British Microbiology Research Journal
13(1): 1-10, 2016, Article no.BMRJ.24360
ISSN: 2231-0886, NLM ID: 101608140

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Ventilator Associated Pneumonia in a Tertiary Care Hospital: Incidence, Risk Factors and Etiological Agents

Nahla Y. Sahlol[1], Lamiaa A. Madkour[1*] and Youssef M. Soliman[2]

[1]Department of Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt.
[2]Department of Chest Diseases, Faculty of Medicine, Cairo University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NYS, LAM and YMS designed the study and wrote the protocol. Authors NYS and LAM managed the literature research. Author NYS performed the statistical analysis and wrote the first draft of the manuscript. Author LAM wrote subsequent drafts. Author YMS collected the clinical samples from patients. Authors NYS, LAM, and YMS managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24360
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Universita, Italy.
Reviewers:
(1) Arturo Abdelnour, National Children´s Hospital, San Jose, Costa Rica.
(2) Charbell Miguel Haddad Kury, Medical School of the Municipality of Campos dos Goytcazes, Brazil.
Complete Peer review History: http://sciencedomain.org/review-history/13297

Original Research Article

Received 16th January 2016
Accepted 1st February 2016
Published 14th February 2016

ABSTRACT

Aims: With an escalating mortality rate reaching 50%, ventilator associated pneumonia (VAP) continues to pose an enormous threat to ICU patients worldwide.
Study Design: Prospective cohort study.
Place and Duration: The study was conducted from March 2014 through February 2015 at Kasralainy University Hospital. Hundred patients who were on Mechanical Ventilation (MV) for more than 48 hours were monitored for the development of VAP.
Methodology: We endeavored to identify the incidence, risk factors, and the most common etiological pathogens of VAP in ICU patients.
Results: Out of the 100 enrolled patients, 34 patients developed VAP. With univariate analysis, it was proven that the duration of MV and trauma were significant risk factors for VAP. The most common isolated pathogens were *Klebsiella, Pseudomonas,* and *Acinetobacter*. Alarmingly, 89.8%

**Corresponding author: E-mail: m_l@kasralainy.edu.eg;*

of the isolated organisms were multi-drug resistant (MDR).
Conclusion: The duration of MV has to be reduced to minimize the incidence and morbidity associated with VAP. Likewise, unnecessary prolonged hospitalization should be avoided. The choice of antibiotics should be judicial and guided by sensitivity patterns of the pathogens. These predictors, however, need further work to validate reliability.

Keywords: VAP; risk factors; nosocomial pneumonia; MDR pathogens.

1. INTRODUCTION

Ventilator-associated pneumonia (VAP) is the most frequent ICU-acquired infection [1]. It can be defined as a pneumonia where the patient has been on mechanical ventilation (MV) for > 2 days on the date of event, with the day of ventilator placement being day 1, and the ventilator was in place on the date of event or the day before [2].

Ventilator-associated pneumonia has been linked to a significant rise of morbidity and mortality; including prolongation of MV [3] and ICU stay [4,5], higher risk of death [4], as well as increased healthcare expenditures [6].

The incidence of VAP ranges from 8-68%. Mortalities may reach 24-50% and even up to 76% when the etiological agent is a multidrug resistant (MDR) pathogen [7]. Meanwhile, there's a noticeable discrepancy in the incidence of VAP in different regions of the world. This is probably attributed to the different diagnostic criteria, the study population, hospital resources, and the type of ICU [1].

Several risk factors associated with VAP have been reported; including the duration of MV, chronic pulmonary disease, acute respiratory distress syndrome (ARDS), trauma, sepsis, neurological disease, prior use of antibiotics, and blood transfusions [8]. Study of these factors would confer prognostic information about the probability of developing VAP in individual patients and populations. This shall be reflected on understanding the mechanisms that predispose to VAP, and allows risk stratification to target high risk patients for prevention strategies [9].

