Study of microbiological and antibiotic sensitivity pattern of ventilator associated pneumonia (VAP) in ICU of a tertiary care hospital in Nepal

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Abstract

Introduction: Ventilator-associated pneumonia (VAP) is the most frequent intensive care unit (ICU) acquired infection among patients receiving mechanical ventilation. Accurate clinical and microbiologic diagnosis of VAP is essential not only for selection of appropriate antimicrobials but also to prevent their misuse. As the organisms and their sensitivity pattern may differ in every ICU, the knowledge of the resident flora and their behaviour should be known for successful treatment. **Methods:** The study was conducted to evaluate the organisms responsible for VAP and their Antibiotic Sensitivity Pattern for the study setting. A prospective, open, epidemiological clinical study was performed in a tertiary care hospital in Nepal. 100 patients admitted to ICU and Mechanically Ventilated were evaluated about VAP. Clinical Pulmonary Infection Score (CPIS) was used to diagnose VAP. **Results:** Among 60 patients ventilated for more than 48 hours, 25 (41.6%) developed VAP. The VAP was caused predominantly by Klebsiella pneumonia in 34.5% of cases, followed by Acinetobacter calcoaceticus baumanni in 27.6%, Acinetobacter wolffi and Pseudomonas aeruginosa in 13.8% each and Escheresia coli in 10.3%. The most sensitive antibiotics were Colistin, followed by Polymyxin B and Amikacin with sensitivity rates of 67%, 60% and 58%, respectively. **Conclusion:** Based on these results, an empiric approach to antibiotic treatment can be made tailored to the specific settings. Given the magnitude of drug resistance and its implicated financial and societal burden, there is an urgent need for broad implementation of Antibiotic Stewardship programs across all health care settings.

Keyword: Antibiotic sensitivity pattern, microbiological profile, VAP

Introduction

Ventilator-associated pneumonia (VAP) is defined as infection of the pulmonary parenchyma in patients exposed to invasive mechanical ventilation for at least 48 h.[1] However, the diagnosis of VAP is often a problem.

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Accurate clinical and microbiologic diagnosis of VAP is essential for selection of appropriate antimicrobials and prevent emergence of multidrug resistant pathogens in the ICU.[2] As the organisms and their sensitivity pattern may differ in every ICU, the knowledge of the resident flora and their behaviour should be known for successful treatment. In this context, we conducted this study to find out the microorganism associated with VAP in our ICU and chart out the antibiotic sensitivity pattern for the same period.

Subjects and Methods

The study enrolled 100 consecutive patients who were admitted to the ICU of a tertiary care referral university hospital over a one-year period. The study was prospective observational and approved by the local institutional review board. After obtaining written informed consent from either the patient or the first degree relative. 100 consecutive patients admitted and mechanically intubated were enrolled. Out of 100, 40 were excluded as they had a diagnosis of Pneumonia at initial presentation, were suspected to have ARDS on admission, died within 48 hours or were transferred from ICU of other centers. Approval from Ethics committee was obtained in January 2012.

From each patient the demographic data and primary diagnosis, co-morbidities, date of admission in hospital and ICU were noted. The study patients were monitored at every third day for the development of VAP using clinical and microbiological criteria until either discharge or death. The relevant data were recorded from medical records, bedside flow sheets, radiographic reports, and reports of microbiological studies of the patients.

Modified CPIS[3,4] criteria [Table 1] were used for the diagnosis of VAP. CPIS at baseline was assessed based on the first five variables, i.e., temperature, blood leukocyte count, tracheal secretions, oxygenation, and character of pulmonary infiltrate. CPIS at 72 h was calculated based on all seven variables and took into consideration the progression of the infiltrate and culture results of the tracheal aspirate. A score > 6 at baseline or at 72 h was considered suggestive of ventilator associated pneumonia.

