

Role of colonizers and value of routine surveillance culture of endotracheal aspirate of patients in the diagnosis of ventilator associated pneumonia



Sana Siddique¹, Fareya Haider², Sharique Ahmad³, Khalid Iqbal⁴, Mastan Singh⁵

¹Senior Resident, ²Assistant Professor, ⁵Professor and Head, Department of Microbiology, ³Professor, Department of Pathology, ⁴Assistant Professor, Department of Cardiothoracic and Vascular Surgery, Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India

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ABSTRACT

Background: Ventilator associated pneumonia (VAP) is considered to be second most common nosocomial infection patients requiring critical care. **Aims and Objective:** The present study was conducted to study the role of colonizers and importance of surveillance cultures of endotracheal aspirate (ETA) in the diagnosis of ventilator associated pneumonia in a tertiary care hospital in Lucknow. **Materials and Methods:** An observational longitudinal study was conducted over a period of 2 years, on a total of 210 critically ill patients on mechanical ventilation for >48hrs, to identify the common isolates from ETA culture. Follow up of such patients was done to know the role of these isolates in causation of Ventilator Associated Pneumonia (VAP). Patients fulfilling both clinical Pulmonary infection score (CPIS > 6) and microbiological criteria were diagnosed as VAP. Those microorganisms with a colony count of less < 10⁵ cfu/ml in both the patients with VAP and those without VAP were considered as colonizers. **Results:** *Klebsiella pneumoniae* (46.2%), *Pseudomonas aeruginosa* (16.2%) and *E.coli* (13.8%) were found to be the commonest colonizers followed by *Acinetobacter baumannii* (8.6%), *Citrobacter koseri* (3.8%), Coagulase Negative Staphylococci (2.9%), *Staphylococcus aureus* (2.4%) and *Proteus vulgaris* (1%). Of the total patients 28 developed VAP out of which 21 had late onset VAP and 7 had early onset VAP. Among the VAP positive patients the causative organism was *Klebsiella pneumoniae* (53.6%) for majority of cases followed by *Pseudomonas aeruginosa* (21.4%) and *Acinetobacter baumannii* (17.9%). **Conclusion:** Prolonged duration of mechanical ventilation increased the chances of colonization by MDR microorganisms leading to nosocomial or Hospital acquired infections (HAI) such as VAP which in turn lead to increased rate of morbidity and mortality. VAP considered to be a leading cause of HAI, routine quantitative surveillance culture of ETA (endotracheal aspirate) will allow prospectively to determine prevalence and progression of colonization in lower respiratory tract, so that strict and prompt preventive measures can be taken rather than cure.

Key words: Ventilator associated Pneumonia; Nosocomial infection; Hospital acquired infections; Multiple drug resistance

INTRODUCTION

Hospital acquired infections (HAIs) are additional risk factors affecting the clinical course of patients admitted to a healthcare facility that are instrumental in increasing

the risk of morbidity, mortality as well as economic costs of healthcare delivery. According to WHO, hospital acquired infections can be defined as "An infection occurring in a patient in a hospital or other healthcare facility in whom the infection was incubating at the time of admission or was absent. This

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Address for Correspondence:

Dr. Fareya Haider, Assistant Professor, Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India. **Mobile No:** +91-9956861059. **E-mail:** fareyahaider@gmail.com

includes hospital acquired infection which appeared after discharge, and also infections among staff working in the healthcare facility".¹ Devices invasive in nature like catheters and ventilators employed in a health care establishment of modern era have an association with these infections.² These devices are more of use in case of patients requiring critical care. Thus, causality of these critical patients is not only from their critical illness but also from secondary processes such as nosocomial infection. Second commonest nosocomial infection found in critically ill patients is Pneumonia. It has been estimated that 86% of nosocomial pneumonias are associated with mechanical ventilation (MIV) and have been termed as ventilator-associated pneumonia (VAP).³ VAP has been defined as pneumonia occurring to those patients who have undergone endotracheal intubation or tracheostomy more than 48 hours, caused by infectious agents incubating or absent at the time of initiation of MIV.⁴ By the joint efforts of CDC and National Institutes of Health, a new guideline for diagnosis of VAP came into being which aimed in improving the diagnosis of VAP as well as the reliability and validity of Surveillance culture.⁵

