A REVIEW ON THE CAUSATIVE AGENTS, RISK FACTORS AND MANAGEMENT OF VENTILATOR ASSOCIATED PNEUMONIA: FROM SOUTH ASIAN PERSPECTIVE

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences
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September 2021

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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Abstract/ Executive Summary

Ventilator Associated Pneumoniae (VAP) is a common hospital acquired pneumoniae in ICU patients. Patients with pneumoniae after 48 hours of mechanical ventilation is considered VAP. INICC found that VAP rates between 2003 to 2008, 2004 to 2009 and 2012 to 2017 as 13.6, 15.8 and 14.1 per 1000 episodes. VAP can be two types, early onset VAP and late onset VAP. The most common pathogens include *Acinetobacter baumannii*, *Pseudomonas Aeruginosa* and *Klebsiella pneumoniae*. Risk factors that increase the mortality of VAP are age, gender, increased in mechanical ventilation, disorder of consciousness, burns, comorbidities, prior antibiotic therapy, invasive operation, gene polymorphism and others. Blind bronchial sampling is used to collect endotracheal aspirates for identification and antibiotic susceptibility. Treatment protocol uses antibiotic therapy depend on the pathogens and their AST. For early onset VAP, cephalosporin (cefotaxime or ceftriaxone), a fluoroquinolone, or piperacillin-tazobactam and for late onset VAP, ceftazidime, ciprofloxacin, meropenem, and piperacillin-tazobactam.

Keywords: Ventilator Associated Pneumoniae; mechanical ventilation; endotracheal aspirates; antibiotic susceptibility; antibiotic therapy

Dedicated to my loving parents, Nawrin Yusuf and A.K. M Amirul Islam.

Acknowledgement

First of all, I would like to express gratitude Almighty Allah for bestowing me with strength and patience to complete the many perils of my undergraduate life. I thank my parents for believing and supporting me when even I had given up on myself. Their advices have helped me go through some of the hard times of growing up.

To my supervisor, Akash Ahmed, Lecturer, Department of Mathematics and Natural Sciences, I would like to thank for all the support and encouragement throughout the times of undergraduate thesis. His kind and friendly approach has helped me overcome my anxiety and work together to complete this thesis.

I thank all my faculties, for inspiring and motivating me, I have learned so much from my years of studying under them. Their advices have helped me overcome some parts of my insecurities and helped me grow as a person.

Finally, I thank all my friends who supported me and cared for me. The good memories we made; I will remember forever.

Table of Contents

List of Tables

List of Figures

List of Acronyms

VAP	Ventilator Associated Pneumonia
HAP	Hospital acquired pneumonia
ATS	Amer-ican thoracic society
IDSA	Infectious Diseases Society of America
ICU	Intensive Care Unit
PICU	Pediatric Intensive Care Unit
INICC	International Nosocomial Infection Control Consortium
CDC	Centers for Disease Control and Prevention
MICU	Medical Intensive Care Unit
CCU	Critical Care Unit
SICU	Surgical Intensive Care Unit
NNIS	National Nosocomial Infection surveillance system
MDR	Multidrug Resistance
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin- susceptible *Staphylococcus aureus*
MV	Mechanical Ventilation
CPIS	Clinical Pulmonary Infection Score
APACHE	Acute physiology and chronic health evaluation

AB	Acinetobacter baumannii
ESBL	Extended spectrum beta-lactamase
TBI	Traumatic Brain injury
MBL	Metallo-β-lactamase
BAL	Bronchoalveolar lavage
PCR	Polymerase chain reaction
CLSI	Clinical and Laboratory Standards Institute
PFGE	Pulsed-field gel electrophoresis
(REP)-PCR	Repetitive extragenic palindromic
AR	Antibiotic Resistance
AST	Antibiotic Susceptibility Test
CF	Cystic fibrosis
NB-BAL	Non-bronchoscopic protected BAL
PPV	positive predictive value
NPV	negative predictive value

Chapter 1

Introduction

Ventilator Associated Pneumoniae (VAP) is a common nosocomial disease considered fatal to the critical care(Modi & Kovacs, 2020). VAP is pneumonia that is very common ICU-borne infection, ranging in occurrence from 11% to 57.14%(Kepekci, 2020). Most common form of infectious complication and mortality among patients in the intensive care unit (ICU) (Gursel & Demirtas, 2006).Without intubation, incidence of pneumonia in 48 hours more after admission is considered hospital acquired/ nosocomial pneumonia (HAP) based on the Infectious Diseases Society of America / American thoracic society (IDSA/ATS) guidelines (2016). Contamination of natural flora through aspiration of gastric or oropharyngeal contents are important for the pathogenesis where oropharynx is the main source for contamination. Further, the continual aspiration of subglottic secretion may also be a cause of VAP as seen in two randomized trails(Bonten et al., 2004). An HAP that occurred after endotracheal intubation for more than 48-72hours is VAP (Bonten et al., 2004). In hospitals around the world, Ventilator-associated pneumonia (VAP) is an important reason of mortality and antimicrobial consumption (Khurana et al., 2017). In Pediatric Intensive Care Units (PICU) , it is the most often caused infection and has a pooled cumulative incidence of 22.8%, hence remained a large cause of morbidity and mortality with high health care related costs (Osman et al., 2020) . 8-28% patients with mechanical ventilation (MV) have VAP complications over time. While infections that are related to organs like urinary tract and skin have a low mortality of 1-4%, VAP mortality rates that range from 24-50% and even as high as 76% in some cases with high infection causing pathogens. The most common organisms involved with VAP include S*taphylococcus aureus , Pseudomonas aeruginosa , Acinetobacter baumannii*, and

Klebsiella pneumoniae. but other factors like amount of patients in ICU, population, length of stay and previous exposure to antimicrobial therapy can also affect (Chastre & Fagon, 2002).

Chapter 2

Prevalence

VAP occurs in 9-27% of mechanically ventilated patient with a mortality rate of 9%. It increases with the number of ICU and duration of stay (Hunter, 2012). VAP mortality rate is between 27 and 76% (Bonten et al., 2004). In the first 5 days the risk is highest during ventilation and has a mean duration of 3.3 days from intubation to the development of the disease (Ashok et al., 2014). With 724 adults, an Italian study showed that there is a 5% increase in incidence from Day 1 of ventilation and this only increases to 69% at the 30th day from receiving ventilation (Bonten et al., 2004). Between March 2011 and March 2012, reports state that a total of 1873 patients were enrolled in 56states in US alone with 502 in 18 sites, Europe (495 at 14), Latin America (500 at 14), and Asia Pacific (376 at 10) (D. S. Xie et al., 2011).

