Aetiological agents of ventilator-associated pneumonia and its resistance pattern – a threat for treatment

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RESEARCH

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Abstract

Background

Ventilator-associated pneumonia (VAP) is a common type of nosocomial pneumonia encountered in intensive care units. There are several aetiological agents which make treatment challenging. Improper antibiotic treatment of ventilated patients may lead to the emergence of multidrug resistant (MDR) pathogens.

Method

A prospective study was performed over a period of 20 months. Our study had two arms: the first, 'Incidence and risk factors of VAP in a tertiary care hospital' was the subject of an earlier publication; we therefore present the second investigative arm in this work. The aetiological agents of patients on mechanical ventilation (MV) were identified by standard bacteriological method. The susceptibility pattern was evaluated by Kirby-Bauer disc diffusion method. Extended spectrum beta lactamase (ESBL) testing was performed by combination disc method, and metallo-beta lactamase (MBL) testing was performed by EDTA disk synergy test (EDS).

Results

Late-onset VAP was associated with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, while early-onset VAP was commonly caused by members of *Enterobacteriaceae*, *Candida albicans* and *Staphylococcus aureus*. 72.2 per cent of VAP patients had monomicrobial and 27.8 per cent had polymicrobial infection. Out of the 24 isolates obtained from patients with VAP, seven (29.2 per cent) were MDR pathogens. ESBL and MBL production was detected in 40 per cent and 20 per cent of *Klebsiella pneumoniae* isolated in our study. Around 50 per cent of isolates associated with late-onset VAP were MDR, while 22.2 per cent isolates obtained from patients with early-onset VAP were MDR.

Conclusion

VAP is a nosocomial pneumonia that is common among ventilated patients. The aetiological agents vary from common organisms to MDR pathogens that are difficult to treat. A proper knowledge of MDR pathogens and early isolation followed by prevention of prolonged antibiotic therapy can reduce the mortality of late onset VAP.

Key Words

Ventilator associated pneumonia, aetiology, drug resistance

Implications for practice

- VAP is a common nosocomial infection. About 29.2 per cent of the isolates from VAP patients were MDR pathogens.
- Most of the MDR pathogens were isolated from late onset VAP compared to early onset VAP.
- A more detailed understanding of MDR pathogens and early detection has the potential to reduce mortality levels currently associated with late onset VAP.

Background

Ventilator-associated pneumonia (VAP) is an inflammation of lung parenchyma caused by organisms acquired after mechanical ventilation (MV),[1,2] and the condition indirectly influences the length of stay, cost of treatment, and mortality of those patients that acquire this condition. There are several aetiological agents that make the

treatment of VAP challenging. The colonisation of the upper respiratory tract is a predisposition for the development of VAP. The less virulent, normal mixed flora of the oropharynx become replaced by endogenous Gram negative organisms.[3] The aetiological agents may be monomicrobial or polymicrobial. Approximately 58 per cent of organisms isolated from various studies were found to be Gram negative bacilli (GNB) and 20 per cent were identified as *Staphylococcus aureus*.[2] There is also a lack of a unanimous diagnostic method for VAP.[4] As a result, presumptive antibiotic treatment of ventilated patients leads to the emergence of multidrug resistant pathogens (MDR).[5] The common MDR pathogens include *Pseudomonas* spp, *Acinetobacter* spp. and certain strains of Enterobacteriaceae which are extended spectrum β lactmase (ESBL), AmpC β-lactamase (AmpC) or metallo-β-lactamase (MBL) producers.[6] The aetiological agents vary according to the patient population and the hospital setting.[2] The challenges associated with the identification of the different organisms causing VAP with various patient populations and the emergence of resistant organisms indicates the necessity for studies to identify organisms in each setting. Knowledge of these organisms would benefit a rational antibiotic therapy and assist the prevention of mortality and morbidity. This study focuses on the aetiological agents of VAP, their antibiotic susceptibility pattern, and the MDR pathogens associated with this pathology. This study is a cohesive part of our earlier study describing the incidence and risk factors of VAP.[7]

Method

Study design

This prospective study was performed over a period of 20 months from November 2009 to July 2011 at a tertiary care teaching hospital.[7] Our study had two arms, the first, 'Incidence and risk factors of ventilator associated pneumonia in a tertiary care hospital' was the subject of an earlier publication.[7] We present the second investigative study in this work. The study was approved by the local ethics committee and informed consent was obtained from all participants.