Meanwhile, the diagnosis of VAP necessitates an alert clinical suspicion, along with bedside examination, radiological examination, and microbiological analysis of respiratory secretions [10]. Prompt detection of the causative pathogens and their antibiotic susceptibilities is of pivotal importance for diagnosis of VAP; thereby reducing the adverse effects of inadequate therapy on the patient's prognosis [11].

In this study, we endeavored to identify the incidence, risk factors, and the most common etiological pathogens of VAP in ICU patients.

2. MATERIALS AND METHODS

2.1 Study Population

This prospective cohort study was conducted from March 2014 through February 2015 at Kasralainy University Hospital; Chest ICU. A total of 100 mechanically ventilated patients were enrolled in this study; including 65 males and 35 females. The patients' ages ranged from 17 to 80 years.

2.2 Ethical Consideration

Before commencement of the study, approval of the protocol was obtained from the Ethics Committee in the Department of Microbiology and Immunology, Cairo University.

2.3 Inclusion Criteria

Patients on mechanical ventilation for more than 48 hours were included in this study.

2.4 Exclusion Criteria

Patients with pneumonia occurring on admission or developing within 48 hours on ventilator were excluded from this study (Fig. 1).

2.5 Data Collection

Patients admitted to the Chest ICU who were intubated and mechanically ventilated with no manifestations of chest infection (no infiltrates on chest X-ray for 48 h after intubation), were monitored at frequent intervals (every 48 hours) for the development of VAP, using clinical and

laboratory criteria till discharge or death. The following variables were obtained: age, sex, provisional diagnosis, date of admission, and duration of MV. Parameters such as fever, leukocytosis and chest X-ray were collected. The possible risk factors of VAP including trauma, smoking as well as comorbid conditions, e.g. chronic obstructive pulmonary disease (COPD), ARDS, heart disease, diabetes mellitus (DM), renal failure, and malignancy were also recorded [12].

2.6 Diagnosis of VAP

VAP was diagnosed in patients who fulfilled both clinical and microbiological criteria, as follows:

2.6.1 Clinical diagnosis

The clinical suspicion of VAP was based on the association of a new or progressive consolidation on chest radiology plus at least two of the following variables: fever >38℃, leukocytosis or leucopenia, and purulent secretions [13].

2.6.2 Microbiological diagnosis

2.6.2.1 Specimen collection

Broncho-alveolar lavage (BAL) specimens were collected by the clinician through wedging the tip of a fiber-optic bronchoscope into a segment of the airway, with instilling sterile saline and aspirating each aliquot [14]. Specimens were collected in dry, sterile, labeled containers and transported as early as possible to the laboratory of the Microbiology and Immunology Department, Cairo University.

2.6.2.2 Direct detection

- Gram's stain was done to identify the morphology of micro-organisms, and the pus cell count; with >10 PMNLs / HPF being suggestive of infection [10,15].
- Ziehl-Nelsen stain was done for the detection of acid-fast bacilli.

2.6.2.3 Culture and identification

Day 1:

BAL was serially diluted in sterile normal saline to reach 1/1000 dilution. Then, 0.01 ml of the 1/1000 dilution was inoculated onto blood, chocolate and MacConkey's agar plates. The plates were incubated for 24 hours at 37℃ [16].

Day 2:

1. Plates were examined for bacterial growth. If no growth was retrieved, the plates were re-incubated for another 24 hours and re-examined.
2. In case of bacterial growth, the colonies were counted and expressed as CFU/ml. The number of CFU/ml equals the number of colonies on agar plate x dilution factor x inoculation factor. Thus, the presence of a single colony on the agar plate after inoculating 0.01 ml of the 1/1000 dilution was interpreted as $>10^5$ CFU/ml [16]. Hence, the diagnosis of VAP was confirmed microbiologically in patients having quantitative culture of $>10^5$ CFU/ml [17].
3. The isolates were identified based on standard bacteriological techniques; including colony morphology, Gram's stain, as well as biochemical reactions.
4. Antimicrobial susceptibility tests were done by performing Kirby-Bauer disc diffusion technique on Mueller-Hinton agar (MHA) plates [18].