International Nosocomial Infection Control Consortium (INICC) conducted surveillance study showed that from January 2004 through December 2009, the intensive care units (ICUs) of 36 countries in Latin America, Asia, Africa, and Europe had overall rate of 15.8 per 1,000 ventilator-days and a crude unadjusted excess mortalities of 15.2% for ventilator- associated pneumonia (Victor D. Rosenthal et al., 2012). From January 2003 through December 2008 in 173 intensive care units (ICUs) in Latin America, Asia, Africa, and Europe, overall rate of ventilator-associated pneumonia (VAP) was 13.6 per 1000 ventilator-days (Victor D. Rosenthal et al., 2010).

Between March 2011 and March 2012, reports state that a total of 1873 patients were enrolled in 56states in US alone with 502 in 18 sites, Europe (495 at 14), Latin America (500 at 14), and Asia Pacific (376 at 10). Centers for Disease Control and Prevention (CDC) stated that in US, there are a mean rate of 3.6 cases of VAP per 1000 ventilator days in ICU. A data from

International Nosocomial Infection Control Consortium surveillance study stated that the cases in developing countries maybe as high as 16.8 cases per 1000 ventilator days (R. Khan et al., 2016).

From January 2012 to December 2017 in 523 intensive care units (ICUs) in 45 countries from Latin America, Europe, Eastern Mediterranean, Southeast Asia, and Western Pacific, data from INICC ICU and CDC-NHSN ICU reported the ventilator-associated pneumonia rate were 14.1 and 0.9 per 1,000 ventilator-days. Although INICC ICU showed a higher rate compared with CDC-NHSN ICU (Víctor Daniel Rosenthal et al., 2020). It can be seen that the report between 2012 to 2017 showed a higher VAP rate per episode. In a US survey done in 2014. VAP and HAP (hospital acquired pneumonia) take up 22% of disease contracted from hospital as shown in 183 US hospitals (Modi & Kovacs, 2020).

Table 1 VAP rates per episode in different countries in Asia

Country	**Study design**	**Type of ICU**	**Criteria to diagnose**	**VAP rate - episodes per 1000 ventilation**	**Reference**
India	Prospective study	MICU and CCU	CDC	14.35 to 8.1	(I. D. Khan et al., 2017)
India	Prospective study	Neurosurgery and Polytrauma	CDC	11.9	(Khurana et al., 2017)
Bangladesh	Prospective cohort	CCU	CDC	35.73	(Mallick et al., 2015)
Thailand	Prospective study	SICU	CPIS	6.3 to 2.8	(Chittawatanarat et al., 2014)
Thailand	Surveillance study	ICU	(N/A)	12.6-13.6	(Reechaipichitkul et al., 2013)

Nepal	Prospective study	MICU and SICU	CDC	21.4	(Parajuli et al., 2017)
Kuwait	Prospective surveillance study	(N/A)	CDC	4	(Al-Mousa et al., 2016)
South Korea	Retrospective study	Cancer ICU	(N/A)	2.13	(Park et al., 2014)
Pakistan	(N/A)	Medical and surgical	(N/A)	26	(Noor & Hussain, 2005)
Saudi Arabia	(N/A)	Medical surgery	(N/A)	16.8	(Memish et al., 2000)
Japan	Cohort study	Medical and surgical	(NNIS)	6.5	(Suka et al., 2014)
China	Prospective study	(N/A)	(N/A)	4.5	(J. Xie et al., 2018)

(N/A) means no usable data found. Abbreviations used: ICU- Intensive Care Unit; MICU-Medical Intensive Care Unit; CCU- Critical Care Unit; CDC-Centers of Disease Control and Prevention; SICU- Surgical Intensive Care Unit; NNIS- National Nosocomial Infection.

Chapter 3

Infectious agents and Antibiotic Susceptibility

There are two classifications of the disease. In the first four days of intubation and mechanical ventilation if the patient is found to have VAP then it is an early onset pneumonia and commonly caused by bacteria that are antibiotic sensitive. Multidrug resistant pathogen usually causes the late onset pneumonia which happens after four days. Early onset pneumonia is usually caused by antibiotic sensitive bacteria such as *Haemophilus spp,* streptococci including *Streptococcus pneumoniae,* and methicillin sensitive *Staphylococcus aureus.* The late onset pneumonia is typically caused by MDR bacteria such as *Pseudomonas aeruginosa, Acinetobacter spp,* and methicillin resistant *S aureus* (Hunter, 2012). In early onset VAP, *S.aureus* is very common compared with late onset VAP while MRSA isolates are more common in late onset VAP compared with early onset. Late onset VAP patients have the higher chances of being infected with Gram-negative bacilli. Although, some studies found no significant differences for specific pathogens like *P. aeruginosa* or *A. baumannii*. It is same for MDR pathogens when comparing early-onset versus late- onset VAP (Restrepo et al., 2013). *Enterobacteriaceae* (66.66%), *P. aeruginosa* 1 (6.67%), *S. aureus* (20%), and Coagulase-negative staphylococci (CONS) (6.67%) were early onset and non-fermenters (50%) including *Pseudomonas spp*. (15.62%), *Burkholderia spp* (3.13%), *Acinetobacter spp*. 10 (31.25%), *Enterobacteriaceae* 13 (40.61%), *S. aureus* (3.13%), *Enterococcus spp.* (3.13%), and *Candida spp*. (3.13%) were common in late onset of VAP in this study (Mahapatra et al, 2019).

3.1 Pathogens

Most of the VAP occurrence were caused by Gram-negative bacilli taking in 41-92% of the VAP episodes. Of them, *Pseudomonas Aeruginosa* made up the most. Some reports showed

Candida spp isolates (Arabi et al., 2008). In one study, the common organisms isolated where *Klebsiella pneumonia* (16%), *Eschereria coli* (8.3%), *Pseudomonas aeruginosa* (2.7%), *Citrobacter* (2.7%), Coagulase negative *Staphylococcus aureus* (2.7%). Imipenem and cefeperazone+sulbactum sensitive and ampicillin resistant gram negatives were isolated as well as cefoxitin sensitive gram positives were isolated. The presence of ESBL in the study was 5.5% (Akhtar et al., 2020).

