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The utility of a modified technique for lower respiratory tract sampling in COVID-19 ICU and review of diagnostic approaches in suspected ventilator associated pneumonia.

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ABSTRACT

INTRODUCTION: Lower respiratory tract (LRT) sampling is an aerosol generating procedure. In COVID 19 pandemic, guidelines have advocated caution against all aerosol generating procedures. However, microbial cultures on tracheobronchial aspirates are important to guide antibiotic usage in ventilator-associated pneumonia (VAP).

MATERIAL AND METHODS: In our tertiary care COVID-19 intensive care unit (ICU), a protocol was set for using closed suction system for timely LRT sampling in VAP and to reduce the risk of exposure to respiratory secretions. Timing of sample collection was as per intensivist discretion following CDC VAP definition. This prospective study was conducted between June to November 2020, to assess the utility of this technique in diagnosis of suspected VAP. Microbiological and clinico-radiological parameters were documented. Heavy growth (>10^5 cfu/mL) on semiquantitative culture was taken as significant.

RESULTS: Total 69 samples generated from 63 patients were analyzed. Mean age 54.48 years and 77.78% of patients had one or more comorbidities. Average duration of invasive ventilation prior to the first culture was 7.14 ± 4.36 days. Progressive radiological worsening at the time of sample collection was in 92.75% (64 of 69 episodes). Microbiological diagnosis of VAP was confirmed in 76.81%. Culture reports guided antibiotic change. Insignificant culture growth in 13.06%. The positivity rate for early and late (>4days) samples were 69.56% and 80.43% respectively. 95% of culture isolates were Gram negative microorganisms. Most common being Acinetobacter baumannii (41.67%) and Klebsiella pneumoniae (31.66%) in both early and late VAP. Around 85% were multidrug resistant organisms. There were no significant adverse events related to sampling technique.

CONCLUSIONS: Lower respiratory tract sampling using closed suction system is easy to execute and minimizes procedure related risk to both patient and health care workers in COVID-19 ICU. Gram negative MDR pathogens are prevalent in both early and late VAP. Need further comparative study to understand effectiveness of this technique against other conventional techniques in VAP diagnosis.

KEY WORDS: Closed suction system, ventilator-associated pneumonia, COVID-19, lower respiratory tract sampling.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) has emerged worldwide creating new challenges in healthcare and high demand for intensive care services. Two highly affected countries with COVID-19 pandemic reported a high burden of infection among healthcare workers [1,2]. In healthcare settings prevention of spread of SARS-COV-2 carried paramount importance. This led to various modifications in respiratory critical care to prevent spread of infection during aerosol generating procedures such as endotracheal intubation, use of non- invasive ventilation and bronchoscopy [3,4]. In severe cases of SARS-COV-2 pneumonia; immune dysregulation, lymphopenia, use of immunosuppressants and prolonged ICU stay make the patient prone for nosocomial infections [5]. Ventilator associated pneumonia is the second most common nosocomial infection reported in mechanically ventilated cases. High prevalence of ventilator associated Pneumonia (VAP) has also been reported in COVID-19 patients [6]. The most accurate technique in diagnosis of VAP is debatable. It's even more challenging in COVID19 ICU [7].

Recent SSC guidelines have mentioned use of endotracheal aspirate as an acceptable sample in diagnosis of VAP in preference to bronchoalveolar lavage [3]. The traditional endotracheal sampling technique is inexpensive and simply performed bedside but carries high risk of aerosolization of respiratory secretions [3]. In view of high viral load in LRT secretions, its suggested to avoid such aerosol generating procedures unless absolutely necessary [3,8]. In severely ill hypoxemic patients on ventilators, there is considerable risk of worsening hypoxemia or hemodynamic instability during tracheobronchial sampling. However, microbial cultures on tracheobronchial aspirates plays an important role in the diagnosis of VAP and usage of appropriate antimicrobial agents. This is one of the important factors determining outcome in critically ill and in prevention of drug resistant superbugs in ICU. Concerns related to timely lower respiratory tract sampling in severely hypoxemic mechanically ventilated patients and associated risk of worsening and risk of aerosolization while using conventional sampling techniques in COVID-19 patients are rarely addressed in VAP studies. To address this concern during the peak of the pandemic, we continued LRT sampling with novel use of an available closed suction system (CSS). In mechanically ventilated patients, closed suction catheter system has become a standard of care to clear tracheobronchial secretions.

