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VENTILATOR ASSOCIATED PNEUMONIA DUE TO *Acinetobacter baumannii* ITS RISK FACTORS AND ITS ANTIBACTERIAL RESISTANCE PATTERN: A CASE CONTROL STUDY

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ABSTRACT

***A.baumannii* is the emerging cause of severe illness and considerable mortality due to its higher capacity to acquired resistance to multiple drugs. This study was performed to identify prevalence of VAP cases, its associated risk factors and antimicrobial resistant pattern in intensive care unit. This case control study was conducted in Kanpur from October 2015 to January 2017. Respiratory samples were collected from six different hospitals of Kanpur. Patients with *A. baumannii* acquisition (colonization or infection) were considered as test cases and controls were without Acinetobacter acquisition. All the clinical details were monitored for Clinical Pulmonary Infection Scoring (CPIS). Endotracheal aspirates or secretions, ET tips and sputum samples were microbiologically analyzed for microbial growth and antimicrobial sensitivity testing was performed according to CLSI guideline. In the study total 200 test cases were observed among these 118 cases were suspected for pneumonia. The incidence of VAP due to *A.baumannii* was 11.02% and rate of VAP infection due to *A.baumannii* was 37.90 per 1000 device days. No significant differences were found in age and gender of test and control group. Prolonged Ventilatory Support (>10 days) had a higher incidence of VAP. Supine position, comatose patients were also found to be risk factors of VAP. Out of the 13 patients 84.46% patients developed the late-onset VAP due to *A.baumannii.* There was statistically significant difference found between test and control group. Other clinical findings like PaO_2/FiO_2 ratio was found to be <242 mmHg in 10 cases. Mortality rate due to VAP-AB was 53.85% which was statistically significant. Only polymyxin and tegicycline were 100% sensitive. In our study, VAP due to a XDR Acinetobacter is one of the most dreadful complications that occur in the critical care setting.**

KEYWORDS: *A.baumannii*, Antimicrobial Resistant Pattern, Risk Factors, Ventilator Associated Pneumonia.

Acinetobacter is gram negative coccobacilli, ornamented as sophisticated nosocomial pathogen in 21st century. Multi-drug resistance (MDR) genetic determinants, tolerance to wide range of pH, sanitary, humidity and unique ability to survive on almost all nutrient sources, this trails selects this pathogen to be ubiquitous in the hospital environment (Thapa B., 2011). This behavior is likely the source for most outbreaks of hospital infections. *A.baumannii* is commonly associated with high morbidity and mortality which includes infections, such as respiratory tract infections, bacteremia, urinary tract infection, meningitis, skin and soft tissue infections especially in patients with severe health conditions (Malhotra S., 2014). Ventilator-associated pneumonia (VAP) refers to pneumonia which occurs in people who have required mechanical ventilation through an endotacheal or tracheostomy tube for at least 48 hours. VAP is a common and severe complication of critical illness that is associated with an increased length of stay in the hospital or intensive care unit and with a high mortality rate. Although number of studies provided evidence that *A. baumannii* infections may be associated with high mortality(Boots R.J., 2005 & Niederman, 2001), but some of them support the possibility that the clinical course of critically ill patients may be influenced by many variables and that subsequently the acquisition of or infection with *A. baumannii* may not independently lead to poorer outcomes. This controversy has caused considerable confusion among clinicians and investigators about the mortality associated with of *A. baumannii* infections.

Here, we report the prevalence of VAP cases due *A.baumannii*, associated risk factors and antimicrobial susceptibility pattern of *Acinetobacter* isolated from intubated patients with pneumonia admitted to intensive care unit. In this case control study we also compare the clinical characteristics and mortality between patients acquired VAP due to *A.baumannii* and patients with same clinical feature associated with other microorganism.

MATERIALS AND METHODS

The study was a prospective study conducted in Department of Microbiology in Rama Medical College Hospital & Research Center, Kanpur from October 2015 to January 2017. The non-repetitive samples were collected from the different ICU patients, sent to the microbiology laboratory for the culture identification and

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sensitivity testing accordingly, under standard protocols and guidelines.

