

# Quantitative correlation of colony forming units of *Acinetobacter baumannii* obtained from endotracheal tube aspirate culture with clinical pulmonary infection score

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## ABSTRACT

**Background and Aims:** Ventilator-associated pneumonia (VAP) is the most common healthcare-associated infection in adult critical care units. The reason behind the different levels of severity and the outcome in different individuals is not established yet. Co-morbidities and age may be a valid reason. In an attempt to find out the reason behind the different levels of severity of VAP in different individuals, we conducted a study with the primary objective being to assess the quantitative correlation between the colony forming units (CFU) of *Acinetobacter baumannii* in the endotracheal tube aspirate and the Clinical Pulmonary Infection Score (CPIS) in mechanically ventilated patients without co-morbidities. The secondary objective was to see whether the severity of VAP depends on the number of CFU ( $>10^5$ /ml). **Methods:** This observational study was conducted in a 27-bedded intensive care unit of a tertiary care hospital in northern India. Endotracheal tube aspirate was collected from patients with suspected VAP. A total of 81 patients without any prior co-morbidity and having *Acinetobacter baumannii* in their endotracheal tube aspirate were included in the study group. Quantification of *Acinetobacter baumannii* was done by counting CFU. The CPIS was calculated for all *Acinetobacter baumannii* positive patients. Correlation between CFU of *Acinetobacter baumannii* and CPIS was assessed using Spearman's Rho correlation coefficient. **Results:** Among 81 patients having *Acinetobacter baumannii* infection, only 61.9% had a CPIS  $>4$ . There was an insignificant correlation between the quantity of CFUs of *Acinetobacter baumannii* and CPIS ( $P$  value = 0.784). **Conclusion:** Number of CFU  $>10^5$ /ml in an endotracheal tube aspirate culture bears no relation to the severity of VAP as predicted by the CPIS.

**Key words:** *Acinetobacter baumannii*, colony forming units, intensive care units, ventilator-associated pneumonia

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## INTRODUCTION

Intensive care unit (ICU) patients have the greatest risk of developing a nosocomial infection from mechanical ventilation. One of the most common ICU infections is ventilator-associated pneumonia (VAP). VAP develops in approximately 20–30% of mechanically ventilated patients.<sup>[1,2]</sup> VAP happens to be the reason behind the high mortality rate (approximately 42%), longer stay and more consumption of resources in ICU patients.<sup>[3,4]</sup> Nonspecific clinical and radiological findings cause difficulty in diagnosing VAP. According to the Centers for Disease Control and Prevention (CDC), a pneumonia

could be defined as VAP when the patient is on mechanical ventilation for  $>$ two calendar days on the date of event, (day of ventilator placement being Day 1)

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and the patient should be on ventilator on the date of event or the day before.<sup>[5]</sup> Besides increased morbidity and mortality, VAP also causes a greater economic burden to patients by prolonging the ICU stay. Common causative organisms for early VAP are *Streptococcus pneumoniae*, *Haemophilus influenzae*, methicillin-sensitive *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and many others. The causative organisms for late-onset VAP include multi drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, *Acinetobacter* species, *Pseudomonas aeruginosa* and extended-spectrum beta-lactamase producing organisms.

Diagnosis of VAP is not an easy task mainly due to a wide spectrum of ventilator-associated events like acute respiratory distress syndrome (ARDS), pulmonary embolism, barotraumas, and pulmonary oedema, which may mimic VAP. The clinical pulmonary infection score (CPIS) is a good tool to diagnose VAP.<sup>[6]</sup> To establish the presence of significant infection, the quantitative cultures of microbial aspirate are often used. The diagnostic threshold for VAP is  $10^5$  colony-forming units per ml (CFU/ml).<sup>[7]</sup> Previous work of many researchers has advocated  $\geq 10^5$  CFU/ml in endotracheal aspirate as a diagnostic threshold for VAP.<sup>[8-10]</sup> In our setup, *Acinetobacter baumannii* is the most common organism (approximately 35%) being isolated from endotracheal tube (ET) culture. Therefore, we decided to make the diagnosis of VAP on the basis of clinical suspicion and investigations. We also calculated the CPIS score of every patient and the number of CFUs of *Acinetobacter baumannii* in the endotracheal aspirate sample ( $\geq 10^5$ ). Currently, CPIS is based on simple criteria that can help in making the diagnosis of VAP. Clinical diagnosis of VAP is made if CPIS  $> 6$ .<sup>[11,12]</sup> The primary objective of this study was to see the quantitative correlation between the CFUs of *Acinetobacter baumannii* in the ET aspirate and CPIS in mechanically ventilated patients. The secondary objective was to see whether the severity of VAP depends on the number of CFUs  $> 10^5$  or not.