Day 3:

1. Conventional biochemical reactions were examined to identify the causative organisms.
2. Antimicrobial susceptibility tests were interpreted by measuring the diameter of each zone of inhibition in mm and reporting the organisms as resistant, intermediately susceptible or susceptible, in accordance with the Clinical and Laboratory Standards Institute guidelines [19].

2.7 Statistical Analysis

All statistical calculations were done using Microsoft Excel 2010 and SPSS version 16 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) for Microsoft Windows.

Univariate analysis of the risk factors was done using Chi-Square test to determine the relation between qualitative variables. In addition, the *t*-test was used for quantitative variables that were expressed as mean and standard deviation. Multivariate analysis was done by logistic regression. Probability value (*P*-value) <0.05 and odds ratio >1 were considered statistically significant.

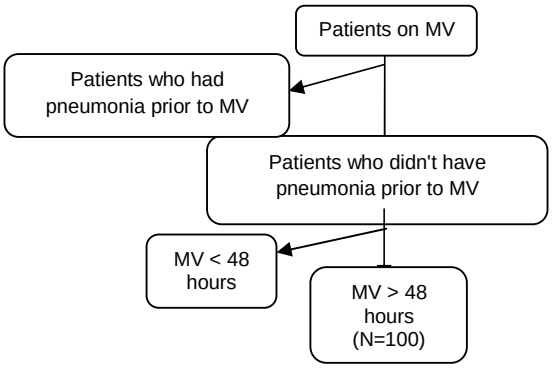

Fig. 1. Inclusion and exclusion criteria; N= number

3. RESULTS AND DISCUSSION

The present study was conducted on 100 mechanically ventilated patients in Kasralainy Chest ICU. The patients' ages ranged from 17 to 80 years. Out of the 100 patients, 65 patients were males, while 35 patients were females (Fig. 2).

Out of the 100 patients, 34 patients developed VAP, of which 22 were males and 12 were females. The patients' ages ranged from 18 to 78 in the VAP group, and from 17 to 80 in the non-VAP group. On the other hand, nearly 60% of the VAP cases occurred between the age of 51 and 70 years (Fig. 3).

Meanwhile, there was no statistically significant difference in age or gender among the VAP and non-VAP patients (Table 1).

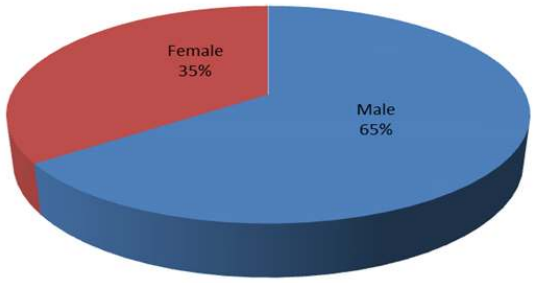

Fig. 2. Gender distribution in the study group

3.1 Risk Factors of VAP in the Study Group

Univariate analysis was used to reveal the risk factors of VAP by comparison between the VAP and non-VAP patients. In addition, multivariate analysis using logistic regression was applied to determine the independent effects of such variables on the development of VAP.

With univariate analysis, it was noticed that the duration of MV was the most significant risk factor in the studied group (*P*-value <0.0001). Likewise, trauma was a significant risk factor (*P*-value= 0.02). However, other variables showed

