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Ventilator-associated pneumonia: role of colonizers and value of routine endotracheal aspirate cultures

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SUMMARY

Objectives: To determine the role of colonizers in the causation of ventilator-associated pneumonia (VAP) and the value of routine pre-VAP endotracheal aspirate (EA) cultures in appropriately treating VAP.
Methods: A prospective observational cohort study was conducted over a period of 15 months. Two hundred patients on mechanical ventilation for > 48 h were studied.
Results: *Acinetobacter spp* (33.7%) and *Pseudomonas spp* (29.8%) were the most common colonizers. Of the 200 patients, 36 developed VAP. In 20 VAP patients, the pre-VAP EA culture-based strategy was not useful. However, in the remaining 16 VAP patients, a pre-VAP EA culture-based strategy would have appropriately treated 13 (81%; 95% confidence interval (CI) 62–100%), in comparison to only nine (56%; 95% CI 32–80%) by the American Thoracic Society (ATS) strategy. The seven patients in whom the ATS guidelines were inappropriate had *Acinetobacter spp* and *Pseudomonas spp* resistant to the higher antibiotics recommended by the ATS for multidrug-resistant pathogens. The positive predictive values of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from pre-VAP EA cultures were 88%, 83%, and 100%, respectively.
Conclusion: VAP patients should be treated based on ATS guidelines, but whenever *P. aeruginosa*, *A. baumannii*, and MRSA are isolated from pre-VAP EA cultures, the initial antibiotic therapy should be extended to treat these.

1. Introduction

Ventilator-associated pneumonia (VAP) is the most frequent intensive care unit (ICU)-acquired infection, occurring in 9–24% of patients intubated for longer than 48 h.[1] It is associated with increased morbidity, prolonged hospitalization, and increased healthcare costs.[2,3] The diagnosis of VAP remains controversial because of the absence of a 'gold standard' for diagnosis.[4] Lung biopsy, which can be considered as the gold standard, is not feasible in the clinical setting.[5] Therefore, the American Thoracic Society (ATS) guidelines recommend a quantitative distal sampling of the lung, by bronchoscopic or non-bronchoscopic approaches, to improve the specificity of diagnostic methods.[4] The diagnostic challenge is further complicated by the need to differentiate between pathogenic microorganisms and colonizing flora.

Upper airway colonization is considered an important predisposing factor for the development of VAP.[6] Secretions of the lower respiratory tract obtained by bronchoscopy may accurately diagnose the pathogens.[7] However, it may do so too late in the course of VAP to reduce the morbidity, as bronchoscopy is usually done at the advanced stages. Therefore, culture of endotracheal aspirates (EA) is more relied on as it is less invasive and can be obtained early in the course of infection.

Routine endotracheal aspirate cultures of critically ill patients in ICUs may be predictive of patients who are at high risk of invasive disease, and may guide the selection of appropriate empirical therapy based on the predominant pathogens identified in these cultures in the event of the development of VAP.[8] But the role and accuracy of such approaches remain controversial. In a study in which 1626 respiratory surveillance samples were collected, surveillance cultures effectively predicted only one episode of VAP and one of tracheobronchitis.[9] But in another study, the tracheal surveillance cultures predicted the pathogen in 67 out of 110 episodes of nosocomial pneumonia.[10] Similarly, in a study from France, routine weekly endotracheal aspirate cultures guided adequate antibiotic therapy in 85% of VAP cases.[11] Since there are

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contradictory reports on the role of routine cultures there is a need to re-evaluate the role of colonizers in predicting VAP pathogens. Failure to treat the potential pathogens increases the morbidity and mortality, while overenthusiastic treatment of the colonizing organisms results in unnecessary exposure to broad-spectrum antibiotics and predisposes to infection with multidrug-resistant (MDR) pathogens.[12]

The primary aim of our study was to determine if pre-VAP EA cultures have any supplementary role to play, along with the ATS guidelines, in the treatment of VAP. The secondary objectives of this study were: (1) to identify the common colonizers and their role in the causation of VAP, (2) to determine the value of routine tracheal aspirate cultures performed before the onset of VAP in predicting the causative microorganisms and selecting effective empirical antimicrobial therapy in the event of subsequent VAP, and (3) to compare the appropriateness of treatment based on the pre-VAP EA strategy with that of the ATS strategy.

