A STUDY ON MICROBIAL ETIOLOGY OF VENTILATOR ASSOCIATED PNEUMONIA

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY ON MICROBIAL ETIOLOGY OF VENTILATOR ASSOCIATED PNEUMONIA"** submitted by **Dr. J.PREETHI** to the Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of M.D degree Branch-IV (Microbiology) is a bonafide research work carried out by her under direct supervision & guidance.

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DECLARATION

I, Dr**. J.PREETHI** declare that, I carried out this work on, **"A STUDY ON MICROBIAL ETIOLOGY OF VENTILATOR ASSOCIATED PNEUMONIA"** at the Institute of Microbiology, Madurai Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D. Degree examination in Microbiology.

Place : Madurai **Dr. J.PREETHI**

Date :

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CONTENTS

INTRODUCTION

Pneumonia is an inflammation of the lung that is most often caused by infection with bacteria, viruses, or other organisms. Occasionally, inhaled chemicals that irritate the lungs can cause pneumonia. Healthy people can usually fight off pneumonia infections. However, people who are sick, including those who are recovering from the flu (influenza) or an upper respiratory illness, have weakened immune systems that make it easier for bacteria to grow in their lungs.

When air is inhaled through the nose or mouth, it travels down the trachea to the bronchus, where it first enters the lung. From the bronchus, air goes through the bronchi, into the even smaller bronchioles and lastly into the alveoli [2].

DEFINING PNEUMONIA BY LOCATION IN THE LUNG

Pneumonia may be defined according to its location in the lung:

- Lobar pneumonia occurs in one part, or lobe, of the lung.
- Bronchopneumonia tends to be scattered throughout the lung.

DEFINING PNEUMONIA BY ORIGIN OF INFECTION

COMMUNITY ACQUIRED PNEUMONIA (CAP). People with this type of pneumonia contracted the infection outside a hospital setting. It is one of the most common infectious diseases. It often follows a viral respiratory infection, such as the flu. Commonest organism causing CAP is Streptococcus pneumoniae. Other pathogens include Haemophilus influenzae, Mycoplasma, and Chlamydia.

HOSPITAL ACQUIRED PNEUMONIA.(HAP) Hospital-acquired pneumonia is an infection of the lungs contracted during a hospital stay. This type of pneumonia tends to be more serious, because hospital patients already have weakened defense mechanisms, and the infecting organisms are usually more dangerous than those encountered in the community. Hospital patients are particularly vulnerable to Gram-negative bacteria and staphylococci. Hospital-acquired pneumonia is also called nosocomial pneumonia.

A subgroup of hospital-acquired pneumonia is Ventilator-Associated Pneumonia (VAP), a highly lethal form contracted by patients on ventilators in hospitals and long-term nursing facilities. Ventilator associated pneumonia (VAP) is defined as nosocomial pneumonia occurring in a patient after 48 hours of mechanical ventilation via a tracheal or tracheostomy tube. It is commonly classified as either early onset (occurring within 96 hours of start of mechanical ventilation) or late onset (>96 hours after start of mechanical ventilation). It is a common condition, difficult to diagnose accurately, and expensive to treat. Its development prolongs a patient's stay in the intensive care unit (ICU), and is associated with significant morbidity and mortality. Most cases seem to result from aspiration of pathogenic material that commonly colonises the oropharyngeal airways of the critically ill. Simple measures to decrease the incidence of aspiration or reduce the burden of colonisation of the oropharynx may aid in the prevention of ventilator associated pneumonia. A favourable outcome seems to be more likely if appropriate antibiotics are given in a timely manner.[42]

EPIDEMIOLOGY

INCIDENCE

The incidence of Ventilator associated pneumonia (VAP) was 7% to 70%. Generally, the rates of VAP in surgical ICU were higher than in medical ICUs, depending on the differences in the patient population, surgical disorders, the proportion of patients that needed MV and the duration of ventilation. The risk of pneumonia increased by the duration of MV and the highest risk was during the first 8–10 days. The need for reintubation, urgent intubation and documented massive aspiration are also associated with high incidence of VAP. In Korea, the incidence of VAP is 3.5 to 7.1 per 1000 ventilator days. In India overall rate of VAP of 8.95 per 1000 ventilator days [119]

MORTALITY

VAP is associated with increases in morbidity and mortality, hospital length of stay, and costs. The mortality rate attributable to VAP is 27% and has been as high as 43% especially when antibiotic resistant bacteria were responsible. Length of stay in the intensive care unit is increased by 5 to 7 days and hospital length of stay 2- to 3-fold in patients with VAP. Mortality is more likely when VAP is associated with certain microorganisms (Pseudomonas, Acinetobacter), blood stream infections, and ineffective initial antibiotics. VAP is especially common in people who have acute respiratory distress syndrome (ARDS). In the Philippines, the crude mortality rate for hospitalized patients with pneumonia was 42.4%, with a mortality rate attributable to infection of 30.1%. India on HAP that found an overall crude mortality of 67.4% in ICU patients

with pneumonia, with 40% of the mortality in these patients attributable to infection alone [119].