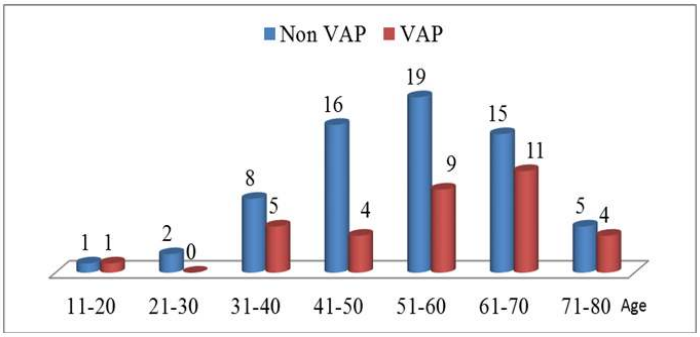

Fig. 3. Age distribution in the study group

Table 1. Gender and age distribution among VAP and non-VAP patients

Gender and age	VAP N= 34	Non-VAP N= 66	*P*-value
Males N =65 (100%)	22 (33.8%)	43 (66.2%)	0.9
Females N=35 (100%)	12 (34.3%)	23 (65.7%)	
Mean age ± SD (years)	55.21±13.88	52.42±12.71	0.3

SD= Standard Deviation

Table 2. Analysis of risk factors of VAP by univariate analysis and multivariate analysis

Risk factors	Univariate analysis			Multivariate analysis and logistic regression	
	VAP N= 34 (%)	Non-VAP N= 66 (%)	*P*-value	*P*-value	Odds ratio
Duration of MV Mean ±SD (days)	21.85±16.4	7.47±4.2	<0.0001	<0.0001	1.5
Trauma	4 (11.8%)	1 (1.5%)	0.02	0.05	4.8
DM	16 (47.1%)	21 (31.8%)	0.14	0.03	8.3
Smoking	12 (35.3%)	20 (30.3%)	0.6	0.2	3.2
Obesity	4 (11.8%)	2 (3%)	0.08	0.4	0.2
Respiratory disease	12 (35.3%)	31 (47%)	0.4	0.6	1.8
Neurological disease	3 (8.8%)	6 (9.1%)	0.9	0.3	4.6
Renal disease	3 (8.8%)	2 (3%)	0.8	0.2	6.7
Cardiac disease	5 (14.7)	13 (19.7%)	0.4	0.5	0.4
Hepatic disease	2 (5.9%)	2 (3%)	0.3	0.4	0.3
Malignancy	2 (5.9%)	3 (4.5%)	0.5	0.8	1.9
Steroid intake	10 (29.4%)	14 (21.2%)	0.4	0.5	0.5

no statistically significant difference. On the other hand, the multivariate analysis showed that the duration of MV (*P*-value <0.0001), trauma (*P*-value 0.05 and odds 4.8), as well as DM (*P*-value 0.03 and odds 8.3), smoking (odds 3.2) and some co-morbidities had significant effects on the development of VAP (Table 2 above).

3.2 Early and Late Onset VAP

In the present study, most of the VAP cases occurred after 4 days or more on MV; where 32 (94%) cases were late onset VAP, and only 2 (6%) cases were early onset VAP.

3.3 Causative Organisms of VAP

Out of the 34 VAP cases, Gram's stain revealed bacterial cells in 32 (94%) cases. Meanwhile, culture revealed that 17 (50%) cases had monomicrobial infection, while 15 (44%) cases had polymicrobial infection. On the other hand, in only 2 (6%) cases, Gram's stain didn't reveal any bacteria, and no bacterial growth was retrieved by culture (Fig. 4).

Noteworthy, both cases of early-onset VAP were monomicrobial and caused by methicillin resistant *Staphylococcus aureus* (MRSA). On the other hand, the common pathogens isolated in late onset VAP were *Klebsiella pneumoniae* which represented 35% of the isolated organisms, followed by *Pseudomonas aeruginosa* which represented 25% of the isolated organisms (Figs. 5 & 6).