2. Materials and methods

2.1. Setting and subjects

A prospective observational cohort study was conducted during a period of 15 months from October 2006 to December 2007, in the departments of microbiology, medicine, and anesthesiology and critical care at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), a tertiary care hospital in Pondicherry, India. All consecutive adult patients on mechanical ventilation (MV) for >48 h in the medicine intensive care unit (MICU) and critical care unit (CCU) were included in this study. Patients with pneumonia prior to MV or within 48 h of MV were excluded. Only the first episode of VAP was evaluated. This study was approved by the research and ethics committees of the Institute, and informed consent was obtained from the patient's next of kin.

2.2. ICU setting

There are eight, well-spaced beds in each ICU, but there is no partition between them. There are three nurses posted to an ICU with a nurse to patient ratio of 1:2.7. The ICU floors are routinely mopped with Lysol and the other surfaces including the trolleys, beds, and window sills are cleaned with ethanol three times a day. The bed covers are changed once every two days or earlier if soiled, and whenever a new patient is admitted. The choice of antibiotics for the treatment of VAP patients was left to the discretion of the attending physician. The physicians were treating the patients on an individual basis using a combination of the ATS strategy, surveillance cultures, presence of risk factors for MDR pathogens, and their knowledge of the local microbial flora in the ICU and their antibiograms, without undue emphasis on any single strategy.

2.3. Data collection

The following data were collected from the patients enrolled in the study: age, gender, underlying illness, duration of hospitalization, duration of mechanical ventilation, and details of prior antibiotic therapy. Other relevant data were recorded from medical records, bedside charts, radiographic reports, and reports of microbiological studies.

2.4. Routine surveillance

EA were obtained every 3 days from all patients included in the study. Quantitative culture of the EA was performed immediately in the microbiology laboratory. EA 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 5% sheep blood agar. After incubation at 37 °C in a 5% CO_2 incubator for 24 h, a colony count was done and expressed as number of colony-forming units per ml (CFU/ml). The number of CFU/ml is equal to the number of colonies on the agar plate × dilution factor × inoculation factor. Therefore the presence of even a single colony on the blood agar after inoculating 0.01 ml of 1/1000 times diluted EA was interpreted as more than 10^5 CFU/ml.[13] The organisms isolated from the clinical specimens were identified based on standard bacteriological procedures.[14] The susceptibility of the isolates to some routinely used antibiotics was determined by the Kirby–Bauer disk diffusion method.[15]

2.5. Diagnosis of VAP

All patients included in this study were monitored at frequent intervals for the development of VAP using clinical and microbiological criteria, until discharge or death. The clinical pulmonary infection score (CPIS) based on six clinical assessments, each worth 0–2 points, including fever, leukocyte count, quantity and purulence of tracheal secretions, oxygenation, type of radiographic abnormality, and results of sputum culture and Gram stain, was used in patients clinically suspected of VAP.[16] Microbiological confirmation was based on a positive Gram stain (>10 polymorphonuclear cells/low power field and ≥1 bacteria/oil immersion field) and quantitative EA culture showing $\geq 10^5$ CFU/ml.[5,17,18] Patients fulfilling both the clinical (CPIS >6) and the microbiological criteria were diagnosed to be suffering from VAP.

Patients developing VAP within the first four days of MV were classified as having early-onset VAP, while those developing VAP at five or more days after the initiation of MV were classified as having late-onset VAP.[4]

2.6. Colonizers

Those microorganisms isolated from the EA of the mechanically ventilated patients at a concentration of less than 10^5 CFU/ml in both the patients with VAP and those without VAP were referred to as colonizers in this study.

2.7. Evaluation of the pre-VAP EA strategy

The bacteria isolated from pre-VAP EA cultures and those present at a concentration of 10^5 CFU/ml in the quantitative EA culture obtained after VAP developed were considered to be same if they belonged to the same species and had similar antibiotic susceptibility patterns. We compared the antibiotic therapy that would have been prescribed based on the pre-VAP EA strategy with that of the ATS strategy.