The main organisms related to the increased rate of mortality in VAP are *Pseudomonas* or *Acinetobacter spp* with high mortality risks. In the study conducted, *Pseudomonas aeruginosa* was seen to take up the highest, causing 22.9% of the infection. There were *Klebsiella pneumonia* and *E. Coli* and *Pseudomonas Aeruginosa* had a significant higher infection in VAP group. (Bonten et al., 2004) In a retrospective study using 49 patients, it was seen that most patients who were isolated with *Klebsiella spp,* died. *Enterobacter spp* and *Psuedomonas Aeruginosa* had a 80% and 70,6% mortality rate respectfully while only one patient had an isolation of *Staphylococcus aureus* (Kepekci, 2020).

VAP caused by *Enterobacteriaceae* among elderly patients are *Escherichia coli* and *Klebsiella* species. No difference was found with high-risk pathogens or polymicrobial pneumonia compared with middle aged patients. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* had no significant difference. For old and very elder patients, *Enterobacteriaceae, E. coli* and *Klebsiella* species seem to cause the most VAP, especially *E. coli* which has been found to significantly cause VAP following aspiration. Other literatures showed that Gram negative bacteria are responsible for 34.1% of pneumonia in patients above 65 years old and 20.5% in patients under 65 years age (Blot et al., 2014).

In burn patients, it's found that microorganisms like *Staphylococcus aureus,* methicillin-resistant *S. aureus* (MRSA), *Pseudomonas Aeruginosa* , and *Acinetobacter baumannii* (AB)

accounted for the majority of organ- isms causing VAP. In the first week, *S. aureus* was the most common while after 2 weeks, *P. Aeruginosa, A. baumannii*, and MRSA were the most commonly cultured organisms. *P. Aeruginosa* and *A. baumannii* combined accounted for nearly 20% of the VAP in the first 2 weeks (Sen et al., 2016).

In the study conducted with 49 patients, it was seen that VAP patients had a longer ICU stay and it was found that five different microorganisms were causing VAP in the ICU, of which *Pseudomonas aeruginosa* was the most common (Kepekci, 2020). Prospective cohert study in china found that the most common isolates from VAP patients were gram negative bacteria (72.7%), gram positive bacteria (15.3%) and fungi(12.0%). The common pathogens were *Pseudo-monas aeruginosa* and *Acinetobacter baumannii* then *Staphylococcus aureus* and *Stenotrophomonas maltophilia* (D. S. Xie et al., 2011).

Table 2 VAP causing pathogens and their resistance to antibiotics

Organism	**Resistance**	**Reference**
Acinetobacter baumannii	Imipenem, meropenem, piperacillin, tazobactam, amikacin, ciprofloxacin, ceftazidime	(Victor D. Rosenthal et al., 2012) (But et al., 2017)(D. S. Xie et al., 2011) (Ashoka Mahapatra, Das, 2019)
Klebsiella Pneumonia	Ceftriaxone, ceftazidime, Imipenem, meropenem, ertapenem, ampicillin	(Victor D. Rosenthal et al., 2012) (Yan et al., 2016)

Pseudomonas Aeruginosa	Fluoroquinolones, Piperacillin, piperacillin-tazobactam, Amikacin, ciprofloxacin, ceftazidime, colistin	(Victor D. Rosenthal et al., 2012) (But et al., 2017) (D. S. Xie et al., 2011)
Escherichia Coli	Ceftriaxone, ceftazidime, Imipenem, meropenem, ertapenem, Fluoroquinolones	(Victor D. Rosenthal et al., 2012)
Staphylococcus Aureus	Oxacillin, Methicillin	(Victor D. Rosenthal et al., 2012) (D. S. Xie et al., 2011) (Ashoka Mahapatra, Das, 2019)
Enterobacteriaceae	Ampicillin,	(Ashoka Mahapatra, Das, 2019)
Extended spectrum beta-lactamases (ESBL)	Ampicillin, ampicillin-sulbactam, cefazolin, ceftriaxone, aztreonam, Imipenem, Ertapenem	(Yan et al., 2016)

3. 2 Antibiotic Susceptibility of VAP causing pathogens.

Studies show that the resistance in VAP for the following organisms are Acinetobacter baumannii for Imipenem or meropenem (66.3%) , *Klebsiella pneumoniae* for Ceftriaxone or ceftazidime (68.9%) and Imipenem, meropenem, or ertapenem (7%), *Pseudomonas aeruginosa* for Fluoroquinolones (46.2%), Piperacillin or piperacillin-tazobactam (40.2%),

Amikacin (28.3%), Imipenem or meropenem (42.7%) and Cefepime (37.5%), *Escherichia coli* for Ceftriaxone or ceftazidime (67.5%), Imipenem, meropenem, or ertapenem (4.2%) and Fluoroquinolones (54.9%); Staphylococcus aureus for Oxacillin(73.2%) (Rosenthal et al., 2012). *Acinobacter Baumanni* strains were found to have a carbapenem resistance of 99.4% in one study and also susceptible to colistin. The strain was found to be resistant rates like 99.7% to meropenem, piperacillin/ tazobactam 99.3%, amikacin 93.1%, ciprofloxacin 99.7%, and ceftazidime 99.3%. *Pseudomonas Aeruginosa* isolated from VAP patients had antimicrobial resistance rates to 54.1% piperacillin/tazobactam 52.7%, amikacin 29.7%, ciprofloxacin 50%, ceftazidime 45.9%, and colistin 1.4% (But et al., 2017).

Meticillin resistant *S.aureus* was reported to be high , about 47.5%. Also imipenem *resistant P aeruginosa* (42.0%), imipenem-resistant A. baumannii (80.3%) and ciprofloxacin-resistant *P. aeruginosa* (58.6%) were found high in China (D. S. Xie et al., 2011). Prospective study in china found that some of the 92 *S. aureus* isolated were methicillin-resistant (MRSA). Although no vancomycin-resistant enterococcus (VRE) or vancomycin-resistant/intermediate *S. aureus* (VRSA/VISA) were found (D. S. Xie et al., 2011). Meticillin resistant *S. aureus* was reported to be high, about 47.5%. Also, imipenem resistant *P aeruginosa* (42.0%), imipenem-resistant *A. baumannii* (80.3%) and ciprofloxacin-resistant *P. aeruginosa* (58.6%) were found high in China.