This study analyses the microbiological yield of lower respiratory tract samples obtained using a closed suction system in VAP suspects in COVID-19 ICU and its radiological correlation.

MATERIAL AND METHODS

Considering the routine implementation and safety of closed suction system in mechanically ventilated patients, during the peak of pandemic a protocol was set to continue lower respiratory tract sampling using this device in COVID-19 ICU. This prospective observational study was conducted in an adult COVID-19 ICU of a tertiary care teaching hospital in Western India between June to November 2020.The aim was to assess the utility of this modified technique in diagnosis of suspected VAP in COVID-19 ICU. The study was approved by the institutional ethical committee (Ref No. *BVDUMC/IEC/28*). Lower respiratory tract aspirates were obtained using a closed suction system (CSS-A) through endotracheal tube (ETT) or tracheostomy tube

(TT) in place. The timing of sample collection was as per the clinical or radiological suspicion of VAP in accordance with CDC VAP definition [9]. As per COVID-19 ICU protocol, patient's relatives were counselled about the need of culture for patient management and safety of sampling procedure as clearing of secretions using closed suction system forms a part of routine care in all mechanically ventilated patients. Informed consent was obtained for data collection maintaining anonymity.

Patient data was collected in a preformed proforma to document age, gender, comorbidities, prior antibiotic usage and duration of invasive ventilation prior to sampling. Serial Chest X ray or HRCT findings were noted. Standard VAP prevention protocol, hand hygiene and infection control practices were followed. The treatment protocol for steroids and Tocilizumab was as per CDC/ICMR recommendations for severe COVID-19 pneumonias.

Protocol for lower respiratory tract sample collection

Two personnel, one intensive care specialist along with a respiratory therapist performed sample collection with due aseptic precautions and following airborne infection control practices.

The closed suction catheter with single or double side ports having length of 54 cm for endotracheal tube and 35 cm for tracheostomy tube was used to collect lower respiratory tract samples. To avoid contamination from colonizers in the catheter, a new closed suction system or the one in place less than 72 hrs. was used for this purpose.

Step 1. First clear the ETT and tracheal secretions with a closed suction catheter in place. Oxygenation was optimized with FiO_2 1.0 during the procedure.

Step2: A new set consisting of a closed suction catheter system attached to mucus trap and disposable suction tubing was prepared and aligned on sterile drape (Figure 1a).

Step 3: ETT was briefly clamped during disconnections and a new set attached in line with the endotracheal tube.

Step 4: Sterile syringe filled with 5ml Normal saline kept ready attached to the irrigation side port as shown in Figure 1b.

Step 5: In supine position, a catheter was slowly advanced till full length and then sampling was done with controlled suctioning. To avoid contamination from artificial airway, suction was not applied while passing in and out of the ETT or TT. Lastly, the catheter was flushed with 2-5 ml of saline only if the secretions were thick in the suction catheter and if the sample in a trap was inadequate.

Samples collected in mucus traps were double packed and immediately transported to microbiology laboratory to process by conventional method according to Clinical and Laboratory Standards (CLSI-NABL). The samples were cultured using semi quantitative method. The heavy growth on culture plates corresponded to $\geq 10^5$ CFU/mL. Sample microscopy and gram stain findings were also recorded.

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Data analysis

The relevant clinical and microbiology data was summarized into tables and analyzed using Microsoft Excel and SPSS software. The type of data is quantitative and qualitative. Continuous variables are reported as means or averages. Categorical variables were reported as frequency (n) and percentages and compared using the Chi-squared test or Fisher's exact test, as appropriate. $P<0.05$ was considered to be significant.