2.8. Statistical analysis

Results were expressed as mean ± standard deviation (SD). Comparison of the mean age of patients with and without VAP was carried out using an unpaired Student's *t*-test. All tests of significance were two-tailed. The Fisher's exact test was done to compare the use of broad-spectrum antibiotics according to the different strategies; SPSS version 16.0 statistics software was used (SPSS Inc., Chicago, IL, USA). The sensitivity, specificity, positive predictive value, and negative predictive value were determined using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA, USA). Likelihood ratios were calculated according to Deeks and Altman.[19] All *p*-values of < 0.05 were considered statistically significant.

3. Results

Two hundred patients on MV for >48 h were prospectively followed in this study. Among them, 36 (18%) were diagnosed to have developed VAP during their ICU stay. The incidence of VAP was 22.94 per 1000 ventilator-days. Among the remaining 164 patients, 17 had a discrepancy between the CPIS score and quantitative EA culture. In six cases CPIS was >6, but the quantitative EA culture was negative. In these six patients, the CPIS was considered to be falsely high, because of an abnormal chest X-ray due to a past episode of tuberculosis, traumatic injury to the lung, or cardiopulmonary edema secondary to underlying cardiovascular disease and/or transient fever and leukocytosis following trauma or surgery and/or poor oxygenation due to underlying hemodynamic instability. However, all these patients were afebrile or only transiently febrile and the CPIS was elevated only for a short period and most of them improved within next few days, ruling out the possibility of VAP. So these six patients were not considered to be suffering from VAP. In another 11 cases, the quantitative EA culture was positive, but CPIS was < 6. All of these were afebrile, their chest X-rays were normal, and the subsequent quantitative EA cultures were negative. They also showed rapid improvement in their general condition without any intervention or change in antibiotics, excluding the diagnosis of VAP. So they were also not categorized as VAP patients.

The mean ± standard deviation (SD) age of patients with VAP was 41.4 ± 14. 7 years (range 15–75 years). The mean ± SD age of patients without VAP was 36.8 ± 16.3 years (range 13–80 years); age of VAP patients vs. non-VAP patients, p-value = 0.1170. In our study, 21 (58.3%) cases were late-onset VAP, while 15 (41.7%) cases were early-onset VAP. The mean ± SD day of onset of VAP was 6.17 ± 4.7 (range 2–24). The demographic data of the patients with VAP are summarized in Table 1. Most cases of VAP were caused by Gram-negative bacteria, which accounted for 80.9% of the causative organisms. *Acinetobacter baumannii* (23.4%) and *Pseudomonas aeruginosa* (21.3%) were the predominant Gram-negative bacteria associated with VAP, and *Staphylococcus aureus* (14.9%) was the most common Gram-positive bacterium among patients with VAP. We studied the proportion of VAP patients who had the important risk factors for MDR pathogens. Of the 36 VAP patients, 31 (86.1%) had received antimicrobial therapy in the preceding 90 days, while 28 (77.8%) were hospitalized for five days or more. Of the 28 non-fermenters, 22 (78.6%) were isolated from patients with late-onset VAP, while six (21.4%) were isolated from those with early-onset VAP. However, all six early-onset VAP patients, from whom the non-fermenters were isolated, had risk factors for MDR pathogens.

Table 1
Demographic data for the 36 VAP patients

Characteristic	Value
Age, years (mean ± SD)	41.4 ± 14.7
Gender	
Male	24 (66.7%)
Female	12 (33.3%)
Underlying diseases	
Poisoning	10
Neuromuscular disorders (GBS, MND, tetanus)	9
Intra-abdominal diseases	4
Snake bite	4
CNS infections (encephalitis/meningitis)	3
Pregnancy-related disorders	2
Trauma (fracture, cerebral hemorrhage)	2
Cardiovascular disease	1
Leptospirosis	1
Median time to occurrence of VAP (25^{th} percentile, 75^{th} percentile)	5 (3, 7) days
Median No. of pre-VAP EA cultures performed before diagnosis of VAP (25^{th} percentile, 75^{th} percentile)	1 (0, 2)
Median delay between pre-VAP EA and onset of VAP (25^{th} percentile, 75^{th} percentile)	4 (3, 8) days

VAP, ventilator-associated pneumonia; SD, standard deviation; GBS, Guillain–Barré syndrome; MND, motor neuron disease; EA, endotracheal aspirate.

