterial flora causing infection, thereby complicating therapy because of alteration of susceptibility pattern.<sup>[10,11]</sup> Contaminated secretions from the oropharynx and the gastrointestinal tract are also a source of VAP through repeated micro aspirations.<sup>[4]</sup>

Respiratory infections in critically ill patients are associated with high morbidity and mortality.<sup>[5]</sup>

Rapid diagnosis and initiation of appropriate antibiotic therapy are essential for better outcomes. Patients who are intubated and mechanically ventilated are further at risk of acquiring respiratory infections due to the complex interplay between the endotracheal tube, host immunity, and virulence of invading bacteria. The etiological agents may vary according to the

population of patients in an ICU, duration of hospital stay, pre-existing illness and prior antimicrobial therapy.<sup>[3]</sup> The common problem however as noted in other studies is that bacterial pathogens from tracheal aspirates tend to be a multidrug-resistant.<sup>[7]</sup> There is much well-documented evidence that hospital personnel and environment are the microbial sources and prolonged hospital stay and overuse of antimicrobial agents has led to multidrug resistance of these microbes.<sup>[9]</sup> In the hospital, endotracheal aspiration for the diagnosis of nosocomial infection is common in patients admitted to the ICU.<sup>[8]</sup> Therefore updated knowledge of local epidemiological and susceptibility profile is recommended for guiding the clinicians regarding the empirical choice of antibiotics and has become mandatory along with adequate clinical diagnosis and bacterial confirmation.<sup>[11]</sup> Hence the aim and objective of the study were to analyze the spectrum of aerobic bacteria and their antibiotic sensitivity pattern isolated from endotracheal aspirates of ventilated patients in Intensive Care Units.

It is very essential for the clinicians to be aware of local bacteriological flora and their susceptibility pattern to encourage rational use of antibiotics. The aim of this study was to identify the microbiological profile of the endotracheal secretions in patients under mechanical ventilation for more than 48 hours and to study their antimicrobial sensitivity pattern.

## Materials and Methods

This was a prospective study done in Saveetha Medical College and Hospital, Chennai for a period of one year from January 2017 to December 2017. A total of 107 samples of endotracheal tube aspirate were received from the ICU department and were processed.

## Endotracheal Aspirate

Sampling was done by introducing a catheter aseptically through the endotracheal tube and secretions aspirated into a sterile syringe. The endotracheal tube tip was cut aseptically into a

sterile container and sent to the Microbiology laboratory.

**Bacterial isolation**

Continuous sample collection technique is used. Endotracheal (ET) aspirates and ET-tip samples were transported to the Microbiology laboratory in a sterile container and processed immediately. After initial Gram staining, samples were plated on blood agar, MacConkey agar, and chocolate agar, and incubated at 37°C for 24 hours. Any significant growth was identified and antibiotic susceptibility test was done by Kirby Bauer disc diffusion method in MHA plate.<sup>[12]</sup>

**Bacteriological identification**

This was done by following standard methods.<sup>[13]</sup> Antimicrobial susceptibility of bacterial isolates

was done following CLSI guidelines.<sup>[12]</sup> ESBL screening was done as per CLSI guidelines and confirmed by combined disc method.

**Results**

Among 107 ET samples, 61 samples were culture positive (fig: 1). Culture positivity was more in samples taken from male (39/61) compared to females (22/61). Age wise distribution of positive cultures was maximum among elderly patients >60 yrs. Out of 61 positive cultures, 59 (96%) showed growth of gram-negative organisms. The remaining 2 cultures showed fungal growth consisting of one case of *Candida albicans* and *Candida nonalbicans* each.