According to guidelines, Gram staining of endotracheal aspirate or BAL showing >25 neutrophils and <10 epithelial cells per low-power field or positive culture from sputum, endotracheal aspirate, BAL, lung tissue as the indicator of possible-VAP and positive culture of endotracheal aspirate $>10^5$ CFU/ml, or positive BAL culture with $>10^4$ CFU/ml, or positive culture of protected brush specimen $>10^3$ CFU/ml was considered to be indicator of probable-VAP.

The diagnosis of VAP is complex as it is difficult to differentiate between pathogenic and colonizing microorganisms. Mechanically ventilated patients are unconscious therefore there is no clearance of the secretions in the oropharynx leading to increase in colonizers which gets collected and is passed along the tracheal tube, also forms a biofilm and reaches the distal airway leading to pneumonia.⁶ The organisms reaching the distal airway overcome the host immune response supported by cofactors like pulmonary edema and previous lung infections favoring bacterial multiplication. Therefore the source of infection in most patients with VAP is either the normal flora or bacteremia.⁷ Bronchoscopically obtained secretions of lower respiratory tract accurately diagnoses the pathogens but it is been considered as an invasive procedure when VAP sets in, hence in such cases if endotracheal aspirate is collected early in the course of infection, it can be used for determining the bacterial pathogen by doing culture.⁸

Ventilator associated pneumonia is responsible for increased rate of multidrug-resistant infections leading to enhanced antibiotic use both in dose and duration,

prolonging the time of MIV, which also increases time period of hospital stay and ultimately to increase in morbidity, mortality and last but not the least inflated healthcare cost.⁹

Considering the above given information this surveillance study was planned, in which those patients admitted in Intensive care unit (ICU) and Critical care unit (CCU) of Era's Lucknow Medical and Hospital were taken who were on mechanical ventilation for >48 hrs. Ultimate aim of routinely carrying out Endotracheal aspirate culture of all patients undergoing critical care was to predict about those patients who were at raised risk of invasive disease, which could lead the clinician to judicious and prompt use of antibiotic therapy based on the most prevalent bacterial species recognized in these cultures in the event of the development of VAP.

MATERIAL AND METHODS

Settings and subjects

This study was carried out for a period of 2 years from November 2016 to October 2018 in collaboration with Microbiology department and Critical Care Units of Era's Lucknow Medical College and Hospital, a tertiary care hospital in Lucknow, U.P, India. Since the study was based on surveillance, therefore it was considered to be an observational study. Patients admitted to Critical care unit as well as to Intensive care unit for more than >48 hrs on ventilatory support were taken for the study. Patients with acute respiratory distress syndrome (ARDS) as well having prior clinical and radiological signs of pneumonia or secondary to pneumonia before initiation of MIV were excluded. This study was approved by ethical committee of institute. Informed consent was also taken from the patient's next of kin.

Data acquisition

The data obtained from the patients enrolled in this study included primarily their underlying illness, duration of stay in hospital as well as total time period on ventilatory support and previous antibiotic therapy record. Other recorded data were included from their medical records, bedside charts, radiographic reports, and reports of microbiological cultures.

Specimen collection

Endotracheal aspirate was obtained by inserting a sterile catheter attached to a mucus extractor through the endotracheal tube for a short distance and mucus was aspirated after instilling sterile normal saline. At least 3-5 ml ETA was collected which was properly labelled so that it can be transported to microbiology laboratory for processing (gram's staining and culture) within 1 hr of collection.