3.1. Colonizers of the respiratory tract in mechanically ventilated patients

Acinetobacter spp (33.7%) and *Pseudomonas spp* (29.8%) were the most common organisms colonizing the respiratory tract in patients on MV. Members of the *Enterobacteriaceae* were present as colonizers in 26.8% of the mechanically ventilated patients (Table 2).

Table 2
Colonizers of mechanically ventilated patients

Colonizer	No. of isolates (N = 205)	Ciprofloxacin resistance (%)	Amikacin resistance (%)	Ceftazidime resistance (%)	Meropenem resistance (%)
Gram-negative bacteria (n = 186)					
Non-fermenters (n = 130)					
Acinetobacter baumannii	56	95	86	96	45
Acinetobacter lwoffii	13	92	92	92	39
Pseudomonas aeruginosa	49	65	43	59	8
Pseudomonas spp	12	67	75	58	17
Enterobacteriaceae (n = 55)					
Escherichia coli	21	95	19	90	0
Klebsiella pneumoniae	19	74	16	63	5
Citrobacter diversus	4	100	25	100	0
Enterobacter spp	2	100	50	50	0
Providencia spp	5	100	60	100	0
Proteus spp	3	67	100	100	0
Morganella morganii	1	0	100	100	0
Other (n = 1)					
Haemophilus influenzae	1	-	-	-	-
Gram-positive bacteria (n = 17)					
MSSA	7	14	-	-	-
MRSA	7	86	-	-	-
Streptococcus pyogenes	1	-	-	-	-
Streptococcus pneumoniae	1	-	-	-	-
Enterococcus faecalis	1	-	-	-	-
Fungi (n = 2)					
Candida spp (non-albicans)	2	-	-	-	-

MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

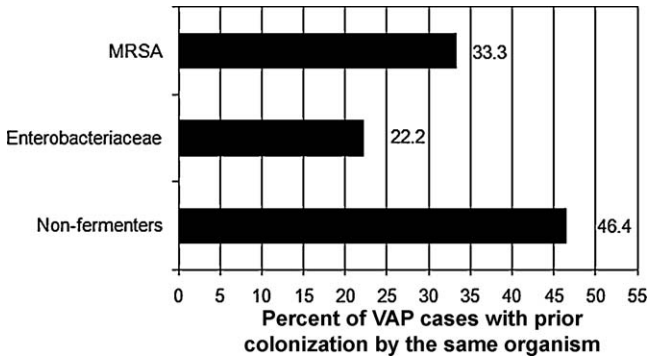

Figure 1. Comparison of the colonization rates of important VAP pathogens.

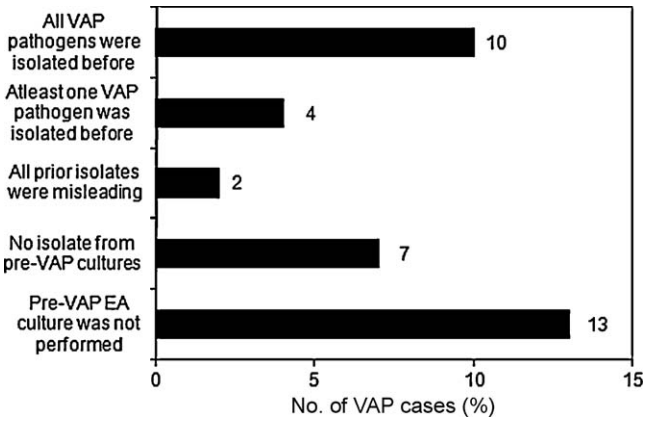

Figure 2. Role of routine serial cultures in predicting VAP pathogens.

Staphylococcus aureus (6.8%) was the most common Gram-positive colonizer, methicillin-resistant *S. aureus* (MRSA) accounting for 50% of these. *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Candida spp* (non-albicans) were the other relatively less common colonizers (Table 2).