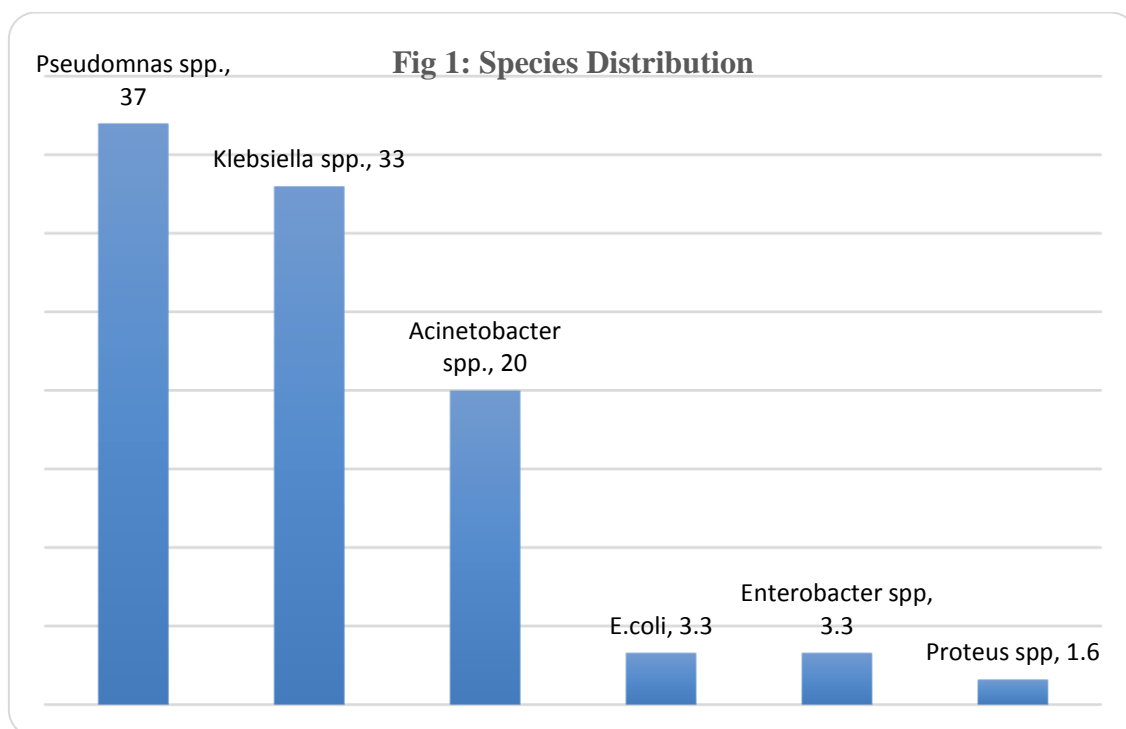


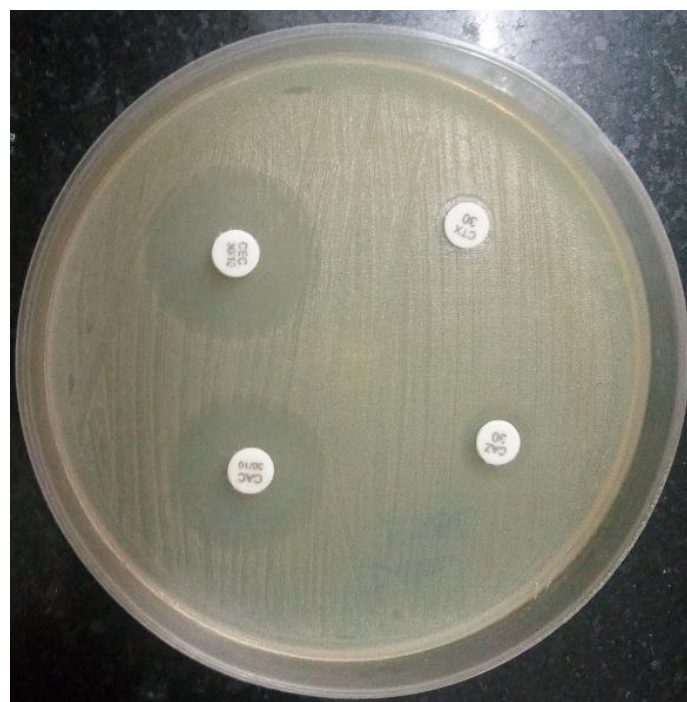
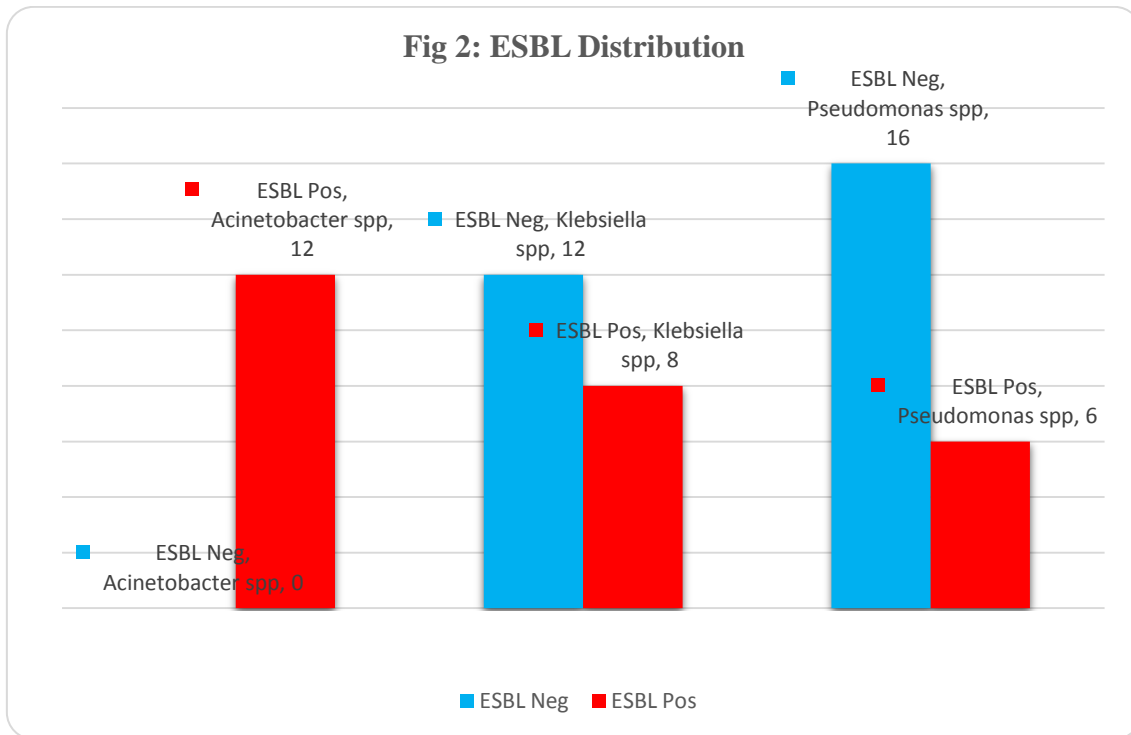
Fig 1 shows predominant species isolated from ET samples. Among gram-negative culture isolates of ET, ESBL rate was highest among *Acinetobacter*