Specimen processing

The ETA sample received in the laboratory was examined by naked eye for amount, appearance, blood stain and purulence. Gram's stain of smear was examined under the microscope for pus cells and presence of bacteria and its morphology for a presumptive diagnosis.¹⁰

Quantitative culture of the ETA followed after serially diluting the aspirate in sterile normal saline to obtain 1/1000 dilution of which 0.01ml inoculated on 5% sheep blood agar and MacConkey agar and incubated for 18-24 hrs at 37°C. A colony count was done when growth appeared on media after overnight incubation which was depicted as number of colony-forming units per ml (cfu/ml calculated by multiplying number of colonies with dilution factor and inoculation factor). On the blood agar (gram positive bacteria) or on MacConkey agar (gram negative bacteria) if even a single colony appeared after inoculating 0.01 ml of 1/1000 times diluted EA was expressed as >105 CFU/ml. Further identification of microorganisms isolated was done on the basis of standard biochemical testing and colony characteristics. EA culture quantitatively showing ≥ 105 cfu/ml and >10 polymorphonuclear cells/low power field as well as ≥ 1 bacteria/oil immersion field in gram stain confirmed microbiological diagnosis.¹⁰⁻¹³

Antibiotic susceptibility assessment

Antibiotic susceptibility was determined by Kirby-Bauer disk diffusion technique. Results were interpreted as per the recommendations of Clinical Laboratory Standard Institute guidelines (CLSI) document 2016.¹⁴

Follow up protocol

Monitoring of these critically ill patients for development of signs of VAP was done at regular intervals till discharge or death. Clinical Pulmonary Infection Score (CPIS) was calculated for every patient on MIV every third day starting from day 0 i.e. after 48 hrs of intubation. VAP was only considered to be present in those ill patients whose CPIS

score was more than 6. Those patients with or without VAP having colony count of less than 105 cfu/ml was considered as colonizers.¹⁵

Diagnosis of VAP

Establishment of VAP diagnosis was achieved by monitoring all patients on the basis of CPIS score (clinical pulmonary infection score) at frequent intervals, until discharge or death. A total of 6 clinical assessments, including fever, total leukocyte count, purulent tracheal secretions (quantity), lung oxygenation, type of radiographic abnormality and results of sputum culture and Gram stain were considered as per CPIS each worth 0-2 points was used in those critical care patients who were clinically found to be suffering from VAP.

Statistical analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 21.0 statistical Analysis Software. The values were represented in Number (%) and Mean \pm SD. Comparison of the mean age of patients with and without VAP was carried out using an unpaired Student's t-test. All p-values of < 0.05 were considered statistically significant.

RESULTS

Among the total (n=210) patients on MIV for >48 hours enrolled in the study the distribution of study population was found to be ranged from minimum of two days to a maximum of 28 days. The mean duration was 7.38 \pm 5.44 days. Most of the patients had a stay of 2-4 days i.e (39%) as shown in Table 1.

On the last day of ventilation, out of 210 patients enrolled in the study only 7 (3.3%) were found to be sterile. *Klebsiella pneumoniae* (46.2%) was the commonest pathogen followed by *Pseudomonas aeruginosa* (16.2%), *E. coli* (13.8%) while less common pathogens were *Acinetobacter baumannii* (8.6%),

Clinical pulmonary infection scoring system (CPIS)			
CPIS points	0	1	2
Tracheal Secretions	Absent	Not Purulent	Abundant and Purulent
Leucocyte count (mm ³)	>4000 and <11,000	<4000 and >11,000	<4000 or > 11,000 + band forms
Temperature (°C)	>36.5 and <38.4	>38.5 and <38.9	>39 or <36
PaO ₂ /FIO ₂ ratio (mmHg)	>240 or ARDS	-	<240 and no ARDS
Chest radiograph	No infiltrate	Diffuse Infiltrate	Localized infiltrate
Culture of tracheal aspirate	Negative	-	Positive
CPIS points	0	1	2
Tracheal secretions	Absent	Not purulent	Abundant and Purulent
Leucocyte count (mm ³)	>4000 and <11,000	<4000 and >11,000	<4000 or > 11,000 + band forms
Temperature (°C)	>36.5 and <38.4	>38.5 and <38.9	>39 or <36
PaO ₂ /FIO ₂ ratio (mmHg)	>240 or ARDS	-	<240 and no ARDS
Chest radiograph	No infiltrate	Diffuse infiltrate	Localized infiltrate
Culture of tracheal aspirate	Negative	-	Positive