3.2. Colonization rates of important VAP pathogens

As 13 patients developed VAP as early as day 2 or 3 of mechanical ventilation, pre-VAP EA cultures could not be performed. Pre-VAP EA cultures were performed only for the remaining 23 VAP cases. Colonization was detected in 14 of the 23 (60.9%) assessable VAP cases. Many of the pathogenic microorganisms causing VAP were initially present as colonizers in the respiratory tract followed by subsequent development of VAP. Colonization rates were relatively higher with non-fermenter and MRSA than *Enterobacteriaceae* (Figure 1).

3.3. Role of routine serial cultures in predicting VAP pathogens

The role of routine serial EA culture in predicting VAP pathogens is shown in Figure 2. In 14 of the 23 assessable cases (60.9%), either one or all of the VAP pathogens were isolated by routine serial culture prior to the diagnosis of VAP, while in seven (30.4%) cases the specimens collected prior to the diagnosis of VAP were sterile.

3.4. Comparison of routine serial EA cultures (pre-VAP) and quantitative culture of EA obtained after the development of VAP

Of the 205 colonizers, only 16 (7.8%) subsequently caused VAP. Of the 16 bacteria belonging to the same species that were isolated from both pre-VAP EA cultures and confirmatory quantitative EA cultures of the respective patients, 13 had exactly the same antibiograms, while the remaining three had slightly different antibiograms. Two *A. baumannii* appeared initially sensitive to amikacin, but later when isolated from confirmatory quantitative EA cultures were resistant to amikacin. Similarly one *P. aeruginosa* isolated from pre-VAP EA culture was sensitive to meropenem, but the isolate from confirmatory quantitative EA culture was resistant. Only 14 of the 31 (45.2%) pathogens causing late-onset VAP and two of the 16 (12.5%) pathogens causing early-onset VAP were isolated previously from pre-VAP EA cultures.

3.5. Diagnostic value of prior colonization in predicting VAP

The diagnostic value of previous colonization by different pathogens in predicting subsequent VAP caused by these microorganisms in terms of sensitivity, specificity, predictive values and likelihood ratios is summarized in Table 3.

3.6. Appropriateness of antibiotic therapy based on pre-VAP EA cultures vs. the ATS strategy

As pre-VAP EA cultures were not performed in 13 VAP cases for the reasons mentioned above and in another seven cases the EA cultures were sterile, we could not apply the pre-VAP EA culture-based strategy to those 20 patients. Hence we evaluated both the ATS strategy and the pre-VAP EA strategy only in the remaining 16 assessable VAP cases. According to ATS guidelines, the antibiotic treatment would have been appropriate in nine of the 16 assessable VAP cases (56%; 95% confidence interval (CI) 32–80%) with a piperacillin–tazobactam and aminoglycoside-based regimen, or in seven of the 16 assessable VAP cases (44%; 95% CI 20–68%) with a carbapenem–aminoglycoside-based regimen. However a strategy based on the pre-VAP EA cultures would have appropriately treated 13 of 16 assessable VAP cases (81%; 95% CI 62–100%). In the majority of the cases in which the ATS strategy would have failed, MDR pathogens such as *Acinetobacter spp* and *Pseudomonas spp* were present, which were resistant to even the higher antibiotics like meropenem, piperacillin–tazobactam, ceftazidime, gatifloxacin and amikacin, recommended by the ATS for the treatment of MDR pathogens. Of the 14 *Acinetobacter spp* isolated from VAP patients, five (36%) and 10 (71%) were resistant to piperacillin–tazobactam and meropenem, respective-

Table 3
Diagnostic value of prior colonization in predicting VAP pathogens

VAP pathogen	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Likelihood ratios	
					LR-pos	LR-neg
Pseudomonas aeruginosa	70 (35–93)	96 (80–100)	88 (47–100)	89 (72–98)	18	0.3
Acinetobacter baumannii	45 (17–77)	96 (80–100)	83 (36–100)	80 (61–92)	11	0.6
MRSA	33 (0.8–91)	100 (89–100)	100 (3–100)	94 (81–99)	∞	0.7
Other[a]	13 (3–34)	52 (30–74)	23 (5–54)	35 (19–55)	0.3	1.7

VAP, ventilator-associated pneumonia; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR-pos, positive likelihood ratio; LR-neg, negative likelihood ratio; MRSA, methicillin-resistant *Staphylococcus aureus*.

[a] Includes all the microorganisms other than *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and MRSA.