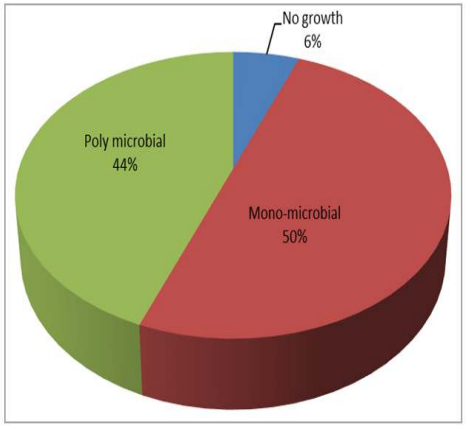

Fig. 4. Results of the bacteriological cultures

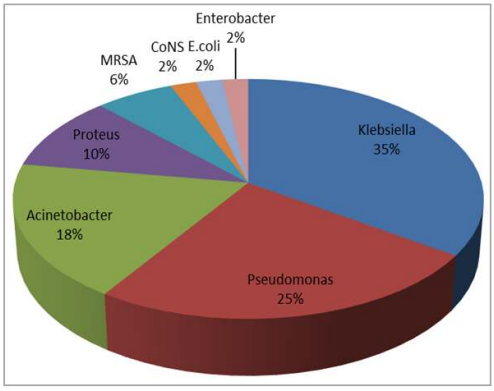

Fig. 5. Causative organisms of VAP in the study group

MRSA = methicillin-resistant Staphylococcus aureus; CoNS = coagulase negative staphylococci

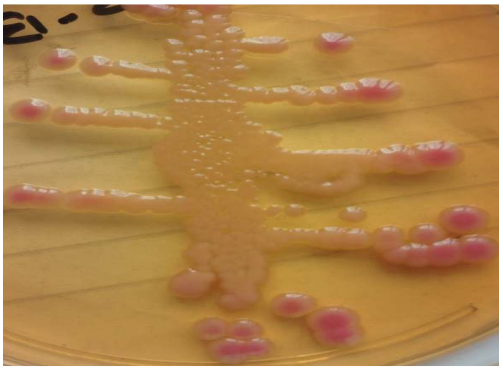

Fig. 6. *Klebsiella pneumoniae* on MacConkey's agar plate

Meanwhile, a pathogen was considered MDR if it was resistant to ≥ 1 agent in ≥ 3 antimicrobial categories [20]. Accordingly, 89.8% of the isolated organisms were MDR pathogens, while only 10.2% were non-MDR pathogens (Table 3).

3.4 Discussion

With the unwelcome rise of VAP, it has become the most frequent nosocomial infection among mechanically ventilated patients. Although VAP can be preventable, its overwhelming impacts on morbidity, mortality, length of hospital stay, and health costs are often enormous [21].

The incidence of VAP reported in the literature is widely variable and ranges from 10% to 65% [22]. Meanwhile, some authors have reported lower incidences between 9% and 27% [3].

In the present study, the incidence rate of VAP was 34%. This relatively high incidence could be owing to the presence of comorbid conditions and to the health-seeking behavior of patients in the developing countries. Owing to the dearth of resources, patients in developing countries seek medical help only when it is absolutely inevitable; when the underlying condition is well advanced and may be irreversible. This may require longer duration of MV, which is directly proportional to the development of VAP. Another important factor leading to the high incidence of VAP in this study may be attributed to the lack of nursing staff. This, in turn, had adverse effects on the ideal patient care [23].

However, the VAP incidence in this study is slightly lower than the incidence of 37% reported by Gadani et al. [24] and 35.14% reported by Golia et al. [25]. As a matter of fact, there is great variability in the incidence of VAP reported from different countries [8,23,25-28] (Table 4).

The variabilities of the incidence in various studies might be due to absence of a standardized diagnostic approach, to the type of ICU, population differences [29] or methodological differences between studies. Even in developed countries, a considerable inter-country variation exists. Meanwhile, infection control in developing countries differs markedly from that in developed countries [30].

In this study, the risk factors for the development of VAP were evaluated. Out of the 100 studied patients, 65% were males and 35% were females. This might reflect the unequal distribution of male and female patients in the ICUs [31].