The organisms cultured and their antibiotic sensitivity pattern were noted in cases diagnosed as VAP. The endotracheal aspirate (ETA) specimens were collected via a sputum suction trap. Tracheal aspirates were categorized as absent, non-purulent or purulent as described by experienced senior sisters involved in daily care of the patients. The ETA samples were transferred to the lab within 1 hour and were used directly for staining and microbiologic culture by semi-quantitative method. The culture media used were Blood, Chocolate, and McConkey agar. After 24 hours the organism grown was again plated on Mueller Hilton agar for antibiotic susceptibility testing by Disc Diffusion method. After 48 hours both the results of culture and antibiotic susceptibility testing were read. Further typing of the organism were done with the help of biochemical testing. The growth was read as light, moderate and heavy as per the discretion of the microbiologist.

Descriptive statistical analysis was done from the demographic data of patients.

Results

Of the 60 patients in the study cohort, using CPIS criteria >6 for the diagnosis of VAP, 25 (41.6%) developed VAP. The overall incidence among the ventilated patients during the given duration was "26 VAPs per 1000 ventilator days" (25 of 976). All patients in the study were on presumptive antibiotic treatment [Figure 1].

Time to the onset of VAP

The onset of VAP was more likely to occur during the first two weeks of Mechanical Ventilation as 80% (20 out of 25) occurred during this period. Early-onset VAP developed in 44% (11 out of 25) of the cases, while the rest were Late-onset VAP.

Microbiological study

Of the 25 cases with VAP, 23 were Culture positive for organisms, however in two cases organisms were not isolated and VAP was diagnosed only based on CPIS score of more than six constituted by the other variables. Of the 23 culture positive cases, in 17 cases single organisms were isolated and in six cases two organisms each were isolated, thus in total from 23 cases 29 organisms were isolated.

The prevalence of isolated organisms is shown in [Table 2]. The most frequent organisms in our study were Klebsiella pneumonia (34.5%), Acinetobacter calcoaceticus baumanni (27.6%), Acinetobacter wolfii and Pseudomonas aeruginosa (13.8% each) and Escheresia coli (10.35).

Antibiotic sensitivity pattern

Colistin, Polymyxin B and Amikacin were the most sensitive antibiotics being sensitive in 67%, 60%, and 58% of the cases, respectively. The sensitivity of Imipenem and Meropenem were less than 50% of the time that they were tested. Cefepime and Ciprofloxacin were sensitive less than 10% of the time whereas Piperacillin Tazobactam was not sensitive in any of the cases [Table 3].

Susceptibility patterns of individual organisms
Klebsiella pneumonia
Among Klebsiella pneumonia (n = 10) isolates, 73% were susceptible to Amikacin. The susceptibility to Polymyxin B was 67% and Colistin 50%. All other antibiotics had rates less than 50% for these isolates [Table 4].

Acinetobacter calcoaceticus baumanni
Acinetobacter calcoaceticus baumanni (n = 8) isolates presented susceptibility rates of 100% for Colistin, 67% for Polymyxin B and 60% for Cefoperazone + Sulbactam. The susceptibility rates for Amikacin, Imipenem, Meropenem were all less than 50% [Table 4].

Pseudomonas aeruginosa
Among Pseudomonas aeruginosa (n = 4), the susceptibility to Colistin was 100% followed by Amikacin at 75% and Cefoperazone-Sulbactam at 67%. However, it was sensitive to Imipenem only 25% of the time [Table 4].

Escheresia coli: Among *E. coli* isolates (n = 3), all were susceptible to Amikacin. However, the isolates were resistant in all cases to Meropenem, Polymyxin B, Colistin, Cefoperazone + Sulbactam [Table 4].