K. pneumonia showed resistance to ampicillin. One of the Extended spectrum beta-lactamase (ESBL) producing strains showed high resistance to ampicillin, ampicillin-sulbactam, cefazolin, ceftriaxone and aztreonam. Some strains are found to be resistant to Imipenem and Ertapenem (Yan et al., 2016). In this study Acinetobacter baumannii was most common and of it 96% were was resistant to carbapenems. Pseudo- monas aeruginosa which were also resistant to carbapenems. The third most common was Escherichia coli (7 isolates, that is 12%), among which 17% was ESBL (+) (Wałaszek et al., 2016).

A. baumannii strains were found resistant to ceftazidime, imipenem but susceptible to colistin and piperacillin/tazobactam. Ceftazidime, piperacillin/tazobactam, imipenem and colistin were found to interfere with the biofilm formation of *A. baumannii*. Specific antibiotic therapy but in low concentrations than that needed to kill *A. baumannii* strains during ventilator-associated pneumonia (VAP) can instead in some cases, stimulate the biofilm formation potential and aggravate the infection. This is why it's important to find not only the strain but also its lethal doses especially when using polymyxins which is the last therapeutic alternative in the treatment of infections for a MDR gram negative bacteria (Imane et al., 2021).

ESBL was produced by 21.74% of Enterobacteriaceae. AmpC β-lactamase was positive in 35.29% non-fermenters and 26.08% Enterobacteriaceae, Metallo-β-lactamase (MBL) was positive in 17.64% non-fermenters and 17.39% Enterobacteriaceae were found in this study. 100% resistance to ceftazidime, amikacin and ciprofloxacin was shown by Acinetobacter spp while 75% of the S. aureus isolates were found to be MRSA (Mahapatra et al, 2019).

Chapter 4

Risk Factors

The risk of VAP is dependent on the length of exposure to the hospital environment as well as factors relating to the host and the treatment methods (Bonten et al., 2004). In a cohort study conducted on the duration of ventilation and risk associated, it was seen the risk of VAP does not remain fixed over the course of ventilation rather it is estimated to increase over time such as 3% per day in the first week, 2% per day in the second week, and 1% per day in the third week and it goes on (Bonten et al., 2004).

4.1 Age

In a study conducted with 417 patients, maximum affected were in the age range between 69.9 ± 15.9 (range: 19–98) years (But et al., 2017) In a multicenter study with 1735 patient, it was found that older age may not increase the risk for VAP. Further, compared with old and middle-aged patients, very old patients had very a smaller number of patients with fever at VAP. Although mortality from VAP was high for elder patients, it did not seem to occur higher among the elderly. (Blot et al., 2014)

4.2 gender

In a retrospective study, it was found that of the 417 patients, 213 (51.1%) males and 204 (48.9%) females. (But et al., 2017) This study with 58 cases of VAP found that the infection was more common among men (43 cases, that is 6%) than in women (15 cases, that is 3%). (Wałaszek et al., 2016) 854 patients with VAP are taken of them, 676 males (79%) and 178 females (21%). The overall incidence of VAP between the genders, male and females were 3.8% and 2.6%. Males developed VAP more than females however it was seen that females had a higher mortality with VAP compared with males (15% vs. 24%). The study also found that females had a higher case of severe episodes compared with males (49% vs. 61%). Females

had greater incidence of early nosocomial VAP and had more VAP cases with MDR and polymicrobial organisms. Females who developed early VAP had a larger mortality than males (43% vs. 74%) (Sharpe et al., 2014)

4.3. Increased mechanical ventilation

The total mechanical ventilation time affects the cumulative incidence of VAP and it was reported that between the 5th and 9th days, the risk of ventilation is high for patients in mechanical ventilation. Therefore, in order to prevent it, there should be great importance to reduce intubation and decrease the invasive mechanical ventilation (MV) exposure (Kepekci, 2020). The longer the stay, more number of patients developed VAP (Apostolopoulou et al., 2003). LOV and LOS is very high in the VAP group which may show that there is a relationship between the two. Duration of MV increases the incidence of VAP. This study found an overall incidence of 20.8 in patients with MV (Abdelrazik Othman & Salah Abdelazim, 2017). Study with 465 patients found that the mean duration for MV for all the patients were 13.4 ±4.4 days and that the duration was important because they statistically demonstrated that the patients with VAP had a longer mean MV duration compared with non VAP patients [Mean ventilation duration, d (mean±SD) 15.1±5.2 vs 13.0±4.1] (Liu et al., 2017). In patients who receive mechanical ventilations, studies show that 28% of the patient get affected by VAP and the rate at with it occurs is dependent on the length or duration (Bonten et al., 2004).

4.4 Disorder of consciousness

Traumatic Brain injury (TBI) a type of head injury, in critical situations, associated with prolonged hospital admission and patients often have intubation and mechanical ventilation (MV) in severe conditions due to airway obstruction, aspiration, or hypoxia caused by TBI.

From the TBI patients with mechanical ventilation for >48hours, 24.3% developed early -onset VAP and 26.4% developed late onset VAP. Patients with greater number of other injuries, had early onset VAP. Hemorrhagic shock, coma and pulmonary contusions were more common with patients with early VAP. Older patients were more affected with late onset VAP. They more frequently with coma on admission and had multiple transfusions. They had an overly more critical clinical condition on admission. This study showed that the VAP incidence in patients with TBI is 49.7% which is very high than average (Jovanovic et al., 2015). Aneurysmal subarachnoid hemorrhage (SAH) is a serious condition that in most cases require mandatory mechanical ventilation (MV) and intensive care unit (ICU) hospitalization. In this study 47% of the patients were positive for VAP. The study showed a significant association between constant sedation and VAP (Cui et al., 2018). From this it can be deduced that disorder of consciousness can be a risk factor.

4.5. Burns

In severely burned patients, VAP can cause morbidity and mortality. Patients with VAP often have large burn injury and suffer from inhalation injury and higher mortality compared to non-VAP patients. This is demonstrated in the study where it shows that mortality was also significantly higher in VAP patients (34% vs 19%) than those who did not (no-VAP). VAP burn patients also have longer duration of ICU, hospital stays and prolonged mechanical ventilation. Inhalation injury may also contribute to the higher risk of VAP in burned patients as VAP rates as high as 55 per 1000 ventilator days. The study also found that patients with VAP had more inhalation injuries than non- VAP (44.6% vs 27%).

The reasoning maybe that pathologic immune, vascular, and organ changes may occur due to severe burning and this may increase the risk of VAP (Sen et al., 2016).