Regarding the age of the studied patients, the mean ± SD was 55.21±13.88 and 52.42±12.71 in the VAP and non-VAP groups respectively. There was no statistically significant difference in the age and gender distribution between the VAP and non-VAP groups. This goes in line with the results provided by earlier studies. Joseph et al. [1] conducted a prospective study on 200 mechanically ventilated patients, of which 59.5% were males and 40.5% were females. The mean ± SD age of the patients was 41.4±14.7 and 36.8±16.3 in the VAP and non-VAP patients respectively. Likewise, there was no statistically significant difference in the age and gender distribution of their patients. In addition, Charles et al. [13] conducted a prospective study on 76 mechanically ventilated patients, of which 73.7% were males and 26.3% were females. Similarly, there was no statistically significant difference in the age and gender distribution of the patients in VAP and non-VAP groups.

In this study, about 60% of VAP cases occurred in the age group of 51 to 70 years. This is in accordance with the study by Golia et al. [25] which showed that patients in the age group of 46-60 years were more prone to VAP. This may be due to the fact that the number of patients exposed to MV > 48 hours was also more in this age group [32].

Meanwhile, by applying univariate analysis in this study, it was observed that the duration of MV and trauma were the only significant risk factors associated with VAP. On the other hand, by applying multivariate analysis and logistic regression, the duration of MV, trauma, as well as DM, smoking, respiratory diseases (e.g. COPD), renal diseases, neurological diseases and malignancy were all independent risk factors for the development of VAP.

These results were generally in accordance with other studies. Hortal et al. [33] reported that the duration of MV, older age and malignancy were independent risk factors of VAP. Meanwhile, Pawar et al. [34] reported that by univariate analysis; COPD, coma, steroid intake and prior antibiotics were significant risk factors of VAP. On the other hand; by multivariate analysis, the authors reported that intermittent positive-pressure ventilation hours and steroid intake had an independent effect on the development of VAP.

Moreover, in a study by Xie et al. [30] the multivariate analysis showed that male sex, coma, COPD, DM, serious illness (including respiratory failure, heart failure, cancer, and dialysis), infection at other sites and prior antibiotic use > 4 days were significant independent risk factors of VAP.

In the present study, most of the VAP cases occurred after 4 days or more on MV; where 32 (94%) cases were late onset VAP and only 2 (6%) cases were early onset VAP. This goes in line with the results of previous studies. In a study by Gadani et al. [24], the incidence of late onset VAP was found to be 73%, while that of early onset VAP was 27%. Another study by Golia et al. [25] concluded that 55.77% of the studied patients had late onset VAP, while 44.23% had early onset VAP.

Out of the 34 VAP cases in this study, Gram's stain revealed bacteria in 32 (94%) cases. Meanwhile, culture revealed that 17 (50%) cases had monomicrobial infection, while 15 (44%) cases had polymicrobial infection. In concordance with our results, Pawar et al. [34] found that 48% of VAP patients had monomicrobial infection while 52% patients had polymicrobial infection. However; Joseph *et al.* [1] reported polymicrobial infection in 27.8% of VAP patients. Moreover; in a study by Golia et al. [25] only 13.46% of cultures were polymicrobial; which is lower than that of the present study. This may be because Golia et al. [25] excluded patients with ARDS, cavitary lung disease, lung cancer, as well as tuberculosis patients, and patients with congenital or acquired immunodeficiency.

Table 3. Resistance profiles of the most common isolated pathogens

Antimicrobial category	Antibiotics	Resistance N (%)		
		Klebsiella **17 (100%)**	***Pseudomonas*** **12 (100%)**	***Acinetobacter*** **9 (100%)**
Penicillins	Ampicillin	17 (100%)	12 (100%)	9 (100%)
	Cefazolin	9 (52%)	0	3 (33%)
Cephalosporins	Cefoxitin	14 (82%)	9 (75%)	9 (100%)
	Ceftazidime	3 (17.5%)	12 (100%)	8 (88%)
	Cefepime	6 (35%)	12 (100%)	3 (33%)
Carbapenems	Imipenem	1 (5.8%)	1 (8.3%)	1 (11%)
Monobactams	Aztreonam	4 (23%)	12 (100%)	4 (44%)
Penicillins + β-lactamase inhibitors	Piperacillin-Tazobactam	4 (23%)	12 (100%)	9 (100%)
	Ciprofloxacin	5 (29%)	2 (16.6%)	6 (66%)
Fluoroquinolones	Ofloxacin	5 (29%)	1 (8.3%)	2 (22%)
	Levofloxacin	1 (5.8%)	11 (91.6%)	1 (11%)
Aminoglycosides	Amikacin	4 (23%)	2 (16.6%)	0
	Gentamycin	7 (41%)	12 (100%)	2 (22%)
Polymyxins	Colistin	2 (11%)	1 (8.3%)	0