Table 1: Modified CPIS Criteria

CPIS points	0	1	2
Temperature (T)	>=36.5 or <=38.4 >=97.7 or <=101.2	>= 38.5 or <= 38.9 >=101.3 or <=102.1	>=39 or <=36 >=102.2 or <=96.8
WBC count (W)	>=4000 or<=11,000	<4000 or >11,000	+band forms >=50%
Pao2/fio2 (O)	>240 or ARDS		<=240 and no ARDS
Tracheal secretions (S)	Absent	Non purulent	purulent
Chest x-ray (X)	No infiltrate	Diffuse (or patchy) infiltrate	Localized infiltrate
Progression of infiltrate (P.I.)	No radiographic progression		Radiographic progression (after CHF and ARDS excluded)
Culture of tracheal aspirate (C)	Pathogenic bacteria‡ cultured in rare or light quantity or no growth	Pathogenic bacteria cultured in moderate or heavy quantity	Same pathogenic bacteria seen on Gram stain, add 1 point

Table 2: Prevalence of Isolated Organisms

Organisms	Growth		Total
	Monomicrobial	Polymicrobial	
Klebsiella pneumonia	7	3	10
Acinetobacter wolfii	2	2	4
Acinetobacter calcoaceticus baumanni complex	6	2	8
Pseudomonas aeruginosa	0	4	4
Escheresia coli	2	1	3
Total	17	12	29

Table 3: Overall Antibiotic Sensitivity Pattern

Antibiotic	Sensitive (n/%)	Resistant (n/%)	Not tested
Amikacin	15 (58)	11 (42)	3
Imipenem	9 (35)	17 (65)	3
Meropenem	6 (26)	17 (74)	6
Polymyxin B	9 (60)	6 (40)	14
Coilistin	8 (67)	4 (33)	17
Cefo + Sulb	6 (32)	13 (68)	10
Cefepime	1 (5)	19 (95)	9
Ciprofloxacin	1 (4)	23 (96)	5
Pip + Taz	0 (0)	25 (100)	4

Acinetobacter wolfii

Acinetobacter wolfii (n = 4) presented susceptibility rates of 100% for Polymyxin B, and 50% for both Imipenem and Meropenem [Table 4].

Discussion

VAP is a major threat to the recovery of patients receiving mechanical ventilation and is one of the most important intensive care unit (ICU)-acquired infections in mechanically ventilated patients. The incidence of VAP ranges from 6 to 52 cases per 100 patients depending on the population studied and patients with underlying lung disease are at high risk of VAP with a risk ratio of 2.78.[2] The mortality rate in patients developing VAP ranges from 33 to 70%.[5]

Endotracheal aspirate (ETA) samples

The analysis of ETA is the most commonly used method of airway sampling in ICUs all over the world. Gram stain, nonquantitative
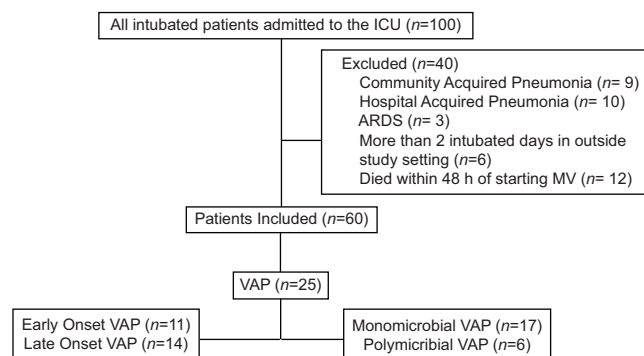

All intubated patients admitted to the ICU (*n*=100)

Excluded (*n*=40)
Community Acquired Pneumonia (*n*= 9)
Hospital Acquired Pneumonia (*n*= 10)
ARDS (*n*=3)
More than 2 intubated days in outside study setting (*n*=6)
Died within 48 h of starting MV (*n*= 12)

Patients Included (*n*=60)

VAP (*n*=25)

Early Onset VAP (*n*=11)
Late Onset VAP (*n*=14)

Monomicrobial VAP (*n*=17)
Polymicribial VAP (*n*=6)