Table 4
Antibiotic(s) used for the treatment of VAP patients according to different strategies

Antibiotic(s)	Number of patients who would receive a particular antibiotic(s) based on:	
	Pre-VAP EA strategy	ATS strategy
Ceftriaxone	0	1
Ceftazidime	1	0
Amikacin + meropenem	2	0
Erythromycin + amikacin	1	0
Meropenem	4	0
Piperacillin–tazobactam	2	0
Ticarcillin	1	0
Vancomycin	1	0
Piperacillin–tazobactam + amikacin	0	35
Cefoperazone–sulbactam + levofloxacin + meropenem	1	0
Colistin + rifampin + meropenem	3	0
No appropriate antibiotic was suggested by the strategy	20	0

VAP, ventilator-associated pneumonia; EA, endotracheal aspirate; ATS, American Thoracic Society.

ly. Similarly, of the 13 *Pseudomonas spp*, three (23%) and four (31%) were resistant to piperacillin–tazobactam and meropenem, respectively.

3.7. Rational use of antibiotics according to different strategies

If the ATS strategy had been used it would have led to prescription of additional unnecessary antibiotics in four of the 36 cases (11.1%). Similarly, the pre-VAP EA culture-based strategy would have led to the use of unnecessary broad-spectrum antibiotics in one of the 16 assessable cases (6.3%). There was no statistically significant difference in the use of broad-spectrum antibiotics according to these strategies (p-value 1.000).

Based on the ATS strategy, higher antibiotics like meropenem, piperacillin–tazobactam, cefoperazone–sulbactam, ticarcillin, colistin, and vancomycin would have been used in 35 of the 36 cases (97%; 95% CI 92–100%). Similarly, according to pre-VAP EA cultures, higher antibiotics would have been used in 14 of the 16 assessable VAP cases (88%; 95% CI 72–100%); p-value 0.2208 vs. ATS strategy (Table 4). The ATS strategy would have led to the use of these higher antibiotics in 35 of the 36 cases, as 21 patients had late-onset VAP and 14 of the 15 early-onset VAP patients had at least one of the risk factors for MDR pathogens.

4. Discussion

In our study a relatively high proportion of the patients developed early-onset VAP. Even in a large US-based study involving 842 VAP cases, about 63% of patients developed VAP at about 48 h of MV.[3] The interaction of several risk factors during the initial days of MV puts these patients at higher risk. Moreover, our hospital, being a tertiary care hospital, most of our patients would have received medical assistance at several primary healthcare centers before approaching us, and hence were probably already colonized with multiple pathogens, which could have contributed to the early occurrence of VAP. *Acinetobacter spp* (33.7%) and *Pseudomonas spp* (29.8%) were identified as the most common organisms colonizing the respiratory tract of the patients on MV. Among the *S. aureus* colonizing the respiratory tract, 50% were MRSA. Colonization of the respiratory tract with *Acinetobacter spp*, *Pseudomonas spp*, and MRSA may originate from endogenous sources such as the oropharynx or the stomach, or from exogenous sources such as contaminated respiratory instruments, infective aerosols from the ICU environment, and contaminated hands and apparel of the healthcare workers. These non-fermenters and MRSA referred to as 'MDR' pathogens, characteristically display high levels of antibiotic resistance and are therefore more difficult to treat in the event of the occurrence of VAP.[4,20] The majority of our VAP patients had risk factors for MDR pathogens, which explains the high rate of colonization by these MDR pathogens.

By repeated assessment of colonization and infection of the lower airways using EA, we found that VAP was preceded by colonization in 60.9% of the 23 assessable VAP cases. The performance of routine quantitative culture of surveillance EA samples allowed us to prospectively and accurately determine the incidence and sequence of lower respiratory tract colonization to infection in patients on MV. Among the VAP pathogens, 46.4% of non-fermenters and 33.3% of MRSA were initially present as colonizers in the respiratory tract followed by subsequent development of VAP. Hence, colonization by these organisms may predispose to VAP. Two *A. baumannii* and one *P. aeruginosa* isolated from pre-VAP EA cultures showed slightly different antibiograms when recovered later from confirmatory quantitative EA cultures. But despite the slightly discordant antibiograms we have considered them to be same, as disk diffusion is not always reproducible even when the same strain is repeatedly tested, and has an inherent weakness of showing difference in antimicrobial susceptibility often related to environmental factors or plasmids.[21] In patients with such discordant antibiograms, the treating physician should ideally discuss with the microbiologist whether the antibiotic showing variable activity against the isolate can be used to treat the patient. The minimum inhibitory concentration (MIC) of that antibiotic should be determined whenever possible and if the isolate is found susceptible, then the patient can be treated with it.