In the study total 200 test cases were observed among these 118 cases were on mechanical ventilator support, suspected for pneumonia, more than 48 hours, were kept in test group and observed for Acinetobacter isolates. From another 200 control cases, 110 patients were on mechanical ventilation for more than 48 hours, observed for other than Acinetobacter isolates. These patients were kept in control group. All the data was collected including admission date to ICU, age, gender, date of initiating mechanical ventilation, total ventilation days and underlying diseases. Patient's vitals, general physical examination, state of consciousness, and position of the patients were monitored regularly. Clinical criteria includes clinical pulmonary infection score (CPIS) (Davis K.A., 2006) which includes temperature, PaO_2/FiO_2 ratio, leucocytes count, chest radiography, tracheal culture. [Table 1]

Table 1: Clinical pulmonary infection scoring system (CPIS)

S.N	CPIS Points	0	1	2
1.	Temperature	≥ 36.5 and ≤ 38.4	≥ 38.5 and ≤ 38.9	≥ 39 and ≤ 36
2.	PaO_2/FiO_2 ratio	>240 or ARDS	-	<240 or no ARDS
3.	Tracheal secretions	Rare	Abundant	Purulent
4.	Leucocytes count (mm^3)	>4,000 and <11,000	<4,000 and >11,000	<4,000 or >11,000 + band forms
5.	Chest Radiography	No infiltrate	Diffused infiltrate	Localised infiltrate
6.	Tracheal culture	Negative		Positive

Microbiological Criteria

Based on the clinical and microbiological criteria, patients were diagnosed for VAP. For microbiological processing, Endotracheal tips, aspirates, suction tips, sputum, and tracheostomy tips were collected aseptically. Endotracheal tip, suction tips or tracheostomy tips were cut by sterile surgical blade and taken into sterile tube or container directly and was sent for culture. Gram stain smear was prepared from samples to determine pus cells and microorganisms. Smear was scored as per Bartlett scoring system. [Table 2] Microbiological confirmation was based on a positive Gram stain (>10 polymorphonuclear cells/low power field and ≥1 bacteria/oil immersion field) and culture. Endotracheal aspirate was serially diluted in sterile normal saline to 1/10, 1/100 and 1/1000 and 0.01 ml of 1/1000 dilution was inoculated on Blood agar and MacConkey agar. After inoculation, a colony count was observed and expressed in cfu/ml. The number of colony forming unit/ml (cfu/ml) is equal to the no. of colonies on agar plate agar plate x dilution factor x inoculation factor.

Other samples were also cultured on Blood agar and MacConkey agar and incubated at 37°C for overnight incubation. After incubation, colony morphology was studied and were identified by standard procedures, which has mentioned in our another study (Nidhi Pal, 2017). Result was expressed as cfu/ml. With quantitative analysis of aspirate and tip, the threshold for diagnosing VAP in this study was considered as 10^5cfu/ml.

Table 2: Bartlett scoring system

Parameters	Grade
No. of neutrophils/ 10x LPF	
<10	0
10-25	1+
>25	2+
Mucus present	1+
No. of epithelial cells/ 10x LPF	
10-25	-1
>25	-2

RESULTS

In this study 200 test cases were studied from October 2015 to January 2017 from six different hospitals of Kanpur. In the study total 200 test cases were observed among these 118 cases were suspected for pneumonia and all of these patients were on Mechanical ventilator. Out of 118 cases, from 24 patients *A.baumannii* was isolated. Of 24 cases, only 13 were acquired ventilated associated pneumonia (VAP) due *A.baumannii* which was clinically as well as microbiologically fulfilling the criteria of VAP. [Table 3] Hence the incidence of VAP due to *A.baumannii* was 11.02% and rate of VAP infection due to *A.baumannii* was 37.90 per 1000 device days.