Setting

This study was performed in the intensive care unit (ICU) setting of our teaching hospital, a six-room facility that has separate cabins for each patient. The patients were admitted for various medical and surgical conditions in our ICU. They were either directly admitted to the ICU or transferred from wards. Proper aseptic precautions were followed while handling each patient in order to prevent the transfer of organisms from one patient to another. For details regarding the inclusion and exclusion of the study participants and the data collection protocol, refer to our previous publication.[7]

Diagnosis of VAP

VAP was diagnosed based on both the clinical and microbiological criteria as previously described.[7]

Antibiotic susceptibility testing

The susceptibility of the clinical isolates to some routinely used antibiotics was determined by the Kirby-Bauer disc diffusion method.[8] The Gram negative organisms were tested with netlimicin, amoxyclav, piperacilin tazobactam, imipenem, ceftazidime, amikacin and gentamicin. Similarly gram positive organisms were tested with erythromycin, clindamycin, amoxyclav, oxacillin, vancomycin, ciprofloxacin, gentamicin. Gram positive organisms resistant to oxacillin were tested with vancomycin, linezolid, teicoplanin and mupirocin. The control organisms *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923) were used.

Gram negative organisms which were resistant to ceftazidime were tested for extended spectrum beta lactamase (ESBL) production. Mueller-Hinton agar plates were inoculated as in the Kirby-Bauer disc diffusion test. After drying, one disc of ceftazidime (30µg) and one disc of ceftazidime-clavulanic acid (30/10µg) were kept on the plate. The plates were incubated aerobically at 35^0C. Five mm or more increase in zone of inhibition of ceftazidime-clavulanic acid disc compared to the ceftazidime disc alone was taken as confirmatory evidence of ESBL production.[9]

A 0.5 M EDTA solution was prepared by dissolving 186.1g of disodium EDTA. $2H_2O$ (REACHEM, Chennai, India) in 1,000ml of distilled water. The pH was adjusted to 8.0 by using NaOH and was sterilised by autoclaving. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a Mueller-Hinton agar (MHA) plate. A 10µg meropenem disc or 30 µg ceftazidime disc (HIMEDIA, Mumbai, India) was placed on the agar. A blank disc (6mm in diameter, Whatmann filter paper no. 1) was kept on the inner surface of the lid of the MHA plate and 10µl of 0.5 M EDTA is added to it. This EDTA disc was then transferred to the surface of the agar and was kept 10mm edge-to-edge apart from the meropenem or ceftazidime disc. After incubating overnight at 37^oC, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production.[10]

Blood culture

Five to ten ml of blood from patients with suspected VAP was collected in 50ml brain heart infusion (BHI) broth and sub-cultured on blood agar and MacConkey after 24h, 48h and one week of aerobic incubation.[11]

Method of analysis

Data entry and analysis were performed using SPSS for Windows, Version SPSS 16.0 (SPSS Inc., Chicago, IL). Percentages were calculated for categorical variables.

Results

Of the 76 patients who were on mechanical ventilation for more than 48 hours, 18 (23.7 per cent) developed VAP. The demographic details of the study participants have been described in detail in our earlier article.[7]

Aetiological agents of VAP

Monomicrobial infection was observed in 13 of the 18 (72.2 per cent) VAP patients, while polymicrobial infection occurred in five (27.8 per cent) patients with VAP. Of these five patients with polymicrobial infection, four were infected with two pathogens, while one was infected by three pathogens simultaneously. The aetiological agents of VAP observed in our study are summarised in the Table 1.