Citrobacter koseri (3.8%), CONS (2.9%), *S. aureus* (2.4%) and *P. vulgaris* (1.0%) as can be observed in Table 2.

Ventilator associated pneumonia was confirmed in the patients having clinical pulmonary infection score >6. Only 28 (13.3%) patients had CPIS >6 during their stay on mechanical invasive ventilation, rest 182 patients did not acquire pneumonia during their stay on MIV as shown in Table 3.

Among 28 patients who acquired VAP during 4 to 14 days of their stay on MIV, median duration of acquiring VAP was 6th day and mean duration was 6.86±2.58 days. Early onset VAP (within 4 days) was among 25.0% of patients while rest 75% was found to be late onset (>4 days) as relevant in Table 4.

Causative organisms in VAP patients on MIV was *Klebsiella* spp. For majority of cases (53.6%) followed by *Pseudomonas*

spp. (21.4%) and *Acinetobacter* spp. (17.9%). In 1 (3.6%) patient each causative organism was *E. coli* and *Enterococcus* spp. as shown in Table 5.

Of total VAP cases, two third (n=19; 67.9%) had same pathogen in the pre-VAP culture and on day of suspected VAP, in only 2 (7.1%) cases specimen was sterile on pre-VAP cultures and rest 7 (25.0%) cases pathogen on day of VAP and before the day of VAP was not same as depicted in Table 6.

Mean duration of MIV among patients who acquired VAP (15.71±6.39 days; range 4-28 days) was found to be significantly higher as compared to those who did not acquire VAP (6.10±3.96 days; range 2-23 days) as shown in Table 7. Total duration of ventilator use of patients enrolled in the study was 1550 days. VAP rate was calculated using

$$\text{VAP rate} = \frac{\text{No of cases} \times 1000}{\text{Total Duration of Ventilator use}}$$

VAP Rate found to be 18.06 per thousand ventilator days.

Mortality was found higher in VAP acquired than no VAP patients as shown in Table 8.

Table 1: Study population distributed on the basis of duration on mechanical ventilation

S.No.	Duration (days)	No. of patients	Percentage
1	2-4 days	82	39.0
2	5-7 days	60	28.6
3	8-10 days	27	12.9
4	>10 days	41	19.5

Mean±SD: 7.38±5.44days; Min-Max (median): 2-28 (6.00) days

Table 2: Distribution of study population according to pathogen isolated on last day of ventilation

S.No.	Diagnosis	No. of patients	Percentage
1	Sterile	7	3.3
2	<i>Acinetobacterbaumannii</i>	18	8.6
3	<i>Citrobacterkoseri</i>	8	3.8
4	CONS	6	2.9
5	<i>E. coli</i>	29	13.8
6	<i>Klebsiellapneumoniae</i>	97	46.2
7	<i>Proteus vulgaris</i>	2	1.0
8	<i>Pseudomonas aeruginosa</i>	34	16.2
9	<i>S. aureus</i>	5	2.4

Table 3: Prevalence of ventilator induced pneumonia among study population

S.No.	Ventilator Induced Pneumonia	No. of patients	Percentage
1	CPIS >6 (VAP Positive)	28	13.3
2	CPIS ≤6 (VAP Negative)	182	86.7

Table 4: Distribution of vap patients (n=28) according to duration of acquiring Pneumonia