Figure 1: Trial Profile of the patients

and semi-quantitative culture of tracheal secretions has the advantage of reproducibility and of requiring little technical expertise and no specialized equipment or technique. The role of serial cultures of endotracheal aspirates is a distinct advantage of this technique over more expensive methods, such as non-bronchoscopic Bronchoalveolar lavage (BAL) or bronchoscopy-derived samples. In a study by Wu *et al.*,[3] quantitative culture of endotracheal aspirate had a sensitivity of 93% and specificity of 80%, using bronchoscopically obtained samples as the standard in patients already receiving antibiotics, a clinical scenario very similar to ours where all the patients are already on antibiotics. However, other studies note that with blind tracheal aspirates, one normally samples the upper respiratory tract and the organisms isolated may just be colonizer.[3,6] Khilnani *et al.*[7] also showed a lower yield of ETA cultures at 52% compared to 68% for non-bronchoscopic protected bronchoalveolar lavage and 80% for bronchoscopic brushing. Till date, the optimal strategy for the diagnosis of VAP remains to be defined. microbiological sampling techniques with quantitative cultures versus non-invasive sampling methods with either quantitative or semiquantitative cultures did not find any differences in patients' outcomes.[8] Working in a resource limited setting, we chose endotracheal aspirates for obtaining respiratory tract specimens.

Culture Results

In 23 out of 25 cases, organisms were isolated. In the other two cases, VAP was diagnosed based on CPIS score of more than 6. Since CPIS considers 7 variables, a diagnosis of VAP is

Organisms Antibiotics	Klebsiella pneumonia (n=10)			Acinetobacter calcoaceticus baumanni (n=8)			Pseudomonas aeruginosa (n=4)			Acinetobacter wolfii (n=4)			E. coli (n=3)		
	S	R	Nd	S	R	Nd	S	R	Nd	S	R	Nd	S	R	Nd
Amikacin	8 (80%)	2 (20%)	-	1 (20%)	4 (80%)	3	3 (75%)	1 (25%)	-	1 (25%)	3 (75%)	-	3 (100%)	--	--
Imipenem	4 (56%)	5 (44%)	1	1 (17%)	5 (83%)	2	1 (25%)	3 (75%)	-	2 (50%)	2 (50%)	-	1 (33%)	2 (67%)	--
Meropenem	4 (56%)	5 (44%)	1	--	4 (100%)	4	--	3 (100%)	1	2 (50%)	2 (50%)	-	--	3 (100%)	--
Polymyxin B	4 (67%)	2 (33%)	4	4 (67%)	2 (33%)	2	--	-	4	1 (100%)	--	3	--	2 (100%)	1
Colistin	2 (50%)	2 (50%)	6	5 (100%)	--	3	1 (100%)	-	3	--	--	4	--	2 (100%)	1
Cefoperazone + Sulbactam	1 (17%)	5 (83%)	4	3 (60%)	2 (40%)	3	2 (67%)	1 (33%)	1	--	3 (100%)	1	--	2 (100%)	1

Table 4: Antibiotic Sensitivity Pattern of Isolated Organisms

S=Sensitive; R=Resistant; Nd=Not done

possible even without isolation of organisms. The sensitivity of Endotracheal aspirates in our study was 92%. In studies on endotracheal specimens, sensitivity ranged from 38 to 100%, and specificity ranged from 14 to 100%.[9] The varied sensitivity perhaps reflects the lack of standardization of the procedure and varying parameters of comparison. Using CPIS for comparison, Khilnani *et al*.[7] had a sensitivity of 52% for Endotracheal specimens. Our study looked at the culture of aerobic bacterial organisms only since anaerobic and fungal cultures were not being routinely done in the study setting.