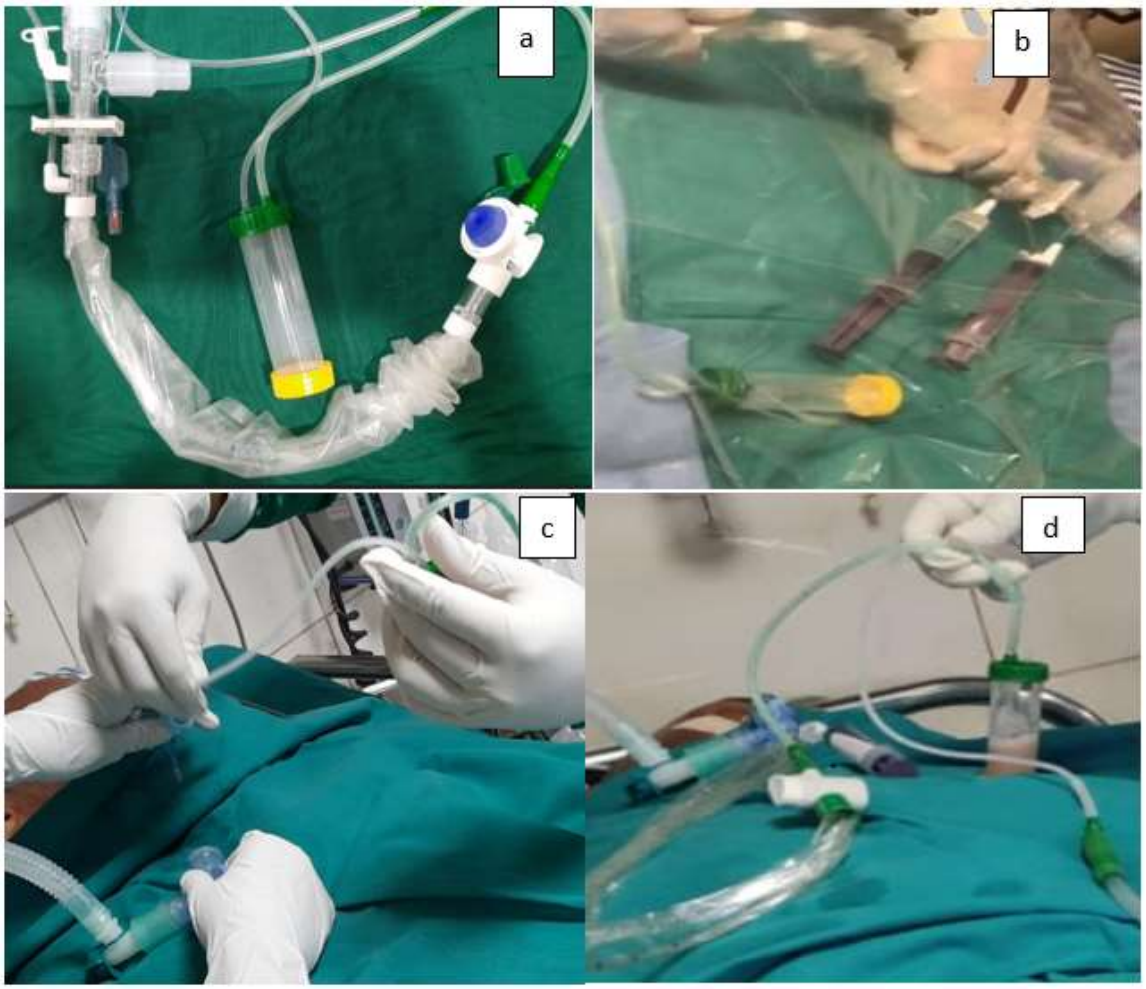

Figure 1. (a): CSS with mucus extractor set-ready to use. **(b)**: Closed suction system (CSS) technique of LRT sampling - No exposure or spillage. **(c)**: Traditional open endotracheal aspirate -direct exposure. **(d)**: Collected sample using CSS

Multimedia 1. Protocol for lower respiratory tract sample collection.

RESULTS

The analysis included a total 69 closed suction system aspirate (CSS-A) samples collected from 63 COVID-19 patients with suspected VAP. 11 patients underwent sampling twice during their stay in the COVID ICU. Increased tracheobronchial secretions provided an adequate quantity of sample in 69 of 74 aspirates attempted. Clinical characteristics of these 63 patients are presented in Table 1. This group was male predominant with a mean age of 54.48 years. Total 77.78% (n-49) patients had one or more comorbid conditions, commonest being hypertension (49.2%), diabetes mellitus (42.8%), COPD (12.7%) followed by morbid obesity (8%). Average duration of invasive ventilation before culture was 7.14±4.36 days.

CSS sample microscopy and culture results are given in Table 2. Microbial cultures were positive with significant growth (>10^5 cfu/mL) in 53 out of 69 (76.81%) samples cultured. (Chart 1) One sample had candida growth on culture. Among all positive cultures, 83% samples had moderate to many (3+/4+) pus cells and 2+ to 4+ organisms on gram stain, while 13.21% (n-7) samples had only few pus cells and few GNB/GNCB on microscopy with significant culture growth of Acinetobacter baumannii (n-6) and Elizabethkingia Meningoseptica (n-1).