Table 4. Incidence of VAP in studies from different countries [8,23,25-28]

Country	Researchers	Number of studied patients	Incidence of VAP (%)
USA	Ibrahim et al. (2001) [26]	880	15
India	Rakshit et al. (2005) [23]	51	47
20 countries	Tejerina et al. (2006) [8]	2897	15
Turkey	Ertugrul et al. (2006) [27]	100	28
India	Golia et al. (2013) [25]	148	35.1
Brazil	Resende et al. (2013) [28]	126	26.2

On the other hand, in only 2 (6%) cases of the present study, Gram's stain didn't reveal any bacteria, and no bacterial growth was retrieved by culture. This may suggest infection with *Legionella,* viruses, fungi or anaerobic bacteria. This result was in agreement with Marik and Careau [35] who drew the attention toward the possibility of these agents as causative organisms of VAP, and highlighted that clinicians should take into account such microorganisms and consider them during empirical therapy.

In the present study, both cases of early onset VAP were caused by MRSA, while the most common pathogens in late onset VAP were *Klebsiella pneumoniae* (35% of the isolated organisms), followed by *Pseudomonas aeruginosa* (25% of the isolated organisms) and *Acinetobacter* spp. (18% of the isolated organisms). Meanwhile, 89.8% of the isolated organisms were MDR pathogens, while only 10.2% were non-MDR pathogens.

Noteworthy, airway intubation is associated with increased frequency of Gram negative bacterial colonization of the upper and lower respiratory tracts, followed by rapid growth of these Gram negative bacteria and pneumonia [36]. Several studies have reported that more than 60% of nosocomial pneumonias were caused by aerobic Gram negative bacilli. *P. aeruginosa* and other Gram negative bacterial species adhere five times better to buccal cells and tracheal epithelial cells of severely ill patients than to cells of normal individuals [37]. This reflects their ability to survive in the hospital environment [36].

Moreover, numerous studies have shown that MDR bacteria, in particular aerobic Gram-negative bacteria, easily colonize the gastrointestinal tract and respiratory tract of hospitalized patients [38]. In addition, it is well known that MDR bacteria are becoming increasingly prevalent in the hospital environment as a result of the extensive use of antibiotics [39]. Consequently, prior use of antibiotics significantly decreased the incidence of VAP caused by Gram-positive cocci or *H. influenzae*, but significantly increased the rate of VAP caused by *P. aeruginosa* [40].

Noteworthy, the etiological agents of VAP may differ according to patients, units, hospitals or countries. The main epidemiological patterns may not only vary from unit to unit, but also in a given unit over the course of time and this is true for their associated susceptibility patterns. Thus, reported differences can frequently be explained by local specificities [40].

4. CONCLUSION

Proper and widespread awareness of VAP epidemiology should occupy an utmost priority in health-care settings; not only in developing countries, but also in developed ones.

This study was attempted to highlight the most significant risk factors of VAP in ICU patients, and to identify the most commonly incriminated pathogens.

Recognition of the risk factors for VAP, combined with prompt clinical diagnosis and identification of the causative agents, can open new avenues for effective preventive strategies, which in turn, can improve the patients' outcomes. Meanwhile, the choice of antibiotic therapy should be guided by the sensitivity patterns of the involved pathogens. The judicial use of appropriate antibiotics may reduce patient's colonization and subsequent VAP with MDR pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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