Organisms isolated

Seventeen episodes of VAP were Monomicrobial whereas six episodes of VAP were polymicrobial. Two organisms each were isolated in all these six cases. Pseudomonas was isolated only from polymicrobial cases of VAP. In the study by Combes *et al*.[10] 52% had monomicrobial infections and 48% had Polymicrobial infection. In most studies the rate of Polymicrobial VAP ranges from 40 to 62%.[11-13] In comparison, our rate of Polymicrobial VAP is 26% among Culture Positive cases. It has been seen that antibiotic therapy prior to VAP onset seems to be associated with dramatically fewer polymicrobial infections,[10] this might be one of the reasons for our results given that prior antibiotics had been administered to all the patients. Microorganisms responsible for VAP may differ according to the population of patients in the ICU, the durations of hospital and ICU stays, and the specific diagnostic method (s) used. In our study, the most isolated organism was Klebsiella pneumonia (34.5%), followed by the Acinetobacter calcoaceticus baumanni. Pseudomonas aeruginosa was isolated from 13.8% of the cases. Interestingly isolates of *Staphylococcus aureus* were not isolated from VAP patients, though there were instances of the growth of *Staphylococcus aureus* in the exclusion group. The data from 24 investigations conducted with ventilated patients, for whom bacteriologic studies were restricted to uncontaminated specimens, confirmed those results: Gram Negative Bacteria (GNB) represented 58% of recovered organisms.[5] The predominant gram negative bacteria were P. aeruginosa and Acinetobacter spp., followed by Proteus spp., Escherichia coli, Klebsiella spp., and H. influenza. A relatively high rate of gram-positive pneumonias was also reported in those studies, with *S. aureus* involved in 20% of the cases.[5] Absence of Methicillin Resistant *Staphylococcus Aureus* (MRSA) as

a cause of VAP is quite puzzling given the abundance of MRSA in other studies. Interestingly, another study in India reports similar findings.[7] Whether the absence is due to a relatively small size of the sample or a trend actually exists will be an important consideration in further studies in our set up. In five cases suspected to have Active tuberculosis, appropriate stains and cultures were deployed however the results were negative.

Organisms in early vs late VAP

It is generally recognized that early-onset VAP (within the first 4 days of hospitalization) in previously healthy patients not receiving antibiotics usually involves normal oropharyngeal flora, whereas late-onset VAP (occurring after at least 5 days of hospitalization) and VAP in patients with risk factors for multidrug resistant (MDR) pathogens are more likely to be due to MDR pathogens.[14] However, MDR pathogens may be isolated in early-onset VAP, mainly in the presence of certain risk factors such as antimicrobial exposure within the preceding 90 days.[14-16] High rates of H. influenzae, S. pneumoniae, Methicillin Sensitive *Staphylococcus Aureus* (MSSA), or susceptible Enterobacteriaceae have been constantly found in early-onset VAP, whereas P. aeruginosa, Acinetobacter spp., MRSA, and multi-resistant GNB are significantly more frequent in late-onset VAP.[17-19] This different distribution pattern of etiologic agents between early and late-onset VAP is also linked to the frequent administration of prior antimicrobial therapy in many patients with late-onset VAP.[20] However, in our study organisms did not differ in either early or late onset VAP. In both the groups, organisms isolated consisted of K. pneumoniae, Acinetobacter spp., Pseudomonas aeruginosa and E. coli. This might be due to the rampant use of antibiotics in many patients developing early-onset VAP before their transfer to the ICU. Similar findings have been reported by Ibrahim *et al*.[21]

Antibiotic sensitivity pattern

Antibiotic resistance is a major problem in ICU. Specially in developing countries, most patients received broad spectrum antibiotics even in outpatient settings. In our study, the antibiotic prescription was on the discretion of the treating physician in the absence of tailored guidelines for our ICU set up. Systematic screening for Extended Spectrum Beta Lactamases (ESBL) was not done in our study.

Overall, Colistin, Polymyxin B and Amikacin were the most sensitive antibiotics. A high level of resistance was documented to third generation Cephalosporins, fluoroquinolones and even to Carbapenems. Interestingly, Piperacillin-Tazobactam, a widely used empiric agent in our ICU till the time, was found to be resistant in all the samples tested.