Table 3: Cases of VAP

Total VAP suspected cases	118
Total *A.baumannii* isolated	24
No. of *A.baumannii* associated with VAP	13
A.baumannii as colonizer	11

Total 13 patients acquired VAP, comprising of 11 male and 2 female who were on mechanical ventilator. [Table 4] Table 5 showed the age wise distribution of VAP patients. Advance age patients mostly acquired VAP. But there were no significant of age between test and control group. In this study it was observed that those requiring prolonged Ventilatory Support (>10 days) had a higher incidence of VAP [Table 6]. Here, no significant difference was found among test and control group. Positions of patients' i.e. Supine position, comatose patients were also found to be risk factors of VAP. Out of the 13 patients who developed VAP, 2 patients developed early-onset (15.38%) VAP and 11 patients developed the late-onset type (84.46%). [Table 7] Here significant difference was found between test and control group. Other clinical findings like PaO_2/FiO_2 ratio was also analysed in VAP patients and was found to be <242 mmHg in 10 cases. In the remaining 3 the ratio was higher (>240 mmHg).[Table 8] In this study out of 13 VAP patients, 7 (53.8%) were die and 2 were taken leave against medical advice (LAMA).[Table 9] Hence, mortality rate due to VAP-AB was 53.85%. this was statistically significant. Antibiotic resistant pattern of A.baumannii associated with VAP infection and colonizer were mentioned in fig 1.

Table 4: Gender wise distribution among VAP patients

	VAP due to A.baumannii (Test group) N=13	VAP due to other than Acinetobacter (Control group) N=28	*p* value
Male	11	17	0.1402
Female	2	11	

Table 5: Age wise distribution among VAP patients

Age	VAP due to A.baumannii (Test group) N=13	VAP due to other than Acinetobacter (Control group) N=28	*p* value
<20	2	3	0.6722
21-40	2	6	0.6509
41-60	5	11	0.9599
>60	4	8	0.9413
Mean Age ± SD	45.84 ± 21.52	47.855 ± 19.224	-

Table 6: Ventilator days among VAP patients

Ventilation days	VAP due to A.baumannii (Test group) N=13	VAP due other than Acinetobacter (Control group) N=28	t test	*p* value
Mean+SD	10.7 ± 4.49	7 ± 3.45	2.901	0.0061*
<=10 days	4	16		
>10 days	9	12		
**p* value = statistically significant				

Table 7: Onset of VAP among VAP patients

Onset of VAP	VAP due to A.baumannii N=13	VAP due other than Acinetobacter N=28	Chi square test	*p* value
Early onset	2	16	6.2859	0.01217*
Late onset	11	12		
*p = statistically significant				

Table 8: PaO2/FiO2 ratio among VAP patients

PaO2/FiO2 ratio	VAP due to A.baumannii N=13	VAP due other than Acinetobacter N=28	*p* value
Mean+SD	242.07+ 35.12	240.12±30.22	0.2211
<240 mmHg	10	16	
>240 mmHg	3	12	

Table 9: Mortality rate due to VAP

Mortality	VAP due to A.baumannii N=13	VAP due other than Acinetobacter N=28	z test	*p* value
Death	7 (53.85%)	6 (21.42%)	2.08	0.038*
LAMA	2 (15.38%)	2 (7.14%)		
Shifted from ICU	4 (30.08%)	20 (71.42%)		
*p = statistically significant				