4.6 Comorbidities

The incidence rates of VAP can differ according to the population involved in the study. For example, cancer patients are reported to have higher VAP rates. Also, patients with major trauma injury patients, chronic obstructive pulmonary disease patients, acute respiratory distress syndrome and patients receiving Extracorporeal membrane oxygenation (Kepekci, 2020). Comorbid diseases are also an important risk factor in causing VAP. The most often comorbid disease associated with VAP are hypertension, Cardiovasuclar diseases, diabetes mellitus, chronic obstructive pulmonary disease , coronary heart diseases and chronic renal failure (But et al., 2017). Renal failure (22.86%), and chronic obstructive pulmonary disease (14.29%) were some of the majority patients with VAP (Mahapatra et al, 2019).

4.7 Prior antibiotic therapy

Multivariate logistic analysis showed that prophylactic antibiotic days is an independent risk for causing MDR VAP. Prior exposure to unrequired antibiotics is the main cause and predictors of development of antibiotic resistance. Hence, by reducing prophylactic antibiotic days can reduce the potentially modifiable factor for the development of MDR VAP in trauma patients (Lewis et al., 2018). This study showed that majority of the patients had prior antibiotic therapy so it may be an independent risk factor (Mahapatra et al, 2019).

4.8. Invasive operation

Endotracheal tubes prevent the actions in the upper airways to effectively protect like coughing and also increase the micro aspiration of infected pharyngeal substances is encouraged. Bacterial biofilm develops on the internal layer of the endotracheal tube over time, resistant to systemic antibiotics, and acts as a nodule for infection. Although biofilm size and the type of bacteria greatly contribute as important risk factors to the formation of infection, the host's strength of immunity will decide whether the ventilator associated pneumonia and parenchymal infection will occur (Hunter, 2012). This study found that invasive medical treatment like

tracheostomy, bronchoscopy, reintubation, enteral and parenteral nutrition, analgose- dation, tube, aspiration, chest drainage had influence on the occurrence of VAP (Wałaszek et al., 2016). Tracheostomy, tube thoracostomy, bronchoscopy, enteral feeding, mean duration of central vein catheterization were found by univariate analysis as risk factors for VAP. Out of the 27 patients the study was conducted on, 16 of them developed VAP after bronchoscopy was performed. Tube thoracostomy was also found to be a risk factor for VAP as cases that were given this all had VAP at least on the lateral lung. This may be because tube thoracostomy influence the lateral lung's ventilation leading to retention of secretions and possible VAP development. Lung parenchyma injury caused by pneumothorax or hemothorax can also be a cause for the development (Apostolopoulou et al., 2003). Tracheostomy was also shown to be higher in VAP and a 20.8% estimated ICU mortality for all mechanically ventilated patients. This study however showed no important difference in mortality for patients with re-intubation or ICU re-admission between the groups (Bonten et al., 2004).

4.9 Gene polymorphism

Some studies showed that single nucleotide polymorphisms within the promoter region of the tumor necrosis factor gene for susceptibility to infections. Any A allele of SNP at 376,308 and 238 loci is related with shorter onset of VAP. As tumor necrosis factor (TNFα) single nucleotide polymorphism(SNP) alleles cause proinflammatory cytokine to be produced. Although this does not predict the severity of the VAP (Kotsaki et al., 2012).

4.10. Other factors

Intra-Abdominal Hypertension was found in 19.5% of the patients with VAP in a study with 123 patients (Papakrivou et al., 2020). Smoking was also found to be a strong predictor of VAP development. A study conducted showed that current smokers were 4.37 times more likely to have VAP than non-smokers (Liu et al., 2017). When compared with patients without

VAP, patients with VAP showed higher chance of severe sepsis/septic shock, ARDS, atelectasis and infection with MDR organisms. Although, occurrence of pneumothorax and tracheo-bronchitis were similar. (Bonten et al., 2004) When compared with patients without VAP, patients with VAP showed higher chance of severe sepsis/septic shock, ARDS, atelectasis and infection with MDR organisms. Although, occurrence of pneumothorax and tracheo-bronchitis were similar (Bonten et al., 2004).

Chapter 5

Diagnosis

A pneumonia can only be considered as VAP if the patient had it after being ventilated mechanically and endotracheal intubated for at least or more than 48hours.The way to clinically diagnose a patient suspected of VAP are pulmonary infections which are fever, purulent secretions, and leukocytosis, with proof of bacterial pulmonary infection and radiology that also confirms the pulmonary infection (Bonten et al., 2004). American Thoracic Society (ATS) guideline suggests sampling should be done noninvasive with semiquantitative cultures when diagnosing for VAP. Suspected VAP patients who has below diagnostic threshold invasive quantitate culture results should be withheld from continuing their antibiotics. Using clinical criteria alone to decide or initiate the antibiotic therapy is recommended (Kalil et al., 2016).

5.1 Clinical empiric diagnosis

Fever at temperatures higher than 38.3°c, leukocytosis > 10000mm3 or leucopenia < 4000 per mm3 are clinical signs for VAP as well as secretions of purulent tracheal and new or continuous radiographic infiltrate. (Akhtar et al., 2020) Acute physiology and chronic health evaluation (APACHE) II is a classification system that works by the idea that acute diseases' severity can be calculated through finding the degree of changes in physiological variables. APACHE II is the revised prototype system of APACHE (Acute physiology and chronic health evaluation) (Gil Cebrian et al., 1987). APACHE score greater than 16 predicted the mortality of patients with VAP. APACHE II scores were significantly higher in non-surviving patients with VAP than patients who survived (Gursel & Demirtas, 2006). Empiric antibiotic therapy involves

creating a regime of antibiotics for the patient that is effective against the pathogens (Swanson & Wells, 2013). In the literature its stated that using only clinical diagnosis to make postmortem studies of VAP suspected patient has a chance of producing 30-35% false negative results and 20-25% false positive results (Hunter, 2012).