Table 1. Demographic and clinical characteristics of severe COVID -19 patients.

Parameter	Findings
Mean age	54.48 Years (23 to 80 years)
Males	69.84% (n-44/63)
Any comorbidity	77.78%
Duration of hospital stay before intubation (<4 days)	74.60 %
Average duration of invasive ventilation before first culture	7.14 ± 4.36 days
Antibiotic usage before first culture	81%
Total Leucocyte count	16000 ± 9400/cmm
Procalcitonin >0.5 (0.51 to 71)	76.19%
Fever > 38.6^0 C	81.16%
PaO_2/ FiO_2 ratio	108 ± 46 mm of Hg
Progressive radiological worsening	92.75% (n-64/69 episodes)
Increased tracheal secretions	82%
Septic Shock	61.90%
Total ventilator days	14.79+ 8.58 days (4 to 48 days)

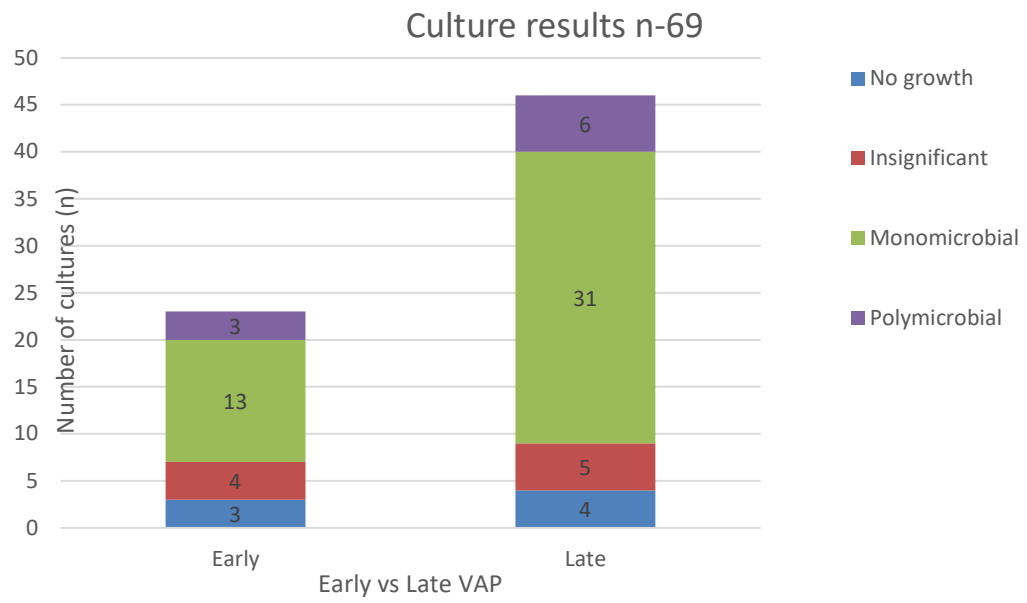

Chart 1. Culture results as per early vs late VAP.

Microbiological profile (Chart 2): Total 60 microorganisms were isolated from 53 cultures with >10^5 cfu/mL (46 monomicrobial and 7 polymicrobial). Acinetobacter baumannii was the most common microorganism isolated (n-25), followed by *Klebsiella pneumoniae (n-19) and Pseudomonas aeruginosa (n-9).* Among all (n-57) gram-negative isolates, 63.16% were carbapenem resistant. 17.54% of *Enterobacteriaceae* were ESBL (-carbapenamase) producing. Acinetobacter baumannii isolated on 2 samples were polypeptide resistant but sensitive to carbapenem and Tigecycline. E. Meningoseptica was resistant to carbapenem, aminoglycoside and polypeptides but sensitive to TMP-SM and ciprofloxacin. Gram positive isolates (5%) were all MRSA. Sampling within 48hrs of postintubation in 6 patients grew pan-sensitive organisms on culture (4 Klebsiella, 2 Pseudomonas).

Table 2. CSS sample microscopy and culture findings.