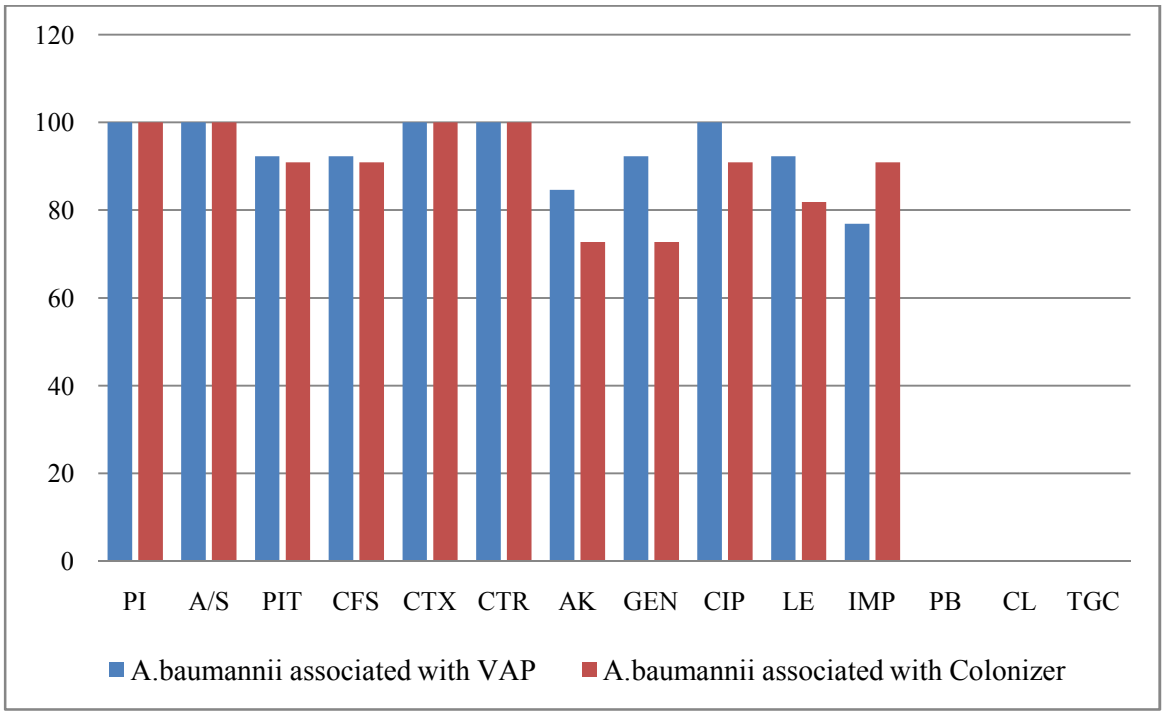

Figure 1: Antibiotic resistant pattern of *A.baumannii* causing VAP

DISCUSSION

VAP is an important Nosocomial Infection or Health Care Associated Infection (HAI) in patients on mechanical ventilation in ICUs. In the study total 200 test cases were observed among these 118 cases, on ventilator, were suspected for pneumonia. The overall incidence of VAP in this study was 21.1%, this was higher than the incidence of 17.5% reported by Cook DJ et.al. (Cook DJ,1998) While R.M. Saldanha Dominic et. al, and N. Ranjan et. al, reported higher incidence rate 38.56% and 57.14% respectively (Gadani H,2010; Ranjan,2014).

Acinetobacter species are common cause of VAP. Since this organism survives in moist and dry conditions for a prolonged period, it often leads to nosocomial outbreaks. In the present study, the incidence of VAP due to *A.baumannii* was 11.02% and rate of VAP infection due to *A.baumannii* was 37.90 per 1000 device days.

Similar to our study Sethi et al. reported, 11.3% VAP cases attributed to *Acinetobacter* (Sethi, 2010). While Baraibar *et.al*. have reported 8.1% VAP cases caused by *Acinetobacter* (Baraibar,1997). This difference in results might be because of different diagnostic methods, ICU environment, compliance of Hand Hygiene and Other Infection Prevention & Control Practices and importantly, the population of the study.

In our study, male predominated (84.61%). Although the incidence of VAP-AB was higher in male but it was statistically not significant. The mean age group of VAP associated with *A.baumannii* was 45 years. It was found that mostly elder aged patients suffered from VAP (Saldanha, 2012). In the present study also advance age patients mostly acquired VAP in both the groups (test and control group) due to lower immune resistance. Hence, age of patients having no relation with occurs of VAP-AB and VAP due to other than Acinetobacter. It was not statistically significant.

In our study, duration of mechanical ventilation (>10 days) was statistically significant risk factors for incidence of VAP and similar to other studies were also in favor (Saldanha, 2012; Ranjan, 2014; Shete, 2010). It was observed that reducing the duration of mechanical ventilation significantly shortens hospital stay and hence subsequent risk of exposure of patients to pathogens. But there were no significant difference in VAP due to *A.baumannii* and VAP due to other than Acinetobacter. It was also observed that patients who developed VAP had longer ICU stays than those who did not.