5.2 Phenotypic/Cultural diagnosis

Bronchoalveolar lavage (BAL), protected specimen brushing and "mini-BAL" (which is method that takes samples from the distal airways through the tracheal tube using a specially designed catheter) are some of the ways to test for VAP . Blind bronchial sampling is a method where a sterile catheter is randomly inserted through the tracheostomy tube and endotracheal aspirates are taken. No introduction of saline must be made during or before the suction. The aspirates can then be tested using Gram's staining to find the phenotypes of the bacteria. Kirby Baeur disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines are often used to determine the Antimicrobial susceptibility of the organism (Akhtar et al., 2020). For *K. pneumoniae*, string test can be used where a strains with mucoviscous string >5 mm can be considered a positive string test. BioMerieux VITEK-2 system can be used to both identify and find the antimicrobial susceptibility for microorganisms including ESBL (Yan et al., 2016). *A. baumanii* MIC (minimum inhibitory concentration) can be calculated by micro- dilution technique using a 96-well polystyrene plate and a serial two-fold dilutions of 50 µL between 0.5 and 512 µg/mL range for each antibiotic (Imane et al., 2021). Endotracheal tube aspirate can be serially diluted and plated on sheep blood agar, chocolate agar, MacConkey agar and Saboraud's dextrose agar (SDA) to test the growth and identify possible isolates present. Then based on that, it can separately test for AST using Kirby Bauer's disk diffusion method. Ceftazidime and ceftazidime + clavulanic acid disk can used in combination disk test to confirm the presence of suspected ESBL organisms (Ashoka Mahapatra, Das, 2019). For finding imipenem susceptibility test, the following minimum

inhibitory concentration are used for detection, ≥2 µg/ml for E. coli or Klebsiella spp; for Enterobacter spp., Serratia spp. and Citrobacter spp ≥4 µg/ml; for Acinetobacter spp, MIC of ≥8 and for P. aeruginosa, an imipenem MIC of ≥16 µg (Khurana et al., 2017). Presence of Carbapenemase can be detected using the Hodge Test. Imipenem–ethylenediaminetetraacetic acid disk synergy test is used to detect the presence of MBL enzymes in microorganisms with carbapenem resistance (Ashoka Mahapatra, Das, 2019).

Carba NP test detects the presence of carbapenem resistant strains. Carba NP test is a biochemical test that is used to detect the presense of carbapenemase production in gram negative bacilli. It is a rapid detection, biochemical test that is based on the in vitro hydrolyzation of imipenem. It detects changes in the pH values using an indicator phenol red (red to orange/yellow). It found to have high sensitivity when detecting of Klebsiella pneumoniae carbapenemase (KPC) and metallo-beta-lactamase (MBL) producers (Pasteran et al., 2015).

Studies show that 15% of VAP patients are bacteremic and there is up to 25% chance that this group will show blood cultures with pathogens from secondary-pulmonary source of infection (Modi & Kovacs, 2020).

5.3 Genetic Diagnosis

Polymerase chain reaction (PCR) can detect presence of resistant genes like carba NP positive strain genes like KPC, NDM, IMP, VIM and OXA48 (Yan et al., 2016). Repetitive extragenic palindromic (REP)-PCR methodology can investigate the clonal profile of Klebsiella isolates through molecular typing (Antoniadou et al., 2007). The presence of the mecA gene confirms the Methicillin resistance and this is performed through PCR. Molecular analysis using pulsed-

field gel electrophoresis (PFGE) can be used to find the genetic profile. (Corne et al., 2005) If there is a positive EDTA-imipenem disc synergy test, it can be further test for presence of blaVIM gene by PCR amplification (Antoniadou et al., 2007). Three-dimensional extract test and AmpC disc tests are used to screen for plasmid-mediated AmpC β-lactamases. For control, Plasmid-mediated AmpC-producing strains of K. pneumoniae HVAMC 39 (high-level ACT-1) and K. pneumoniae UMJMH14 (low-level DHA-1) and phenotypically β-lactamase-negative E. coli ATCC 25922 may be used. E. coli or Klebsiella spp with plasmid-mediated AmpC genes are detected using multiplex PCR (Khurana et al., 2017).

Chapter 6

Management/Prevention

For early onset infections, British Society for Antimicrobial Chemotherapy guidelines state to recommend co-amoxiclav or cefuroxime if patients don't previously prescribe antibiotics or have any risk with multi drug resistant patient. For patients with early onset infection that received antibiotics and have other risk factors, may be prescribed third generation cephalosporin (cefotaxime or ceftriaxone), a fluoroquinolone, or piperacillin-tazobactam. Other treatment antibiotics can be ceftazidime, ciprofloxacin, meropenem, and piperacillin-tazobactam. Vancomycin or linezoid can added if methicillin resistant S. aureus is present. In randomized controlled trials it showed that both have similar effects. In late onset pneumonia, the most common MDR is P. aeruginosa. Acceptable treatment regime include ceftazidime, ciprofloxacin, meropenem, and piperacillin-tazobactam although theres no specific or superior management. Vancomycin or linezolid are possible treatment for meticillin resistant S aureus. Although linezoid is found to be able to penetrate lung tissues better, studies found no difference in results with vancomycin (Hunter, 2012).

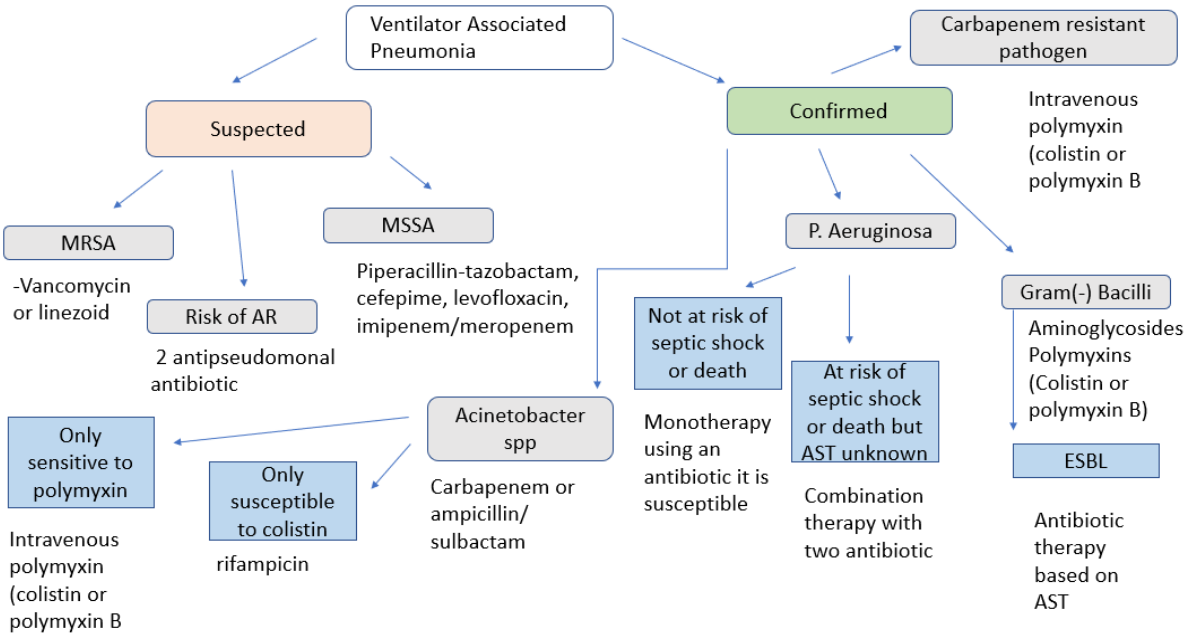

Figure 1 American Thoracic Society guideline to antibiotic therapy for ventilator associated pneumonia