Parameter		Findings n (%)
Microscopy (n-69)		
• Few pus cells		22
• Moderate to Abundant pus cells		47
Gram stain smear		
• Few GNB/GPB		
• Moderate to Many GNB/GNCB ± few GPB		23
• Many GPB		42
		4
CSS-A culture growth (n-69)	>10^5	53 (76.81%)
	<10^5	9 (13.04%)
	No growth	7 (10.14 %)
Monomicrobial		44 (83.02%)
Polymicrobial		9 (16.98 %)
% Positive of Early samples (up to 4 days)		16/23 (69.56%)
Late samples (>4 days)		37/46 (80.43%)

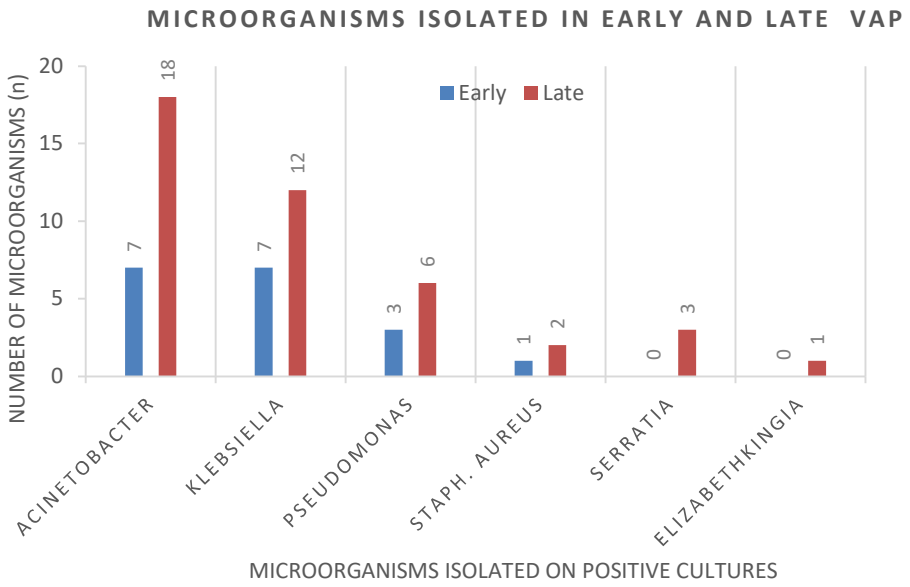

Chart 2. Microorganisms isolated on positive cultures.

Radiological data collected at the time of sampling showed progressive worsening with dense lobar or segmental opacities in 49, diffuse worsening in 15 and persistent non-resolving patchy areas of consolidation in 5. Necrotizing pneumonia and cavitation with or without empyema was secondary to Klebsiella pneumonia (n-4), Klebsiella plus Acinetobacter (n-1) and MRSA (n-1). There was a significant correlation (p value-0.009) between positive microbial culture and radiological suspicion of VAP (Table 3), though none of this was compared with standardized BAL culture for VAP diagnosis in our study. 18.75 % of radiological suspects were culture negative (Table 3). Considering the safety of this technique for LRT sampling, there were no significant adverse events during the procedure.

Table 3. Correlation between microbiologically confirmed and radiologically suspected VAP.

The Fisher exact test		Radiological VAP suspect Yes	No	Total	**p-value**
Microbial culture positive	Yes	52	1	53	
(>10^5cfu/L)	No	12	4	16	0.009
Total		64	5	69	

DISCUSSION

The diagnosis of a ventilator associated pneumonia based on clinical signs and symptoms lacks both sensitivity and specificity. Selection of diagnostic procedure is still debatable [10].

1.Need of a modified technique for lower respiratory tract sampling in COVID-19 ICU.

In COVID 19 pandemic, uncertainty about treatment and high mortality in severely ill cases pressed for the highest measures of airborne infection control and droplet precautions in health care settings.

In all techniques of tracheobronchial sampling such as endotracheal aspirates, bronchoalveolar lavage (BAL) or blind mini- BAL there is a risk of aerosolization of respiratory secretions. Splattering of secretions from the ventilator circuit during disconnections can create numerous infective droplets. Such disconnections are to be avoided in severely hypoxemic patients due to risk of worsening. To obtain bronchoalveolar lavage (BAL), a trained endoscopist with availability of bronchoscope is a prerequisite and carries risk of worsening hypoxemia and hemodynamic instability in severely hypoxemic patients [11]. In addition to this, bronchoscopy is an aerosol generating procedure and in COVID-19 pandemic guidelines have advocated caution against its routine use. The traditional method of obtaining tracheal aspirate is shown in Figure 1c. A study on environmental contamination during suctioning has shown that the air within 100–200 cm of an open endotracheal suction site is contaminated [12]. These negative effects of open suctioning are taken care of by a closed suction system. Most importantly it helps to prevent worsening of hypoxemia by maintaining positive end-expiratory pressure and reducing loss of volume during clearing of secretions in critically ill hypoxemic patients [13]. The closed suction catheter system has become a standard of care in mechanically ventilated cases. Changing inline closed suction system weekly vs daily was not associated with significant increase in VAP [14,15].