Risk factors for ventilator-associated pneumonia

- Duration of mechanical ventilation
- Aspiration of gastric contents
- Chronic obstructive pulmonary disease
- Histamine type-2 receptor antagonist
- Nasal intubation and/or sinusitis
- Use of positive end-expiratory pressure
- Reintubation
- Intracranial pressure monitoring and/or depressed consciousness

- Winter season
- Daily ventilator circuit changes
- Thoracic or upper abdominal surgery
- Age
- Multiple organ system failure
- Prior antibiotic administration
- Supine head positioning (i.e., head of bed not elevated)
- Duration of hospitalization prior to mechanical ventilation

PATHOPHYSIOLOGY

Ventilator associated pneumonia (VAP) primarily occurs because the endotracheal or tracheostomy tube allows free passage of bacteria into the lower segments of the lung in a person who often has underlying lung or immune problems. Bacteria travel in small droplets both through the endotracheal tube and around the cuff. Often, bacteria colonize the endotracheal or tracheostomy tube and are embolized into the lungs with each

breath. Bacteria may also be brought down into the lungs with procedures such as deep suctioning or bronchoscopy.

Whether bacteria also travel from the sinuses or the stomach into the lungs is, as of 2005, controversial. However, spread to the lungs from the blood stream or the gut is uncommon.

Once inside the lungs, bacteria then take advantage of any deficiencies in the immune system (such as due to malnutrition or chemotherapy) and multiply. A combination of bacterial damage and consequences of the immune response lead to disruption of gas exchange with resulting symptoms. [91]

Pathogenic mechanisms for infection of the lower Respiratory tract

MICROBIOLOGY

The microbiologic flora responsible for Ventilator associated pneumonia (VAP) is different from that of the more common community-acquired pneumonia (CAP). In particular, viruses and fungi are uncommon causes in people who do not have underlying immune deficiencies. Though any microorganism that causes CAP can cause VAP, there are several bacteria which are particularly important causes of VAP because of their resistance to commonly used antibiotics. These bacteria are referred to as multidrug resistant

(MDR).

- Pseudomonas aeruginosa is the most common MDR Gram-negative bacterium causing VAP. Pseudomonas has natural resistance to many antibiotics and has been known to acquire resistance to every antibiotic except for polymixin B. Resistance is typically acquired through up

regulation or mutation of a variety of efflux pumps which pump antibiotics out of the cell. Resistance may also occur through loss of an outer membrane porin channel (OprD)

- Klebsiella pneumoniae has natural resistance to some beta-lactam antibiotics such as Ampicillin. Resistance to cephalosporins and aztreonam may arise through induction of a plasmid-based extended spectrum beta-lactamase (ESBL) or plasmid-based ampC-type enzyme. Enterobacter, Citrobacter and also Serratia marcescens as a group also have an inducible ampC gene, which can be induced by exposure to antibiotics such as cephalosporins. Thus, culture sensitivities may initially indicate appropriate treatment which fails due to bacterial response. They may also develop resistance by acquiring plasmids.

- Stenotrophomonas maltophilia and Acinetobacter often colonizes people who have endotracheal tubes or tracheostomies but can also cause pneumonia. They are often resistant to a wide array of antibiotics but are usually sensitive to co-trimoxazole.

- Burkholderia cepacia is an important organism in people with cystic fibrosis is often resistant to multiple antibiotics.

- Methicillin-resistant Staphylococcus aureus is an increasing cause of VAP. As many as fifty percent of Staphylococcus aureus isolates in the

intensive care setting are resistant to methicillin. Resistance is conferred by the mecA gene. [106]

Nosocomial virus and fungal infections are uncommon causes of HAP and VAP in immunocompetent patients. Fungal pathogens causing VAP are Aspergillus species and Candida albicans.