Acinetobacter calcoaceticus baumanni showed high level of resistance to Amikacin (80%), Imipenem (83%) and Meropenem (100%). It also showed 100% resistance to Cefepime, Ciprofloxacin and Piperacillin-Tazobactam. The most sensitive antibiotic was Polymyxin B, Colistin and Cefoperazone-Sulbactam. This reflects that the antibiotic armamentarium for treatment of *Acinetobacter* is limited because of native resistance to many classes of antibiotics.

Klebsiella pneumoniae was most sensitive to Amikacin (Resistance rate 27%), followed by Polymyxin B (resistance rate 33%). Imipenem and Meropenem showed resistance rate of 56%. The isolates were uniformly resistant to Ciprofloxacin and Piperacillin-Tazobactam whereas Cefepime showed resistance rate of 90%.

Pseudomonas aeruginosa was uniformly resistant in all cases to Meropenem, Cefepime and Piperacillin-Tazobactam. The level of resistance to Ciprofloxacin and Imipenem were 75%. It was most sensitive to Colistin, Amikacin and Cefoperazone-Sulbactam. This pattern reflects the intrinsic ability of P. aeruginosa to develop resistance to all known classes of antibiotics.[6]

Escheresia coli showed 100% resistance to Meropenem, Polymyxin B, Colistin, Cefoperazone-Sulbactam, Piperacillin-Tazobactam, Ciprofloxacin and Cefepime. However, it was 100% sensitive to Amikacin. This might reflect small size (*n = 3*) of the isolate. Though a very high level of resistance was seen to both the third generation and Fourth generation Cephalosporins.

Acinetbacter wolfii similarly showed 100% resistance to Cefepime, Ciprofloxacin and Piperacillin-Tazobactam. It was most sensitive to Polymyxin B (100%) and then to Meropenem and Imipenem (at 50% each).

As shown, most organisms show a very high degree of resistance to the usually used Cephalosporins and even the Carbapenems. Polymyxin B and Colistin, two antibiotics uniformly sensitive in most of the cases has just recently been made available in our country. Amikacin was sensitive against most Multi-Drug Resistant pathogens except for Acinetobacter species. The results of the study guided the development of empiric antibiotic protocol for the study setup.

The study is limited by its small study population. Since the best method of diagnosis of VAP is a subject of much debate, the use of CPIS criteria in our study in not exempt from such debates. In the absence of guidelines regarding the use of empiric antibiotics tailored to our setup, the initial antibiotic coverage might have been inadequate which is reflected in the high rates of VAP and the isolation of only Multi-Drug Resistant pathogens.

In summary, based on studies of prevailing pattern of microorganisms causing VAP and their antibiotic sensitivity pattern, an empiric approach to antibiotic treatment can me made tailored to the specific settings. Similar studies and protocol must be made in all ICUs and repeated from time to time to reflect the dynamic changing patterns of the microorganisms. As the empiric antibiotic results in VAP by multi-drug resistant organisms even in early VAP, it is imperative that the primary care physician, as the first contact of the patient with pneumonia, understand and implement the principles of antimicrobial stewardship.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Papazian L, Klompas M, Luyt CE. Ventilator-associated pneumonia in adults: A narrative review. Intensive Care Med 2020;46:888-906.

2. Zilberberg MD, Shorr AF, Micek ST, Mody SH, Kollef MH. Antimicrobial therapy escalation and hospital mortality among patients with health-care-associated pneumonia: A single-center experience. Chest 2008;134:963-8.

3. Wu CL, Yang DI, Wang NY, Kuo HT, Chen PZ. Quantitative culture of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. Chest 2002;122:662-8.