The positions of the patients' i.e. Supine position and level of consciousness of the patients' i.e. comatose patients were the risk factors of VAP in this study (Gadani H., 2010). Higher incidence of VAP was found in supine position as compared with the semi-recumbent position because it may facilitate aspiration, which may be decreased by a semi-recumbent positioning. This outcome was similar to other studies when position is considered as a risk factor (Davis K, 2001). Infection in patients in the supine position was strongly associated with the simultaneous administration of enteral nutrition. Thus, intubated patients should be managed in a semi-recumbent position, particularly during feeding.

Out of the 13 patients who developed VAP due to *A.baumannii* two patients developed early-onset (15.38%) VAP and 11 patients developed the late-onset type (84.61%). Golia S et al. also reported 14.2% of *A.baumannii* responsible for late onset VAP (Golia S, 2013). Mallick et al. also observed Acinetobacter was the commonest organism isolated from late-onset VAP (Mallick, 2015). This bacteria has the unique property to persist in the adverse environment for longer duration and after longer duration bacteria express certain enzymes and cause infections. Early onset VAP-AB in the present study was 15.38% while in control group it was found to be 57.14%. Here 84.61% of patients were suffered from late onset VAP due to other than Acinetobacter. These results show statistically significant ($p=0.012$), which indicates if patient acquire VAP after 4 days or late onset, there would be more chance of acquire VAP-AB.

The PaO2/FiO2 ratio was assessed during the course of ventilatory support. In the present study, PaO_2/FiO_2 ratio was found to be <240 mmHg in 10 cases which was statistically significant risk factor for VAP. Other studies [Thapa et al., 2011] also analyzed most no. of cases have PaO_2/FiO_2 ratio <240mmHg that decline PaO_2/FiO_2 ratio (<240mmHg) indicates towards the increasing oxygen demand and risk of onset of VAP (Ranjan, 2014). In this study, no significant difference was found between VAP due to *A.baumannii* and other than Acinetobacter.

Pneumonia due to Acinetobacter species is usually associated with a high mortality rate as compared to other than Acinetobacter. In our study the mortality rate was 53.8% due VAP-AB (Test group) and 21.4% in

control group. This difference in the mortality rate in the two groups was analysed statistically using fissure's exact z test. The value of z test was 2.08, which is statistically significant (p=0.038). This emphasizes higher mortality rate in VAP-AB and the role of chance factor is negligible. Prashanth and Badrinath, 2006 also documented 50% mortality rate in VAP patients, which was of major concern (Prashanth K., 2006). Fagon et. al., 1996, reported the mortality rate among patients with pneumonia due to Acinetobacter species was 73% (Fagon, J.Y., 1996); this rate was significantly higher than that among patients with the same clinical condition at admission who received ventilatory support.

Assessment of VAP after 48 hours is needed to prescribe whether antibiotics should be continued. It should be assessed by repeated CPIS, as the change in CPIS can guide clinical condition of patients to treat. The judicious selection of patients for antibiotic therapy needs to be emphasized. *A.baumannii* strains associated with VAP were resistant to commonly used antimicrobial agents. Still piperacillin tazobactum and cefoperazone sulbactum were 92.31% each resistant. 84.62% of Acinetobacter were resistant to Amikacin. Here, 76.9% (10/13) were XDR which was associated with VAP infection while 90.91% (10/11) of colonizer were XDR. That may become a probable pathogenic microorganism of respiratory tract. Gladstone et. al., 2005, from Vellore (India) reported 14.2% imipenem resistant Acinetobacter from patients with respiratory tract infections in ICU (Gladstone P, 2005).

The reason for multi-drug resistance in this organism has been attributed to the intrinsic impermeability of its outer membrane and to its close relationship to the soil and aquatic environment which has made it possible for this organism to acquire highly effective resistance determinants in response to multiple challenges

CONCLUSION

VAP due to a Acinetobacter is one of the most dreadful complications that occur in the critical care setting. This study conclude that VAP due to *A.baumannii* cause higher mortality that other causative agents. This poses serious problems in choosing the right antibiotic for the treatment of sick patients admitted into the ICU. Various strategies such as strict infection control measures, judicious prescribing of antibiotics, antibiotic resistance surveillance programs and antibiotic cycling are crucial to control infections due to these MDR pathogen in patients admitted to ICU.

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