## METHODS

This observational study was conducted in a tertiary care hospital of northern India between January and June 2019. After approval from the hospital research and ethics committee and registering the study in the Clinical Trials Registry-India (CTRI/2019/02/017533), 81 patients without any prior co-morbidity were included based on previous prevalence studies. The

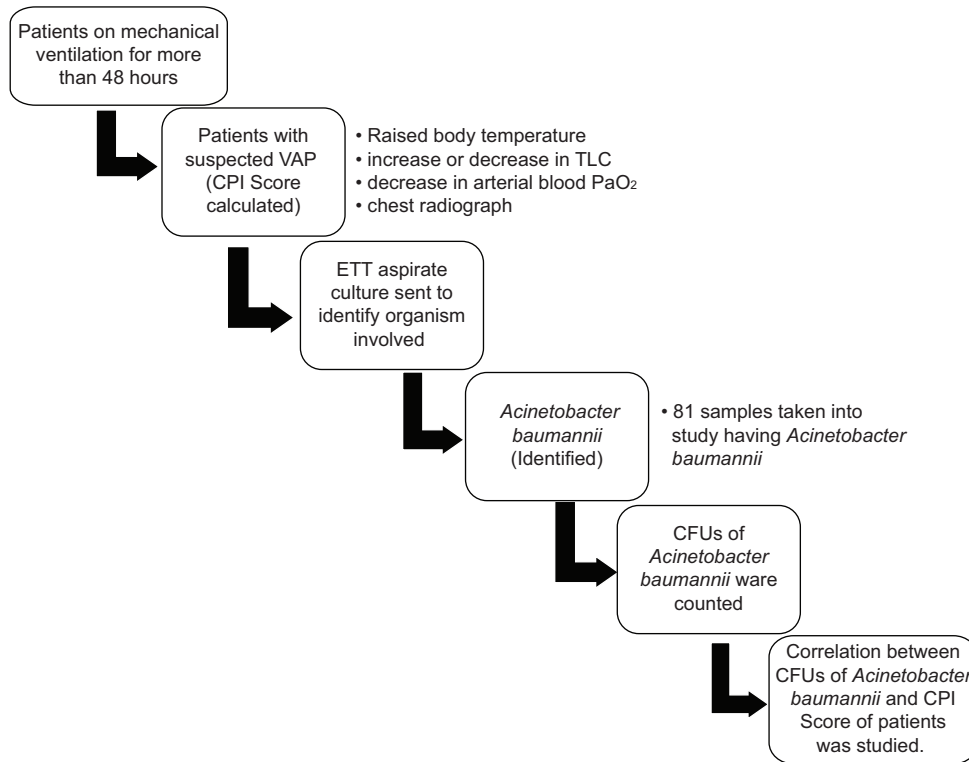
demographic data was subsequently collected, for example -age, sex, cause and duration of mechanical ventilation and ICU stay. After a minimum of 48 hours of ICU stay, if there was a suspicion of pneumonia, peripheral body temperature, total leucocyte count and arterial blood oxygenation were recorded and chest radiography was done. If this was suggestive of VAP, using all aseptic precautions, a 22-inch 12-F suction catheter with a mucus extractor was gently introduced through the ET up to a distance of approximately 25–26 cm and gentle aspiration was performed by the registrar on duty. The collected samples were sent to the microbiology laboratory for culture with proper identification details of patients. Samples were immediately processed for Gram stain and cultures. The organisms were identified up to the species level with the help of the bacterial colony characteristics and different biochemical reactions. MacConkey agar, which is an established selective and differential medium, was used for the isolation of acinetobacter. After seeing the growth of the *Acinetobacter baumannii*, CFU were counted. Spearman's correlation coefficient was used to assess the correlation between CPIS and CFU count of *Acinetobacter baumannii* [Figure 1].  $P$  value  $< 0.05$  was considered statistically significant. All collected data were entered into a Microsoft excel database and analysed using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) Version 17 for Windows.

## RESULTS

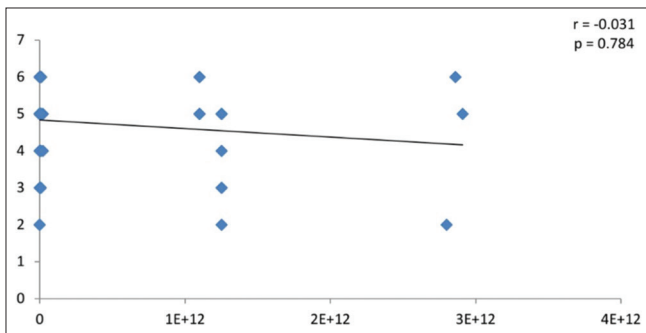
Totally, 81 cases having *Acinetobacter baumannii* as a single isolated organism in their endotracheal aspirate were selected. The demographic data showed a comprehensive collection of subjects of all age groups with a greater presence of males than females [Table 1]. ET aspirates of all patients showed more than 105 CFU/ml aspirate on microbiological culture. About 30.9% of total patients showed CPISs of less than four and 69.1% patients showed CPISs of more than four [Table 2]. There was no significant correlation between duration of mechanical ventilation and CPIS ( $r = -0.18$ ,  $P = 0.876$ ). No correlation was observed between CFUs of *Acinetobacter baumannii* and CPIS ( $r = -0.31$ ,  $P = 0.784$ ) [Figure 2].