Table 1: Aetiological agents of VAP

Aetiological agent	Frequency	Percentage
Pseudomonas aeruginosa	8	33.3
Klebsiella pneumoniae	5	20.8
Escherichia coli	2	8.3
Candida albicans	2	8.3
Staphylococcus aureus	2	8.3
Citrobacter koseri	1	4.2
Proteus mirabilis	1	4.2
Acinetobacter baumannii	1	4.2
Burkholderia pseudomallei	1	4.2
Stenotrophomonas maltophilia	1	4.2
Total	**24**	

Aetiological agents of early-onset VAP and late-onset VAP

Late-onset VAP was associated with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, while early-onset VAP was caused by many members of *Enterobacteriaceae*, *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter baumannii*, Burkholderia *pseudomallei* and *Stenotrophomonas maltophilia* (Table 2).

Antibiotic susceptibility of the VAP pathogens

Kirby Bauer disc diffusion method

The antibiotic susceptibility pattern of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are summarised in Table 3.

Both the *Escherichia coli* isolates were resistant to ceftazidime, while one of them was susceptible to amoxycillin-clavulanic acid, ciprofloxacin, gentamicin and amikacin and both were sensitive to netilmicin, piperacillin-tazobactam and imipenem. The *Proteus mirabilis* isolate was susceptible to all the antibiotics tested. *Citrobacter koseri* and *Acinetobacter baumannii* were resistant to amoxycillin-clavulanic acid, but were susceptible to the other antibiotics tested. *Stenotrophomonas maltophilia* was susceptible to all the antibiotics tested except imipenem. *Burkholderia pseudomallei* was resistant to gentamicin, amikacin and ceftazidime, but was sensitive to amoxycillin-clavulanic acid, ciprofloxacin, piperacillin-tazobactam and imipenem. Both the *Staphylococcus aureus* were sensitive to ciprofloxacin, gentamicin, clindamycin, erythromycin, amoxycillin-clavulanic acid, vancomycin and teicoplanin.

Table 2: Aetiological agents of early-onset VAP and late-onset VAP

Organism (n =24)	Number of isolates	
	Early-onset VAP	Late-onset VAP
Pseudomonas aeruginosa	5	3
Klebsiella pneumoniae	3	2
Escherichia coli	1	1
Candida albicans	2	-
Staphylococcus aureus	2	-
Citrobacter koseri	1	-
Proteus mirabilis	1	-
Acinetobacter baumannii	1	-
Burkholderia pseudomallei	1	-
Stenotrophomonas maltophilia	1	-
Total	**18**	**6**

MDR pathogens associated with VAP

Non-fermenters and *Enterobacteriaceae* with resistance to more than two different groups of antibiotics, ESBL and MBL producers and MRSA were considered as multi-drug resistant pathogens. In our study, of the 24 isolates obtained from patients with VAP, seven (29.2 per cent) were MDR pathogens. Of these seven MDR pathogens, two *Klebsiella pneumoniae*, two *Escherichia coli* and one

Pseudomonas aeruginosa were ESBL producers, while one *Klebsiella pneumoniae* produced MBL.

Table 3: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Antibiotic	No. of susceptible isolates (%)	
	***Pseudomonas aeruginosa* (n = 8)**	***Klebsiella pneumoniae* (n = 5)**
Amoxycillin-clavulanic acid	4 (50.0)	-
Piperacillin-tazobactam	8 (100)	5 (100)
Ciprofloxacin	6 (75.0)	3 (60)
Gentamicin	6 (75.0)	3 (60)
Amikacin	5 (62.5)	3 (60)
Netilmicin	7 (87.5)	5 (100)
Ceftazidime	5(62.5)	0
Imipenem	8 (100)	5 (100)

Antibiotic susceptibility of early onset and late onset VAP pathogens

Around 50 per cent of isolates associated with late-onset VAP were MDR, while 22.2 per cent isolates obtained from patients with early-onset VAP were MDR.

Blood culture

The pathogen causing VAP was isolated from blood in 2 of the 18 cases (11.1 per cent) of VAP. The organisms that were recovered from blood include *Pseudomonas aeruginosa* and *Burkholderia pseudomallei*.