S.No.	Duration (days)	No. of patients	Percentage
1	Early onset (within 4 days)	7	25.0
2	Late onset (>4 days)	21	75.0

Mean±SD: 6.86±2.58days; Min-Max (median): 4-14 (6.00) days

Table 5: Distribution of VAP patients (n=28) according to organism involved for VAP

S.No.	Diagnosis	No. of patients	Percentage
1	<i>Acinetobacter</i> spp.	5	17.9
2	<i>E. coli</i>	1	3.6
3	<i>Enterococcus</i> spp.	1	3.6
4	<i>Klebsiella</i> spp.	15	53.6
5	<i>Pseudomonas</i> spp.	6	21.4

Table 6: Comparison of VAP and Pre-VAP pathogens in routine culture

S.No.		No. of patients	Percentage
1	VAP and Pre-VAP pathogens same	19	67.9
2	VAP and Pre-VAP pathogens not same	7	25.0
3	No colonizers on Pre-VAP	2	7.1

Table 7: Association of VAP and duration of MIV of study population

Group	No. of patients	Min.	Max.	Mean	S.D.
VAP	28	4	28	15.71	6.39
No-VAP	182	2	23	6.10	3.96
Total	210	2	28	7.38	5.44

t'= 10.886; p<0.001

Table 9 shows the Antibiotic resistance pattern of Gram-negative bacteria as well as Non-fermenters and Gram-positive bacteria.

Table 10 shows positive predictive value of organisms found during surveillance culture to that of found during VAP leading to diagnostic accuracy.

DISCUSSION

This particular study was done to know the common colonizers present in respiratory tract of patients on MIV admitted in that unit of a tertiary care hospital in Lucknow where critically ill patients are treated, by doing surveillance culture of the endotracheal aspirate and to see whether the culture can predict the organisms in an event of VAP. Ventilator associated pneumonia in our study was confirmed in those patients having clinical pulmonary infection score >6. Only 28 (13.3%) patients had CPIS >6 during their stay on mechanical invasive ventilation, rest 182 patients did not acquire pneumonia during their stay on MIV.

Table 8: Association of VAP and outcome of study population

S.No.	Outcome	VAP (n=28)		No-VAP (n=182)		Total (N=210)	
		No.	%	No.	%	No.	%
1	Discharged	15	53.6	100	54.9	115	54.8
2	Expired	13	46.4	82	45.1	95	45.2

$\chi^2 = 0.018$ (df=1); $p=0.892$

In our study 28 patients were diagnosed of VAP, among these early onset VAP were (25%) and late-onset VAP were (75%), whereas in a study by Ahmed *et al.* all the cases were of late-onset VAP.¹⁶ A lower percentage of early-onset VAP (25%) was seen in our study, which was similar to that reported by Michel *et al.* in (29%) of patients,¹⁷ whereas Joseph *et al.* reported (41.7%) of early onset VAP case.¹⁸ The early-onset VAP cases in our study may be due to the fact that our hospital is a tertiary care center and some patients have already received some form of primary treatment at the Primary Health Center level and then referred, so probably they were colonized prior to admission which could be a contributor to occurrence of early-onset VAP. The common organisms responsible for VAP in our study was *Klebsiella pneumoniae* (53.6%) followed by *Pseudomonas aeruginosa* (21.4%), *Acinetobacter baumannii* (17.9%) *E.coli* (3.6%) and *Enterococcus* spp (3.6%). According to Joseph *et al* *Pseudomonas aeruginosa* (21.3%) and *Acinetobacter* spp. (23.4%) predominated.¹⁸ Prior colonization by same organism was observed in 67.85% of VAP positive cases. In pre-VAP surveillance culture 53.57% among these belonged to Enterobacteriaceae 28.57% were non-fermenters. Same kind of study conducted by Hayon *et al* showed that all these pathogens causing VAP were previously recovered from 35% of the pre-VAP surveillance culture.¹⁹ Hence colonization by these organisms play an important role in development of VAP. In this study, pre-VAP EA cultures predicted 67.85% (19/28) VAP cases, concordant to Joseph *et al* where the pre-VAP EA cultures were able to predict the VAP pathogens in 60.9% cases.¹⁸ In the present study the sensitivity of prior colonization in predicting VAP for