## DISCUSSION

The major finding of this study was that there was no correlation between the number of CFUs of *Acinetobacter baumannii* in ET culture and CPIS with



**Figure 1:** Flow diagram of methodology; CPI: Clinical pulmonary infection; ETT: Endotracheal tube; VAP: Ventilator-associated pneumonia; CFU: Colony forming units; TLC: Total leucocyte count



**Figure 2:** CPI score, CPI: Clinical pulmonary infection. X axis = showing colony forming units, Y axis = showing CPI score,  $P = P$  value ( $<0.05$ , Nonsignificant),  $r =$  Spearman Rho coefficient, E = Exponent

a Spearman Rho coefficient of 0.31 ( $P$  value = 0.784). This is in concordance with the study of Dennis *et al.*<sup>[13]</sup> who demonstrated that critically injured trauma patients developed a significant bacterial burden ( $>10^5$  CFU) without exhibiting symptoms that suggested the presence of VAP. In our study, 31% of patients did not fulfill CPIS diagnostic criteria of VAP but they had a positive culture (CFU  $>10^5$ ). However, several studies have shown that increased colony count is associated with worse clinical outcomes.<sup>[14,15]</sup> The explanations behind our finding might be the use of prophylactic antibiotics as per the antibiotic policy of our institute in ICU patients.

Table 1: Gender-wise distribution		
	Frequency (No.)	Percentage
Sex		
Male	64	79
Female	17	21
Total	81	100

Data represented as number and percentage of patients

Table 2: Mean values of study parameters			
Parameter	Minimum	Maximum	Mean±SD
Age (years)	17	65	37.94±15.20
Duration of Mechanical Ventilation (days)	2	14	4.27±2.38
CFU/ml	$6.8 \times 10^7$	$2.9 \times 10^{12}$	$2.8 \times 10^{11} \pm 6.6 \times 10^{10}$
CPI Score	2	6	4.77±1.16

Data represented as mean±Standard Deviation. SD: Standard deviation, CFU: colony forming units, CPI score: Clinical pulmonary infection score

The crude mortality rate of VAP caused by *Acinetobacter baumannii* has been estimated to be between 40 and 70%.<sup>[16]</sup> Due to such a high incidence of mortality, we often treat colonising *Acinetobacter baumannii* inspite of increased resistance and development of multi-drug resistant pathogens. As a result of its increasing resistance to many currently available antibiotics, *Acinetobacter baumannii* is now one of the most difficult-to-treat pathogens implicated in VAP.<sup>[17]</sup> Coronavirus disease (COVID)-19 patients can develop pneumonia, ARDS and multiorgan

dysfunction. It has been observed that approximately 5% of the total COVID-19 infected patients who came to hospital were put on mechanical ventilation.<sup>[18]</sup> The duration of mechanical ventilation was more and hence the incidence of VAP also increased. It is said that co-infection with microorganisms such as *Acinetobacter baumannii* can aggravate the progression and worsen the prognosis of the disease.<sup>[19]</sup> Mortality in those with moderate to severe disease put on mechanical ventilation is reported to be high<sup>[20]</sup> and bacterial coinfection has been found to contribute to this mortality. Nevertheless, further research is needed on bacterial coinfections in COVID -19 patients.

The most common organism which was isolated from ET culture was *Acinetobacter baumannii* in our ICU. We did not include patients with any prior morbidity as that could be a reason for the different levels of severity of VAP in different individuals. In our institution, we usually perform tracheostomy on those individuals who are supposed to be on mechanical ventilation for prolonged duration. We do it after 7 days of mechanical ventilation. In this period, we usually send an ET culture in most of the patients, and that is why we did not include tracheostomised patients in our study.

This study is an observational study that follows standard critical care management and monitoring protocols and is done in a closed ICU setup. To the best of our knowledge, this is the first study done till date, that compared the number of CFUs of *Acinetobacter baumannii* and CPIS as a quantitative indicator of the severity of VAP. Some notable limitations of this study are that it is a single centre study, which is significantly limited by the sample size and the inherent heterogeneity of ICU patients. Further studies with larger sample size may give some concrete conclusion about this hypothesis.

## CONCLUSIONS

In conclusion, the findings of this study indicate that there is no statistically significant correlation between the number of CFUs of *Acinetobacter baumannii* and CPIS. CPIS is a good tool to diagnose VAP but if we analyse the severity of VAP based on this score, the number of CFUs of *Acinetobacter baumannii* is not corroborative.

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## Conflicts of interest

There are no conflicts of interest.

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