In a study by Hayon et al., all the organisms ultimately responsible for VAP were previously recovered from only 35% of the respiratory secretions, emphasizing the limitations of serial culture.[22] In the above study, it was also shown that of the 220 microorganisms responsible for VAP, only 21(10%), 17 (8%), 8 (4%), and 7 (3%) were isolated from catheter tip, routine surveillance cultures (nasal, throat and skin swabs), urine, and blood, respectively.[22] However, in a study by Jung et al., the results of pre-VAP EA cultures were concordant with the results of bronchoalveolar lavage (BAL) in 72% of cases.[11] Similarly in a study by Michel et al., pre-VAP EA had identified the same microorganisms (with the same antibiotic resistance patterns) in 83% of the VAP cases.[8] In our study, pre-VAP EA cultures predicted the VAP pathogens in 60.9% of assessable VAP cases. There was a relatively high occurrence (41.7%) of early-onset VAP in our study in contrast to only 29% early-onset VAP in the study by Michel et al. As a result of the increased number of early-onset cases, only a few or no pre-VAP EA specimens were available for many cases, resulting in the low recovery of VAP pathogens in our study. The other possibility for the lower recovery of VAP pathogens in pre-VAP EA could be the early administration of broad-spectrum antibiotics in most of our patients.

In a study by Depuydt et al., the sensitivity of tracheal surveillance cultures to predict MDR VAP pathogens was 69%.[23] Though in our study the sensitivity of pre-VAP EA culture to predict *A. baumannii* was relatively low (45%), the sensitivity for the prediction of *P. aeruginosa* was 70%, which is comparable with the study by Depuydt et al.

As prior colonization by *P. aeruginosa, A. baumannii*, and MRSA had a very good specificity (96–100%) and a high negative predictive value (80–94%), it can accurately exclude most patients without infection by these MDR pathogens; however, as their sensitivity (33–70%) is low, failure to retrieve these organisms does

not rule out the possibility of VAP by these pathogens. All these organisms also had very good positive and negative likelihood ratios, suggesting that the presence or absence of colonization by these organisms can provide strong evidence to rule in or rule out VAP pathogens. Although another recent study also noted that the specificity of these pathogens was high,[24] there are no studies to support antibiotic treatment of these colonizers to prevent VAP. However, a randomized, multicenter study has conclusively proved that antimicrobial treatment of ventilator-associated tracheobronchitis (VAT) is associated with a reduction in the number of days of MV and also lower rates of VAP.[25] Craven et al. in their clinical opinion have also suggested that targeted antibiotic therapy for VAT may be a new paradigm for the prevention of VAP.[26] Colonization by microorganisms other than *P. aeruginosa, A. baumannii*, and MRSA had very poor sensitivity, specificity, PPV, NPV and likelihood ratios. So colonization by these organisms will not be useful in predicting the subsequent occurrence of VAP by these organisms.

In the majority (55.6%) of the VAP cases, treatment based on the pre-VAP EA culture results was not useful in guiding empirical antimicrobial therapy, as the pre-VAP specimens were sterile or pre-VAP EA specimens could not be collected. But whenever one or more microorganisms were retrieved from the pre-VAP EA specimens, the treatment was appropriate in 81% of cases based on this strategy. Michel et al. have also shown that routine EA performed twice a week is useful in prescribing adequate antibiotic therapy in 95% of the patients in whom a VAP is ultimately diagnosed by BAL culture.[8]