Table 9: Percentage resistance of various antibiotics against bacterial isolates

Colonizers	No. of isolates	PIT	AMC	CTR	CPM	CIP	LEVO	AK	IPM	MRP	TOB
Gram Negative bacteria											
<i>K.pneumoniae</i>	97	79.4	100	89.7	89.7	89.7	79.4	79.4	69.1	59.8	69.1
<i>E. coli</i>	29	51.7	69.0	75.9	75.9	75.9	75.9	41.4	58.6	34.5	41.4
<i>Citrobacterkoseri</i>	8	100	100	100	100	100	75	75	87.5	100	75
<i>Proteus vulgaris</i>	2	0	100	100	100	100	100	100	0	0	100
Non-fermenters											
<i>P. aeruginosa</i>	34	17.6	–	76.5	50.0	–	26.5	32.4	32.4	26.5	32.4
<i>Acinetobacterbaumani</i>	18	100	100	100	100	100	100	100	88.9	94.4	100
Colonizers	No. of isolates	VAN	DO	LZ	LE	AMP	AK	CD	CX	HLG/ GEN	TEI
Gram Positive bacteria											
<i>Entero- coccus</i> spp.	4	60.0	0.0	0.0	100	100	40.0	0.0	–	20.0	40.0
CONS	6	0.0	66.6	0.0	33.3	33.3	33.3	33.3	33.3	33.3	33.3
<i>S. aureus</i>	5	0.0	60.0	100	60.0	100	60.0	60.0	40.0	60.0	100

Table 10: Prediction of VAP pathogens by determining prior colonization leading to diagnostic accuracy

Organism	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
<i>Acinetobacterbaumani</i>	3	0	23	2	60.0	100.0	100.0	92.0	92.9
<i>E. coli</i>	1	0	27	0	100.0	100.0	100.0	100.0	100.0
<i>Enterococcus</i> spp.	0	1	26	1	0.0	96.3	0.0	96.3	92.9
<i>Klebsiella pneumoniae</i>	11	2	11	4	73.3	84.6	84.6	73.3	78.6
<i>Pseudomonas aeruginosa</i>	4	1	21	2	66.7	95.5	80.0	91.3	89.3

Acinetobacter baumannii was (60%) and that for *Pseudomonas spp.* was (66.7%) has compatibility with the study done by Depuydt *et al* where ET aspirate surveillance culture showed sensitivity of 69% in predicting VAP pathogens.²⁰ This study showed an incidence of VAP to be 18.06 per 1000 ventilator days. A similar study conducted in Delhi had a comparatively lower incidence of VAP i.e. 11.5 per 1000 ventilator day¹⁶ but in another study conducted at Pondicherry by Joseph *et al*, comparatively higher incidence of 22.94 per 1000 ventilator days.¹⁸ Rate of mortality in our study among VAP patients was 46.4% compared to non-VAP patients (45.1%) which is not of much significance. Ahmed *et al.*, found the crude mortality rate of VAP patients to be 33.3%.¹⁶ Whereas Joseph *et al.* had a mortality rate of 16.2%.¹⁸ However Rocha *et al.*, Rodrigues *et al.* found higher mortality rates between 32.1% and 70.9%.^{21,22} A high degree of antibiotic resistance was observed by Gram negative as well as Gram positive isolates. Most of our VAP patients were found to have risk factors for Multi drug resistant microorganisms, explaining the high rate of colonization by these pathogens.