These important features of CSS stressed its usefulness in the SARS-COV-2 pandemic and narrowed down our search for a safe technique to obtain lower respiratory tract samples. The length of CSS for an adult endotracheal tube is 54 cm and for tracheostomy tube it is 31-35 cm. The protective outer sheath avoids direct handling of the inner catheter. The major advantage was ease in obtaining tracheal aspirates distal to artificial airway without major disconnections or direct exposure to patient's secretions. This also provided a better quantity of secretions (Figure 1d). Other advantages and limitations of this technique are discussed in Table 4. This technique cannot be an alternative to bronchoscopic sampling where site specific bronchoalveolar lavage with endobronchial visualization is required.

Table 4. Advantages and disadvantages of closed suction system for LRT sampling in patients on mechanical ventilator.

Advantages	Disadvantages
1. Reduced exposure to infective secretions 2. Reduced environmental contamination 3. Reduces the risk of contamination of sample from outside pathogens 4. Less invasive and easy way of sampling 5. Longer catheter length compared to mucus extractor catheter 6. Reduced volume loss/ loss of PEEP during suctioning 7. Low risk of worsening hypoxia and hemodynamics during sampling	1. Risk of contamination with colonisers from artificial airway 2. Poor sample quantity if no secretions 3. No comparison with BAL/ n-BAL as it's a proximal airway sample 4. Non-directed sampling 5. Added cost of changing closed suction catheter for sampling

2.Comparative evaluation of different sampling and culture techniques in diagnosis of VAP.

Quantitative culture of bronchoalveolar lavage (BAL) is advocated for optimum diagnosis and management of VAP [16]. But there are many limitations for its routine implementation. In many ICU's, direct tracheal aspirate is preferentially performed. Previous studies comparing BAL and tracheal aspirate cultures revealed variable concordance in microbiological yield [17,18,19]. There were no differences in clinical outcome or antibiotic usage in VAP when different culture techniques were compared [16,17].

In a comparative study, the overall agreement between the protected n-BAL and traditional technique (tracheal aspirate) was 72.5% for microorganisms isolated and colony count [20]. In another study comparing different sampling techniques in VAP diagnosis in Indian setting, the microbial cultures were positive in 84% and 68% of bronchoscopic brush or BAL and tracheal aspirates respectively [21]. In our study, 76.81% (n-53/69) of CSS-A samples were positive at $>10^5$ cfu/mL / heavy growth on semiquantitative culture. The traditional procedure for obtaining tracheal aspirates has a drawback of false positive cultures from biofilm which may not represent presence of lung parenchymal infection or of false negatives if inadequate sampling. In a study by Sara Gil-Perotin et al. endotracheal aspirates and biofilm culture grew the same microorganism in 56% cases [22]. In our technique, to avoid colonizers, the fresh sterile closed suction catheter was used. The sample was obtained distal to the artificial airway and no suction was applied in the tube. Additionally, patients had clinic-radiological signs as VAP suspect and on semiquantitative culture recommended higher threshold of heavy growth reported as $>10^5$ was taken as significant.

In a study comparing semi quantitative to quantitative cultures of tracheal aspirates for the yield of culturable respiratory pathogens, there was variable concordance (52-80%) between the two techniques [16]. Quantitative cultures are complex to perform, while in semiquantitative there is less handling of infected secretions and saves time [16,17]. CDC VAP guidelines suggests use of positive quantitative culture or corresponding semi-quantitative culture result from minimally contaminated LRT specimen [9].

3.Early vs late VAP and microbiological profile.