4. Marquette CH, Georges H, Wallet F, Ramon P, Saulnier F, Neviere R, *et al.* Diagnostic efficiency of endotracheal aspirates with quantitative bacterial cultures in intubated patients with suspected pneumonia. Comparison with the protected specimen brush. Am Rev Respir Dis 1993;148:138-44.

5. Chastre J, Fagon J. State of the art ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;1997. doi: 10.1164/rccm.2105078.

6. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388-416.

7. Khilnani GC, Arafath TKL, Hadda V, Kapil A, Sood S,

Sharma SK. Comparison of bronchoscopic and non-bronchoscopic techniques for diagnosis of ventilator associated pneumonia. Indian J Crit Care Med 2011;15:16-23.

8. Berton DC, Kalil AC, Teixeira PJZ. Quantitative versus qualitative cultures of respiratory secretions for clinical outcomes in patients with ventilator-associated pneumonia. Cochrane Database Syst Rev 2014;2014. doi: 10.1002/14651858.CD006482.pub4.

9. Cook D, Mandell L. Endotracheal aspiration in the diagnosis of ventilator-associated pneumonia. Chest 2000;117 (4 Suppl 2):195-7.

10. Combes A, Figliolini C, Trouillet J-L, Kassis N, Wolff M, Gibert C, *et al.* Incidence and outcome of polymicrobial ventilator-associated pneumonia. Chest 2002;121:1618-23.

11. Baker AM, Meredith JW, Haponik EF. Pneumonia in intubated trauma patients. Microbiology and outcomes. Am J Respir Crit Care Med 1996;153:343-9.

12. Chastre J, Trouillet JL, Vuagnat A, Joly-Guillou ML, Clavier H, Dombret MC, *et al.* Nosocomial pneumonia in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med 1998;157:1165-72.

13. Fagon JY, Chastre J, Domart Y, Trouillet JL, Pierre J, Darne C, *et al.* Nosocomial pneumonia in patients receiving continuous mechanical ventilation. Prospective analysis of 52 episodes with use of a protected specimen brush and quantitative culture techniques. Am Rev Respir Dis 1989;139:877-84.

14. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, *et al.* Executive summary: Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 2016;63:575-82.

15. Khan R, Al-Dorzi HM, Tamim HM, Rishu AH, Balkhy H, El-Saed A, *et al.* The impact of onset time on the isolated pathogens and outcomes in ventilator associated pneumonia. J Infect Public Health 2016;9:161-71.

16. Martin-Loeches I, Deja M, Koulenti D, Dimopoulos G, Marsh B, Torres A, *et al.* Potentially resistant microorganisms in intubated patients with hospital-acquired pneumonia: The interaction of ecology, shock and risk factors. Intensive Care Med 2013;39:672-81.

17. Campbell GD, Niederman MS, Broughton WA, *et al.* Hospital-acquired pneumonia in adults: Diagnosis, assessment of severity, initial antimicrobial therapy, and preventative strategies: A consensus statement, American Thoracic Society, November 1995. Am J Respir Crit Care Med 1996;153:1711-25.

18. Rello J, Sa-Borges M, Correa H, Leal SR, Baraibar J. Variations in etiology of ventilator-associated pneumonia across four treatment sites: Implications for antimicrobial prescribing practices. Am J Respir Crit Care Med 1999;160:608-13.

19. Trouillet JL, Chastre J, Vuagnat A, Joly-Guillou ML, Combaux D, Dombret MC, *et al.* Ventilator-associated pneumonia caused by potentially drug-resistant bacteria. Am J Respir Crit Care Med 1998;157:531-9.

20. Rello J, Ausino V, Ricart M, Castella J, Prats G. Impact of previous antimicrobial therapy on the etiology and outcome of ventilator-associated pneumonia. Chest 1993;104:1230-5.

21. Ibrahim EH, Ward S, Shermand G, Kollef MH. A comparative analysis of patients with early-onset nosocomial pneumonia in the ICU setting. Chest 2000;117:1434-42.