American Thoracic Society has some recommendation for the management and treatment of patients suspected of VAP. If Methicillin-resistant *Staphylococcus aureus* (MRSA) is indicated, use of vancomycin or linezoid for treatment. If methicillin-susceptible Staphylococcus aureus (MSSA) is indicated by the empiric treatment, then a regimen including piperacillin-tazobactam, cefepime, levofloxacin, imipenem, or meropenem. If Oxacillin, nafcillin, or cefazolin are used for treatment of MSSA than the empiric treatment is not needed. Empiric treatment of suspected VAP patients with 2 antipseudomonal antibiotics from different classes can only be done if the patient has risk factors like for MDR, it is patients with Prior intravenous antibiotic use within 90 d, septic shock at time of VAP ARDS preceding VAP, before VAP spend 5 or more days in hospital, Acute renal replacement therapy prior to VAP onset. For MRSA and MDR, *Pseudomonas spp* its Prior intravenous antibiotic use within 90 d. Patients without risk of antimicrobial resistance but are empirically suspected VAP with *P. aeruginosa* can received one antibiotic against it if they are in an ICU where ≤10% of gram-negative isolates are resistant to the agent being considered for mono- therapy. Amino-

glycosides should be avoided if gram negative activity was present. Instead colistin can be used for the treatment. Inhaled antibiotics can be used for treatment patients with VAP due to gram-negative bacilli that are susceptible to only aminoglycosides or polymyxins (colistin or polymyxin B). The choice of antibiotic therapy for definitive therapy for patients with VAP due to *P. Aeruginosa* should be based on the results of antibiotic susceptibility test and should include the sensitivity assessment of the *P. aeruginosa* isolate to polymyxins (colistin or polymyxin B) in situations that have a high prevalence of extensively resistant organisms. Aminoglycoside therapy should not use for treatment against P. Aeruginosa. For patients with VAP due to P. aeruginosa with unknown AST who are not in septic shock or at a high risk for death, monotherapy can be done rather than combination therapy and patients who remain in septic shock or at a high risk for death and have the results of AST known, combination therapy can be suggested. No monotherapy with aminoglycoside. If the VAP patient has ESBL-producing gram- negative bacilli isolate, the results of antimicrobial susceptibility testing and patient-specific factor should be used to create a definitive therapy. For Acinetobacter species, treatment with carbapenem or ampicillin/ sulbactam can be used if the isolate is susceptible to these agents. If sensitive to only polymyxin then intravenous polymyxin (colistin or polymyxin B) should be given. If sensitive to only colistin than adjunctive rifampicin can be used for treatment. Tigecycline should not be used against Acinetobacter species. Intravenous polymyxins (colistin or polymyxin B) can also be used for treatment of patients with carbapenem-resistant pathogen (Kalil et al., 2016). In one study, Cefuroxime has shown to reduce the occurrence of VAP in patients with head injury (Bonten et al., 2004).

Chapter 7

Current and futuristic approach to combat VAP

With the increase in multidrug resistance pathogens, new alternatives are being sought out. One such can be bacteriophage treatment which uses bacterial viruses to treat of patients (Prazak et al., 2019). Bacteriophage therapy or phage therapy involves the usage of live, lytic bacteriophage in the treatment of infections for bacterial infections. Lytic bacteriophage uses bacterial cell lysis to treat infection and the bacteriophage does this by specifically attaching to the bacterial cell wall then, injecting its DNA to produce progeny and finally lysis of the bacterial cell (Reindel & Fiore, 2017). Phages remain localized and do not spread to other parts of the body (Prazak et al., 2020). Animal models with VAP were used to test the efficacy of bacteriophage treatment of *S. aureus*. The test showed significant decrease in the mortality in rats treated with anti-S aureus phage cocktail when compared with placebo group. The treatment was similar to antibiotic treatment for controlling MRSA VAP. 58% of the animals treated with phages survived at the end of the experiment and lived at least 12hours after being infected (Prazak et al., 2019). In another experiment, rat models with VAP were treated with prophylactic application of a nebulized phage. The animal models that lived had a significant reduction in bacterial load in lungs and less lung tissue damage (Prazak et al., 2020). A 15-year-old patient was treated with bacteriophage treatment. The patient had comorbidities of pancreatic insufficiency, insulin-dependent diabetes, cystic fibrosis (CF)-related liver disease, Nissen fundoplication and gastrostomy, CF-related osteoporosis and was expected for a lung transplant. Prior to the transplantation the patient was treated for *Pseudomonas aeruginosa* and *M. abscessus* for 8 years with anti-NTM (non-tuberculosis mycobacterium) treatment. After transplant, patient was administered immunosuppressive drugs and multiple intravenous (iv) antibiotics. After one week of stopping intravenous antibiotic, patient was found to be infected with *M. abscessus*. The patient was then treated a cocktail of phage, a single topical test and iv therapy every 12 hours for at least 32 weeks. The patient didn't show any adverse side effects throughout the treatment. After 6 months of treatment using phage, the patient clinically improved with slow healing of wounds and skin lesions. This is the first case where bacteriophage was used for treatment (Dedrick et al., 2019).

VAP Care bundle can be adopted in hospitals. Bundles are a group of evidence-based clinical methods that when performed individual, was found to be effective for treatment (Wip &

Napolitano, 2009). Head of bed elevation to 30-45 degrees except in cases like log-roll protocol, pelvic fractures, morbid obesity, prone position, intra-aortic balloon pump, and an unstable spine not cleared by neurosurgery. Oral care using chlorhexidine solution can be included. Adequate endotracheal tube cuff pressure (20-30 mmHg) and Endotracheal tube with and in-line suction system and subglottic suctioning (R. Khan et al., 2016). A randomized trail stated in the literature showed a reduction in occurrence of VAP of 3 -fold if the treatment were performed in a semi recumbent position compared to supine position (Bonten et al., 2004). After compliance with VAP prevention bundle from 2010 to 2012, the VAP rate per 1000 days decreased from 15.4 ± 11 in 2008 to 9.1 ± 10.9 in 2012 (Sen et al., 2016).