What this study adds

This study provided ample information about the relation between the colonizers (microorganisms) of upper respiratory tract obtained by surveillance culture of ET aspirate on the day of admission of patient in Critical Care Unit as well as at regular intervals thereafter which was found to be of the same species when VAP sets in. Therefore, when VAP occurs, previous knowledge about organisms, can be used to administer drugs found to be effective for that particular species, thus preventing drug resistant and leading to cure.

CONCLUSION

The three most common colonizing organism isolated from patients on MIV were found to be *Klebsiella pneumoniae* (46.2%), *Pseudomonas aeruginosa* (16.2%) and *E. coli* (13.8%) followed by *Acinetobacter baumannii* (8.6%), *Citrobacter koseri* (3.8%), CONS (2.9%), *S. aureus* (2.4%) and *P. vulgaris* (1.0%). Among the VAP positive patients, prior colonization was observed with same organism in 67.85% patients, suggesting role of ETA in aiding the diagnosis of VAP.

Thus, can be concluded that prolonged duration of mechanical ventilation, poses an increased chance of colonization by MDR microorganisms in the otherwise sterile lower respiratory tract, leading to HAI such as VAP. Routine quantitative surveillance culture of ETA samples allows prospectively to determine the incidence and sequence of LRT colonisation to infection in patients on MV.

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REFERENCES

1. WHO. Report on the burden of endemic health care-associated infection worldwide. World Health Organization, Geneva, 2011.
2. CDC. Types of healthcare-associated infections. Healthcare-associated infections (HAIs), 2016
3. Koenig SM and Truitt JD. Ventilator-Associated Pneumonia: Diagnosis, Treatment, and Prevention. *Clinical Microbiology Reviews*. 2006;19(4):637-657. <https://doi.org/10.1128/CMR.00051-05>
4. Morehead RS and Pinto SJ. Ventilator-associated pneumonia. *Arch Intern Med*. 2000;160:1926-1936. <https://doi.org/10.1001/archinte.160.13.1926>
5. Chastre J and Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med*. 2002;165:867-903. <https://doi.org/10.1164/ajrccm.165.7.2105078>
6. Sottile FD, Marrie TJ, Prough DS, Hobgood CD, Gower DJ, Webb LX, *et al.* Nosocomial pulmonary infection: possible etiologic significance of bacterial adhesion to endotracheal tubes. *Crit Care Med*. 1986; 14:265-270. <https://doi.org/10.1097/00003246-198604000-00001>
7. Craven DE, Lichtenberg DA, Goularte TA, Make BJ and McCabe WR. Contaminated medication nebulizers in mechanical ventilator circuits. Source of bacterial aerosols. *Am J Med*. 1984;77(5):834-838. [https://doi.org/10.1016/0002-9343\(84\)90520-5](https://doi.org/10.1016/0002-9343(84)90520-5)
8. Michel F, Franceschini B, Berger P, Arnal JM, Gannier M, Sainty JM, *et al.* Early antibiotic treatment for BAL-confirmed ventilator-associated pneumonia: a role for routine endotracheal aspirate cultures. *Chest*. 2005;127:589-597. <https://doi.org/10.1378/chest.127.2.589>
9. Van Vught LA, Klownberg PM, Spironi C, *et al* for the MARS Consortium. Incidence, risk factors, and attributable mortality of secondary infections in the intensive care unit after admission for sepsis. *JAMA*. 2016;315(14):1469-1479. <https://doi.org/10.1001/jama.2016.2691>
10. Mackie TJ and McCartney JE. *Practical medical microbiology*, 14th ed., New York: Churchill Livingstone; 1996.
11. Baselski VS, el-Torky M, Coalson JJ and Griffin JP. The standardization of criteria for processing and interpreting laboratory specimens in patients with suspected ventilator-associated pneumonia. *Chest* 1992;102:571S-579S. https://doi.org/10.1378/chest.102.5_Supplement_1.571S
12. Porzecanski I and Bowton DL. Diagnosis and treatment of ventilator-associated pneumonia. *Chest*. 2006;130:597-604. <https://doi.org/10.1378/chest.130.2.597>
13. Wu CL, Yang DI, Wang NY, Kuo HT and Chen PZ. Quantitative culture of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. *Chest*. 2002; 122:662-668. <https://doi.org/10.1378/chest.122.2.662>
14. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved

- standard, 9th ed. Document M2-A9. Wayne, PA: CLSI; 2016.
15. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD and Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic 'blind' bronchoalveolar lavage fluid. *Am Rev Respir Dis.* 1991;143:1121-1129.
https://doi.org/10.1164/ajrccm/143.5_Pt_1.1121
 16. Ahmed NH, Hussain T and Biswal I. Antimicrobial resistance of bacterial isolates from respiratory secretions of ventilated patients in a multi-specialty hospital. *Avicenna J Med.* 2015;5:74-78.
<https://doi.org/10.4103/2231-0770.160233>
 17. Michel F, Franceschini B, Berger P, Arnal JM, Gainnier M, Sainty JM, *et al.* Early antibiotic treatment for BAL-confirmed ventilator-associated pneumonia: a role for routine endotracheal aspirate cultures. *Chest.* 2005;127:589-597.
<https://doi.org/10.1378/chest.127.2.589>
 18. Joseph NM, Sistla S, Dutta TK, Badhe AS and Parija SC. Ventilator-associated pneumonia: role of colonizers and value of routine endotracheal aspirate cultures. *Int J Infect Dis.* 2010; 14(8): e723-e729.
<https://doi.org/10.1016/j.ijid.2010.02.2248>
 19. Hayon J, Figliolini C, Combes A, Trouillet JL, Kassis N, Dombret MC, *et al.* Role of serial routine microbiologic culture results in the initial management of ventilator-associated pneumonia. *Am J Respir Crit Care Med.* 2002;165:41-46.
<https://doi.org/10.1164/ajrccm.165.1.2105077>
 20. Depuydt P, Benoit D, Vogelaers D, Claeys G, Verschraegen G, Vandewoude K, *et al.* Outcome in bacteremia associated with nosocomial pneumonia and the impact of pathogen prediction by tracheal surveillance cultures. *Intensive Care Med.* 2006; 32: 1773-1781.
<https://doi.org/10.1007/s00134-006-0354-8>
 21. Rocha Lde A, Vilela CA, Cezario RC, Almeida AB and Gontijo Filho P. Ventilator associated pneumonia in an adult clinical surgical intensive care unit of a Brazilian university hospital: Incidence, risk factors, etiology, and antibiotic resistance. *Braz J Infect Dis.* 2008;12:80-85.
<https://doi.org/10.1590/S1413-86702008000100017>
 22. Rodrigues PM, Carmo Neto Ed, Santos LR and Knibel MF. Ventilator-associated pneumonia: Epidemiology and impact on the clinical evolution of ICU patients. *J Bras Pneumol.* 2009;35:1084-1091.
<https://doi.org/10.1590/S1806-37132009001100005>

Author's contributions:

SS-Concept and design of the study, interpreted the results, prepared first draft of manuscript and critical revision of the manuscript; **FH**- Statistically analysed and interpreted, reviewed the literature and manuscript preparation; **SA**- Preparation of manuscript and revision of the manuscript; **KI**- Design of the study, statistically analysed and interpreted; **MS**- Concept and coordination of the overall study.

Work attributed to:

Department of Microbiology, Era's Lucknow Medical College and Hospital, Era University, India.

Orcid ID:

Dr. Sana Siddique - <https://orcid.org/0000-0003-1314-1729>
 Dr. Fareya Haider - <https://orcid.org/0000-0002-9267-4867>
 Dr. Sharique Ahmad - <https://orcid.org/0000-0002-9637-8838>
 Dr. Khalid Iqbal - <https://orcid.org/0000-0002-9460-5806>
 Dr. Mastan Singh - <https://orcid.org/0000-0002-4543-2152>

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