*spp* 100 % followed by *Klebsiella spp* 40% and *Pseudomonas spp* (27%) (Fig 2) and (table: 1).

**Table 1: ESBL Distribution**

Organisms	ESBL Producers	ESBL non-producers
<i>Acinetobacter spp</i>	12	0
<i>Klebsiella spp</i>	8	12
<i>Pseudomonas spp</i>	6	16

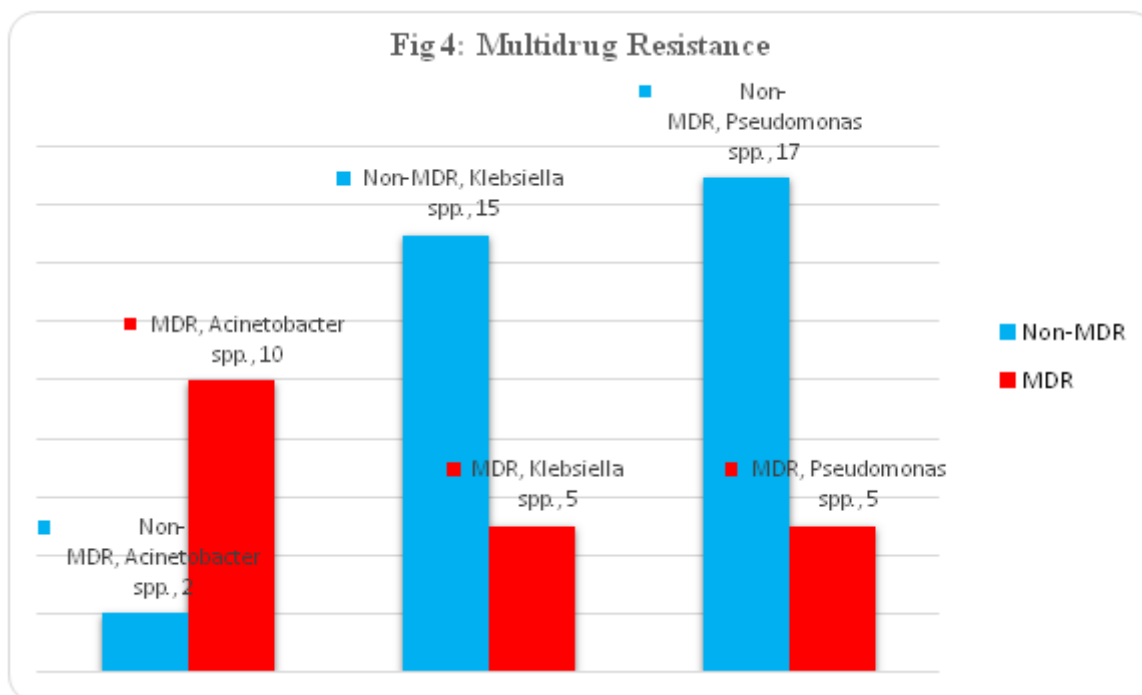
All the ESBL positive isolates (fig: 2, 3) were recovered from patients with a prolonged hospital stay of more >3 days.



**Fig 3: ESBL production by combination disc method**

In this study, most of the multidrug-resistant (fig: 4) *Acinetobacter spp*, *Klebsiella spp*, and *Pseudomonas spp*. were sensitive only to colistin and tigecycline (fig: 3,4).

All the MDR organisms were isolated from patients with multiple comorbid conditions and on hospitalization >4 days.