Initial data published during COVID-19 pandemic reported high rates of VAP from 48-86 % [23-25]. When compared to non-COVID, COVID-19 patients were at high risk of developing VAP.[23] No difference in distribution of causative bacteria between COVID and non-COVID [23]. In a small cohort from China, secondary infections were reported after mean duration of 4.5 (1–19) days of tracheal intubation with gram-negative bacteria in 71.43% [25]. Pre-COVID data on VAP for Indian population shows similar causative organisms as in our study group of severely ill COVID- 19 patients [26,27]. Acinetobacter baumannii was most common pathogen in both early and late onset VAP [26,28]. In our data analysis, the culture isolates within 2 days of intubation (2 Pseudomonas, 4 Klebsiella) in patients with comorbidities such as DM, COPD were pan-sensitive, representing community acquired pathogens as coinfections with SARS-COV-2. Indication for sampling was atypical Chest X ray, baseline leukocytosis and increased tracheal secretions in patients with DM (n-6) and DM plus COPD (n-1). These patients were intubated within 24hrs. of

hospitalization and duration of illness was >4 days before hospitalization. In simultaneous cultures of blood and other respiratory samples wherever obtained, organisms isolated on blood (n-17), bronchoalveolar lavage (n-4) and pleural fluid (n-3) were the same as on CSS-A culture suggesting etiological significance. Total 13.04% samples had insignificant growth- mainly of Acinetobacter baumannii, Burkholderia cepacia and Stenotrophomonas maltophilia. Prior use of broad-spectrum antibiotics, inadequate samples and sampling of colonizers have shown to reduce culture yield. The isolated microorganisms in this study represents the microflora in our hospital environment similar to non-COVID ICU. Early in COVID pandemic overburdened ICU along with compromised hand hygiene due to heavy fulltime PPE and gloves added to the risk of cross infections in immune dysregulated patients with severe COVID-19.

4.Chest radiology in COVID-19 Pneumonia.

Chest radiography alone has poor sensitivity in diagnosis of VAP [29]. As per experience in COVID pneumonia from multiple centers the radiological findings are relatively homogenous with uncommon patterns in less than 10%. [30] Presence or new development of atypical infiltrates, nodular, cavitating or lobar consolidation can provide clues for suspecting secondary infections (Figure 2).