Discussion

VAP is a frequent nosocomial infection that is acquired in ICU following MV. The aetiological agents include both Gram negative and Gram positive cocci. In our study the major isolates were *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which were responsible for 33.3 per cent and 20.8 per cent cases of VAP respectively. It was followed by *Escherichia coli* and *Candida albicans* and *Staphylococcus aureus* each 8.3 per cent. In most reports, *Pseudomonas aeruginosa* and other gram negative organisms were the common isolates followed by *Staphylococcus aureus*.[12] *Pseudomonas aeruginosa* was the common isolate in our study which correlates with other studies from India.[13,14] In a study performed at a tertiary care hospital referral hospital in our area (India), *Pseudomonas* spp and *Acinetobacter* spp were reported to be the most common causes of late-onset VAP, while the members of Enterobacteriaceae and *Acinetobacter* spp were observed to be the common agents causing early-onset VAP.[6] However, the aetiological agents may vary according to the patients, units and countries.[12]

Around 72.2 per cent of VAP patients in our ICU had monomicrobial and 27.8 per cent had polymicrobial infections, a higher rate than found in a similar study in Europe, where 52 per cent of VAP infections were found to be monomicrobial and 48 per cent polymicrobial.[15] Knowledge of susceptibility pattern of the local pathogens is necessary for choosing the appropriate antibiotics. *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* were sensitive to piperacillin tazobactam and imipenem. Among these isolates 62.5 per cent of *Pseudomonas aeruginosa* were sensitive to ceftazidime which most of the *Klebsiella pneumoniae* and *Escherichia coli* were also resistant to. Therefore, piperacillin tazobactam and imipenem can be used for initial empirical treatment of suspected cases of VAP and later narrow spectrum drugs based on the sensitivity pattern of the isolates can be used. Forty per cent of *Klebsiella pneumoniae* and both of the two isolates of *Escherichia coli* recovered in our study were ESBL producers. Another similar study showed 50 per cent of *Escherichia coli* and 67 per cent of *Klebsiella pneumoniae* were ESBL producers.[6] This denotes that ESBL producers are more common among this group of patients.

In the present study, 29.2 per cent of the isolates from VAP patients were MDR pathogens. Depuydt et al. (2008) have reported around 27 per cent of VAP patients with MDR pathogens.[16] The prevalence of the MDR pathogens is known to vary depending on the patient population and the hospital. The prevalence of MDR pathogens in the hospital setting is a threat for the treatment of the patients. The second line drugs available for these pathogens have to be tested. Earlier detection and treatment can reduce the mortality associated with these organisms.

Blood culture of all patients was performed, and post-mortem examination of two samples revealed blood that was found to be positive in one patient with *Pseudomonas aeruginosa* infection and another with *Burkholderia pseudomallei* infection. This suggests that blood culture may aid in early diagnosis of VAP. However, the role of blood culture in diagnosis of VAP is limited, as the spread to the blood occurs in less than 10 per cent of VAP as observed in the present study.[2] The Infectious Disease Society of America (IDSA) has commented that the yield of blood cultures in patients with pneumonia is low. However, the IDSA recommends performing blood cultures in patients with suspected pneumonia when there are certain

indications such as ICU admission, cavitary infiltrates, pleural effusion and leukopenia.[17]

Findings indicate that most of the MDR pathogens were isolated from late onset VAP compared to early onset VAP. The reason for this observation could be because a prolonged stay in the hospital and associated prior antibiotic therapy indirectly influences the development of MDR pathogens. This also correlates with other studies which report the prior antibiotic use to be an associated risk factor for VAP.[6]

Although this study was conducted in a resource-poor setting with a small patient sample, the aetiological agents and the MDR pathogens isolated with this patient population does have implications for the treatment of VAP. The findings of this study also correlate well with other studies conducted in India.[6,13,14]

Conclusion

To conclude, VAP is a nosocomial pneumonia that is common amongst ventilated patients. The aetiological agents vary from common organisms to MDR pathogens whose treatment is difficult. A proper knowledge of the MDR pathogens and early isolation followed by prevention of prolonged antibiotic therapy has the potential to reduce mortality levels currently associated with late onset VAP. Further multicentre studies on VAP are required to determine whether our results are congruent with other countries and settings.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.