## Discussion

There have been sporadic reports of ESBL in India and some of them have recorded the incidence to be as high as 60-68%. ESBL rate in our study was 43% (26/61) which is almost similar to various other studies done elsewhere.<sup>[14]</sup> The comparatively lower rate of our hospital can be justified by the fact that good infection control practices are followed here.

We observed a higher rate of culture positivity from the samples of male patients than those obtained from the female patients. Similar findings were observed by Morehead et al<sup>[15]</sup> in which it was found that culture positivity was more common in elderly male patients as they have more pre-existing lung diseases and incidence of smoking is more in males compared to females.

The duration of stay differed between stable patients and the patients with comorbid conditions. Patients with comorbid conditions like diabetes, asthma, chronic obstructive pulmonary disease, heart diseases, trauma, and patients with mechanical ventilation had a longer hospital stay of 10-15 days. All the ESBL positive isolates were recovered from patients with a prolonged hospital stay of more than 3 days.

In our study, gram-negative bacilli were the most commonly isolated pathogen from ET aspirates which is similar to studies done by Fagon et al<sup>[16]</sup> and Şimşek et al<sup>[17]</sup>. In our study, *Pseudomonas spp* 37% (22/61) was the commonest organism isolated followed by *Klebsiella spp* 33% (20/61) and *Acinetobacter spp* 20% (12/61), which is similar to Summaiya et al<sup>[18]</sup> who found that *Pseudomonas aeruginosa* and *Acinetobacter* species were the most common organisms isolated in endotracheal tube. Trilok Patil et al<sup>[19]</sup>, also reported that in their study, *Pseudomonas aeruginosa* was the most commonly isolated organism, followed by *Klebsiella pneumoniae*. Panda et al<sup>[20]</sup> in their study noted that *Acinetobacter spp* was the most common organism (43%) to cause VAP and this was followed by *Pseudomonas spp* (34%). The predominance of *Pseudomonas spp* can be explained by the fact that these patients on the ET tube had more chances of infection by biofilm producing bacteria like *Pseudomonas spp*. This is quite similar to the study by George et al<sup>[21]</sup> where they found that *Acinetobacter spp* was the most common isolate (37.5%), followed by *Pseudomonas spp* (21.8%) and *Klebsiella spp* (15.6%). Amikacin, Gatifloxacin, and Imipenem

were the common sensitive antibiotics in their study.

Chatterjee et al<sup>[22]</sup> found that out of twenty-one isolates of *Acinetobacter baumannii*, four (19%) were MDR and as high as fourteen (67%) were XDR. Though these strains were isolated from samples of various sources, the majority was tracheal samples and predominance of MDR was in tracheal samples. In their study, Trilok Patil et al<sup>[19]</sup> reported that *Pseudomonas aeruginosa* was the most commonly isolated organism, followed by *Klebsiella pneumoniae*.

Mohammad H. Afify et al<sup>[14]</sup>, found that *Klebsiella spp* was the most common organism isolated followed by *Acinetobacter spp* and *Pseudomonas spp*. Among these 60% were extended spectrum beta-lactamase (ESBL) positive and 40% were Metallo beta-lactamase (MBL) positive. Fungal isolates consisted of one case of *Candida albicans* and *Candida nonalbicans* each.

In our study, among gram-negative culture isolates of ET, the rate of ESBL positivity was highest in *Acinetobacter spp* (100%) followed by *Klebsiella spp* (40%) and *Pseudomonas spp* (27%). These findings are quite comparable to earlier study results as mentioned above.

### Conclusion

Appropriate antimicrobial therapy is possible only with proper specimen collection, culture and sensitivity. Multidrug-resistant organisms are becoming increasingly associated with nosocomial infections. There is a need to improve Hospital Infection Control protocols in high-risk settings such as in all intensive care units. Gram-negative bacteria especially *Pseudomonas spp*, *Klebsiella spp*, *Acinetobacter spp* are most commonly encountered pathogens isolated in endotracheal samples of ventilator-associated pneumonia patients in the pre-specified clinical setting. Among these pathogens, *Acinetobacter spp* shows very high ESBL positivity and imipenem-resistance, while these characteristics are moderately expressed in *Klebsiella spp* and less commonly in *Pseudomonas spp*. Multidrug

resistance also followed the same trail among these common pathogens. The occurrence of resistant pathogens should be considered in formulating robust antibiotic policies. An updated antibiogram and susceptibility is essential to guide initial empirical therapy. In this regard, the VAP bundle checklist is to be implemented strictly to all the ICUs. It contains five components viz elevation of the head end of the bed to 30°, daily 'sedation vacation' and daily assessment of readiness to extubate, daily oral care with chlorhexidine, peptic ulcer disease prophylaxis, and deep venous thrombosis prophylaxis.

**Conflict of Interest:** There is no conflict of interest.

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