There is a delay of 24–48 h before EA or BAL quantitative culture results and antibiotic sensitivity profiles become available to the treating physician. Therefore, the critical care physicians generally use a combination of anti-pseudomonal cephalosporin or carbapenem or β-lactam/β-lactamase inhibitor with anti-pseudomonal fluoroquinolone, with or without vancomycin, according to the ATS guidelines, for the treatment of patients with risk factors for MDR pathogens.[4] However, this empirical regimen recommended by the ATS may not be effective against MDR *A. baumannii* and MDR *P. aeruginosa* resistant to carbapenem and piperacillin–tazobactam. Our study showed that though pre-VAP cultures were not available in the majority of our cases, whenever they were positive, this could guide more appropriate therapy than the ATS strategy. So, knowledge about the susceptibility pattern of the isolates from pre-VAP EA cultures may guide the clinician to appropriately treat such potential MDR pathogens. Combination regimens using colistin, polymyxin, and tigecycline have been reported to be useful in treating such infections.[27,28] Moreover, the PPV of MDR organisms such as *P. aeruginosa*, *A. baumannii*, and MRSA isolated from pre-VAP EA cultures in predicting the VAP pathogens were high enough to justify extending the spectrum of initial antibiotic therapy to deal with these MDR pathogens. So, based on our results, we suggest that VAP patients may be treated as widely practiced, based on the ATS strategy, but that whenever the above-mentioned MDR pathogens are isolated from pre-VAP EA cultures, the antibiotic therapy should be extended to treat them. This modification will be especially useful in settings such as ours where MDR pathogens not responding to the routine higher antibiotics are prevalent. The treatment recommended for meropenem-resistant MDR *Acinetobacter spp* is intravenous colistin combined with rifampin with or without imipenem or tigecycline.[27,29] Similarly the preferred treatment for MDR *P. aeruginosa* resistant to meropenem and piperacillin–tazobactam is colistin or levofloxacin in combination with imipenem or ceftazidime/cefoperazone with sulbactum.[28,30] Pirracchio et al., found high specificities and likelihood ratios for upper airway samples to predict the microorganisms involved in VAP. So, like us, they have also suggested that upper airway samples might provide adjunctive assistance in selecting the therapy for VAP.[31]

Hayon et al. observed that MDR organisms such as MRSA, *P. aeruginosa*, and *A. baumannii* isolated from surveillance cultures had low positive predictive values of 62%, 52%, and 24%, respectively, in predicting the occurrence of VAP by these pathogens.[22] Therefore, the treatment based on surveillance cultures appeared to expose many patients to unnecessary broad-spectrum antibiotics. However, in our ICUs with a relatively high prevalence of these MDR pathogens, we found that MRSA, *P. aeruginosa*, and *A. baumannii* isolated from pre-VAP EA cultures had high positive predictive values of 100%, 88%, and 83%, respectively. Moreover, we observed that there was no significant increase in the use of broad-spectrum antibiotics with the pre-VAP EA strategy compared to the ATS strategy. Therefore, we suggest that in ICUs with a high prevalence of MDR pathogens, treatment based on pre-VAP EA cultures will not expose patients to unnecessary broad-spectrum antibiotics. Similarly, Depuydt et al. also found that the surveillance cultures performed in an ICU with a high prevalence of MDR pathogens contributed to high rates of early appropriate antibiotic therapy with limited use of broad-spectrum antimicrobials.[23]

As the study was conducted in a resource-limited setting, only a small number of patients with VAP were studied, which is the main limitation of our study. The small numbers of pathogens led to very large 95% CI for predictive values, limiting the accuracy of the results. Therefore, the results of our study need to be further confirmed by larger clinical trials, as this may have a major impact on the treatment of VAP, which is a great challenge for critical care physicians, especially in developing countries. The other limitation of our study is that we did not perform quantitative culture of bronchoscopically collected samples like BAL for the confirmation of VAP.

5. Conclusions

To conclude, *Acinetobacter spp* and *Pseudomonas spp* were the most common organisms colonizing the respiratory tract of the patients on MV. VAP patients should be treated based on ATS guidelines, but whenever MDR pathogens like *P. aeruginosa*, *A. baumannii*, and MRSA are isolated from pre-VAP EA cultures, the initial antibiotic therapy should be extended to treat these. Thus pre-VAP EA cultures could be a useful adjunct to the ATS strategy for ensuring appropriate treatment of VAP patients, especially in places where resistance to carbapenems and other second-line antibiotics is fairly rampant.

Conflict of interest: No conflict of interest to declare.

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