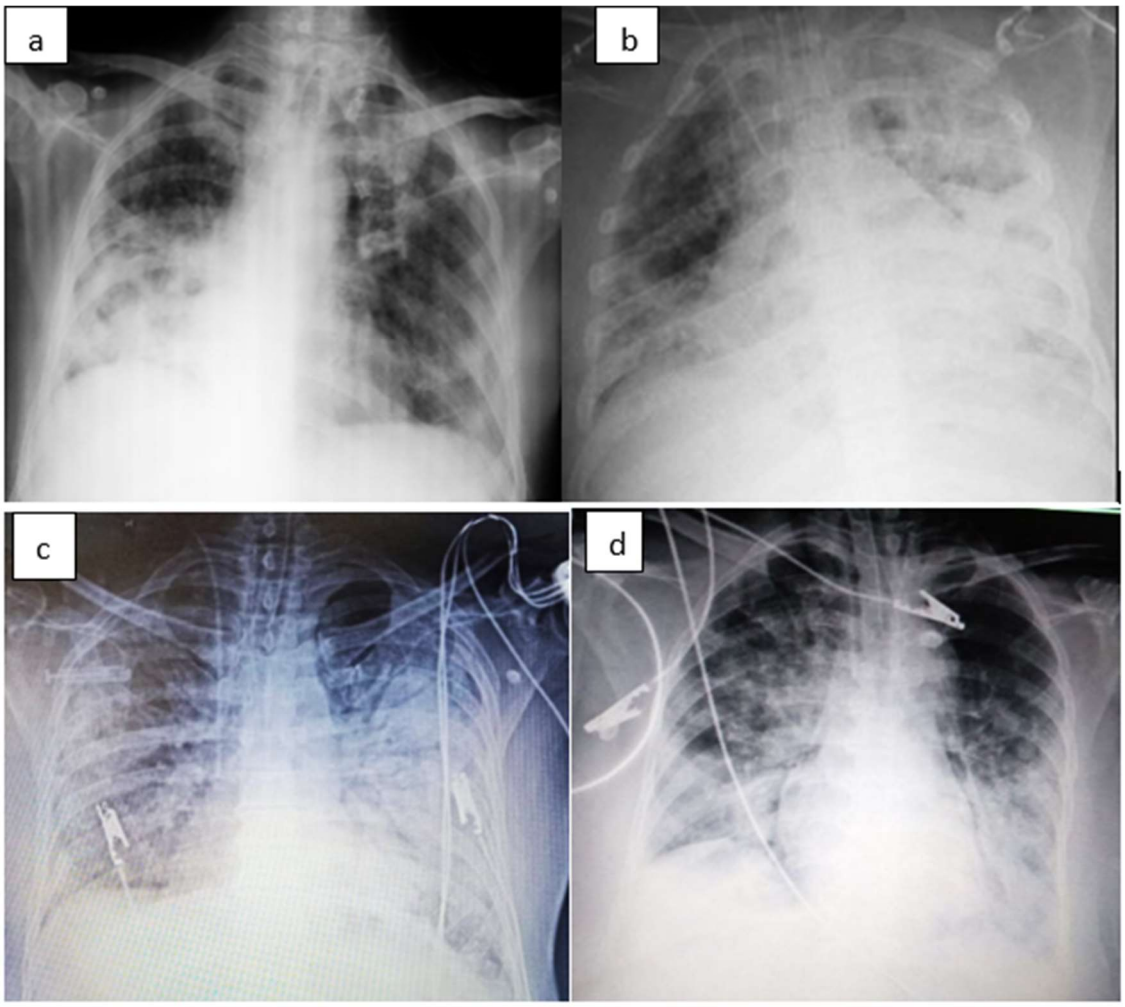

Figure 2. Chest X rays- COVID pneumonia with secondary infection in ICU.
(a), **(b)**: Necrotising pneumonia with cavitation. **(c)**: Bilateral > Left lung consolidation.
(d): Worsening bilateral lower lobe pneumonia with new dense right upper lobe opacities

Worsening of primary COVID-19 as organizing pneumonia, poor quality of chest X-ray in ICU, worsening ARDS or fluid overload makes it challenging to differentiate between various causes. In our study, radiological worsening consistent with secondary bacterial pneumonia was in 92.75% (n-64), out of which 81% (n-52/64) were culture positive.

5.Clinical Pulmonary Infection Score (CPIS) in VAP diagnosis.

The available evidence does not support use of CPIS as a diagnostic or therapy guiding tool [31]. Though serial change in CPIS score has shown to have better predictive value in VAP. It's even more challenging considering primary lung involvement and variable progression of the COVID19 pneumonia/ ARDS, leukocytosis due to steroids and fever as a feature of cytokine storm. Hence suspecting the secondary infection early and isolating the organism on culture is of prime importance [6]. The aim of reducing exposure to patients' respiratory secretions, has directed us to effectively use the available resources without compromising both healthcare worker and patient safety. This technique helped us to obtain lower respiratory tract samples and guide the antibiotic therapy as per the antimicrobial resistance pattern. This technique can also be used to obtain an early LRT sample in COVID-19 suspects with severe pneumonia or ARDS where nasopharyngeal swab is negative.

Limitations of this study. This study lacks the comparison with respect to microbiological yield and procedure related complications with simultaneous conventional sampling techniques of open tracheal aspirate or standard bronchoalveolar lavage (BAL) or n-BAL. Though this cannot be regarded as a major limitation in its implementation in COVID-19 pandemic where minimizing the risk to health care workers and optimizing management of severely ill in overwhelmed critical care settings is of paramount importance. We couldn't compare the benefit of this novel approach to sample collection in risk reduction for healthcare workers to conventional methods of sample collection. We suggest one innovative device based on this closed suction system specifically for lower respiratory tract sampling as a closed blind mini- BAL set. Longer length of catheter to reach distal subsegments will help to obtain appropriate LRT sample. The 2 side ports provided at present in some CSS, the proximal one irrigates the catheter when withdrawn out. The distal MDI port can be used to flush the endotracheal tube from inside which may help to clear secretions and increase sample quantity but at the same time it will disperse the biofilm and colonizers to the periphery of the lung and is not recommended. So, in a suggested modification of CSS for LRT sampling if one port opens up inside the distal end of long protected inner catheter, it will help to give lavage directly in distal lung subsegments as in blind-mini-BAL and obtain uncontaminated samples without major disconnections and direct contact with secretions.

CONCLUSIONS

The closed suction system facilitates timely lower respiratory tract sampling even in severely hypoxemic patients on ventilators, reducing procedure related risk to healthcare workers in COVID-19 ICU. Gram negative MDR pathogens are prevalent in both early and late VAP. Need further comparative study to understand effectiveness of this technique against other conventional techniques in VAP diagnosis.

SUPPLEMENTARY INFORMATION

Funding: *This research received no external funding.*
Institutional Review Statement: *The study was conducted according to the guidelines of the Declaration of Helsinki.*
Informed Consent Statement: *Informed consent was obtained from all subjects involved in the study.*
Data Availability Statement: *The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.*
Conflicts of Interest: *The authors declare no conflicts of interest.*
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