Bacterial load of digestive tract can be reduced through selective decontamination of the digestive tract and oral. Antiseptics such as chlorhexidine can be used for oral decontamination and can help reduce ventilator associated pneumonia. Oral decontamination can also involve the intravenous administration of broad-spectrum antibiotic and also the oral and gastric nonabsorbable oral antibiotics such as typically used polymyxin, tobramycin, and amphotericin B. A study showed that endotracheal tubes which was silver coated tend to have a risk reduction of 35.9% (3.6%-69%) to causing ventilator associated pneumonia when compared with the normal endotracheal tubes as silver has a broad-spectrum antimicrobial activity and is involved in the reduction of bacterial growth and biofilm formation. The length of time spent in tracheal intubation may also help in the reduction of occurrence of pneumonia (Hunter, 2012).

Topical oropharyngeal antimicrobial prophylaxis has shown to reduce the chance VAP. As oropharynx is known to be a source of microbes, having a continuous aspiration in the subglottic secretion showed reduced occurrence of VAP in two randomized studies as stated in the literature (Bonten et al., 2004).

Circuit colonization of bacteria can also be reduced through use of passive humifiers whether it has or does not have a filtering capacity through there is no significant proof that reduces the occurrence of VAP and changing it more than every 48hours did not show any significant improvement in controlling the infection (Bonten et al., 2004).

Treatment using histamine-2-receptor blockers and proton pump inhibitors reduce the acid production which in turn allow the pathogens to grow on the oropharynx and endotracheal tube. This is elevated due to aspiration (Modi & Kovacs, 2020).

Discussion

Developing countries tend to have a higher rate for VAP when compared to developed, varied from 10- 41.7 per 1000 MV-days (D. S. Xie et al., 2011). From table, it can be deduced that developing countries seem to show a higher VAP rate per 1000 episodes. As it can be seen that developing countries like Bangladesh, Nepal, India, Pakistan showed a higher VAP rates of 35.73, 21.4,11.9 and 26 per 1000 respectively. While developed countries like Japan, South Korea and Kuwait showed lower VAP rates per 1000 episodes which are 6.5, 2.13 and 4 respectively.

In the diagnosis, most of the papers used bronchoscopic BAL and blind BAL as the procedure to collect endotracheal aspirates which were then further tested for identification and the antibiotic susceptibility of the pathogens. Non-bronchoscopic protected BAL (NB-BAL) is simple, none invasive procedure that is easy and cost effective compared to bronchoscopic BAL which requires more resources and experienced operators. As qualified operators and other resources like fiberoptic bronchoscopes are not easily available, NB-BAL can be a good alternative as it is not only less invasive but also requires less compromise of oxygenation than B-BAL and is in a good microbiologic concordance with bronchoscopic BAL in distal airway sampling for suspected VAP. In the study conducted found that Non-bronchoscopic protected BAL sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 89%, 75%, 77% and 88% respectively and Bronchoscopic BAL had a sensitivity of 85% and specificity of 77%, PPV and NPV were 74% and 82% respectively (Afify et al., 2016).

VAP is mainly diagnosed through empiric treatment. Beginning the empirical antibiotic therapy as soon as the patient was found to have VAP, was considered the right approach but the problem is that there aren’t any diagnostic techniques that quickly identifies the affected

patient (Swanson & Wells, 2013). Diagnosing and treating VAP is difficult because patients who are given ventilation in ICU are normally already treated prior with antibiotics for other diseases. Broad antibiotics are often used before to treat the intubated patients as most have con-comitant infections (Ausina et al., 1993). So the patient's natural flora is already resistant to them. There is great ecologic impact, especially in ICU patients where broad spectrum antibiotic therapy is used, as it can change the microflora in the patient's body. Antibiotic pressure is found to be the main reasons leading to most nosocomial outbreaks in patients in ICU (Ausina et al., 1993). VAP is often caused by patients' own natural flora (Safdar et al., 2005). Which means it can resistant to the previously used antibiotics. Different patients will have different antibiotic treatments that are available to that locality. So, for every patient a unique antibiotic regime has to be made.

When APACHE II was tested with CPIS to check the discrimination and calibration for predicting 30-day mortality in patients with VAP, APACHE II showed promising results. The possible reasons can be due to the fact that APACHE II was designed to classify the severity of a disease while CPIS was developed for clinical diagnosis. So, when taking data, APACHE II include values like acute physiology score, age points, and chronic health points while CPIS uses six parameters like temperature, white blood cell count, tracheal secretions, PaO2/FiO2, chest radiography, and microbiology which are related to the disease. So testing mortality using CPIS for VAP may not be a good option since many of the patients may also die from other factors like in this case, multiple organ failure (Zhou et al., 2015).

Conclusion

Ventilator associated pneumoniae is one the frequent causes of mortality in intensive care units. It is a hospital acquired pneumoniae that occurs after 48 hours in endotracheal intubation. Prevalence data from INICC were collected from 2003 to 2017 and they found the following VAP rates globally, 13.6,15.8 and 14,1 per 1000 episodes. From the table, it can be understood that developing countries have a higher VAP rate per 1000 episodes. The classification of VAP is of two types, early onset pneumonia and late onset pneumoniae. Early onset pneumoniae are caused by antibiotic sensitive bacteria while late onset pneumoniae is caused by multidrug resistant pathogens. The majority of VAP is caused by gram negative bacilli and the most common are A. *baumannii, P. Aeruginosa* and *K. pneumoniae* with *P aeruginosa* being the most commonly found. In one study, *P. aeruginosa* is found to be resistant to colistin. ATS suggests empirical diagnosis VAP and immediately start antibiotic therapy. APACHEII scores higher than 16 can predict mortality of the patient. Endotracheal aspirates are test for identification (Vitek 2, Gram's staining, Media culture) and antibiotic susceptibility test (imipenem-EDTA test, disc diffusion, micro- dilution, Hodge test, PCR, PFGE) For treatment, a range of antibiotics can be used to treat based on the type of VAP. Other approaches to combat VAP can be the use of VAP bundle which has found to reduce VAP rate. Bacteriophage treatment can be one approach to the treatment of MDR patients. Some methods to reduce occurrence can be selective decontamination of digestive tract and oral, topical oropharyngeal antimicrobial prophylaxis, use of passive humifers and use of histamine-2 receptor blockers and proton pump inhibiters.

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