DIAGNOSIS

The diagnosis of pneumonia in mechanically ventilated patients is difficult, and still there is no "gold-standard" diagnostic method. It is usually based on the combination of clinical, radiological, and microbiological criteria defined by Centers for Disease and Control (CDC)

CDC criteria for ventilator associated pneumonia

Three or more of the following criteria:

- Rectal temperature >38°C or <35.5°C
- Blood leucocytosis (>10.103/mm3) and/or left shift or blood leukopenia (<3.103/mm3)
- More than ten leukocytes in Gram stain of tracheal aspirate (in high power field)
- Positive culture from endotracheal aspirate
- New, persistent, or progressive radiographical infiltrate

But these criteria have low sensitivity and specificity. The systemic signs fever, leukocytosis, etc.) of infection can be seen by any condition in ICU pulmonary edema, pulmonary infarction, after surgery, trauma, devascularized tissue, open wounds, etc.). Investigators reported that the clinical diagnosis of VAP is

associated with 30–35% false-negative and 20–25% false-positive Results. And also, ICU patients do not always have systemic signs of infection due to their underlying disease (chronic renal failure, immunosuppression, etc.). Radiological infiltration has limited value, mimicking by cardiogenic pulmonary edema, noncardiogenic pulmonary edema, adult respiratory distress Syndrome(ARDS), atelectasis, pulmonary contusion, which are not uncommon in ICU.

The upper respiratory tract of patients is colonized with potential pulmonary pathogens a few hours after intubation .Consequently, isolation of pathogens from tracheal secretions do not always indicate pulmonary infection. But a positive Gram's stain may guide the initial antibiotic therapy. However prior antibiotic and corticosteroid therapy can reduce the sensitivity of this technique

CLINICAL PULMONARY INFECTION SCORE (CPIS)

This score combine the seven variables (temperature, leukocytes, tracheal aspirate volume and purulence of tracheal secretions, chest X-ray, oxygenation-PaO2/FiO2- and semi quantitative culture of tracheal aspirate) for the diagnosis of VAP, defined as clinical pulmonary infection score (CPIS)

Clinical pulmonary infection score		
Temperature, °C	≥ 36.5 and ≤ 38.4	0 point
	≥ 38.5 and ≤ 38.9	1 point
	≥ 39.0 and ≤ 36.0	2 point
Blood leucocytosis, mm^3	≥ 4000 and ≤ 11 000	0 point
	<4000 and >11 000	1 point
	+band forms ≥ 500	+ 1 point
Tracheal secretions	<14+ of tracheal secretions	0 point
	≥ 14+secretions	1 point
	+purulent sputum	+1 point
Oxygenation: PaO_2/FiO_2, mmHg	>240 or ARDS	0 point

	≤ 240 and no ARDS	2 point
Chest X-ray	No infiltrate	0 point
	Diffused, or patchy infiltrate	1 point
	Localized infiltrate	2 point
Culture of tracheal aspirate (semi-quantitative: 0-1-2 or 3+)	≤ 1 or no growth	0 points
Pathogenic bacteria cultured	>1+	1 point
	>1+ and same pathogenic bacteria seen in Gram stain	2 point

The score varied from 0 to 12 points and was reported that a CPIS of more than six was associated with a sensitivity of 93% and a specificity of 100% for the diagnosis of pneumonia. However, the original scoring system has some limitations; that it requires 24–48 hours for the results of tracheal aspirate cultures, and also identifying pulmonary infiltrates progression depends on intensivist experience. Modified CPIS (calculated at baseline from the first five clinical variables, and CPIS at 72 hours was based on all variables of the score) that antibiotics were stopped in patients with a persistent low score (<6) after 3 days of therapy, avoiding unnecessary use of antibiotics, and all patients who discontinued the therapy improved. The modified CPIS does not perform better when the clinical suspicion of pneumonia is high, so they proposed incorporating the results of specimens gram stain (by adding two more points when gram stains were positive) to modified CPIS to increase the sensitivity of the score and the physicians' diagnostic accuracy.

Qualitative cultures of tracheal aspirate (TA) is not a specific diagnostic method because of the lower respiratory tract colonization and a high percentage of false-positive results .However, investigators reported that

quantitative cultures of TA have equal diagnostic accuracy to the other invasive techniques . Although, quantitative cultures of TA is non-invasive, inexpensive and a simple method, it has some risks, that if the cut-off value ≥ 10^6 cfu/mL is used, sensitivity will be low and some patients with VAP may not be identified or when the cut-off value ≥ 10^5 cfu/mL is used, unnecessary antibiotic treatment will be given because of low specificity. [96]

INVASIVE TECHNIQUES FOR THE DIAGNOSIS OF VAP

1. Protected-specimen brush (PSB)
2. Bronchoalveolar lavage (BAL)
3. Blood or pleural fluid

In PSB, 0.001 mL of secretions are collected and the presence of >10^3 cfu/mL bacteria have 80–90% sensitivity and 95% specificity for the diagnosis of VAP. In BAL, larger proportion of lung can be sampled and the diagnostic threshold is >10^4 cfu/ml. The sensitivity and specificity of BAL are 86–100% and 95–100%, respectively .[102]

The disadvantages of these invasive techniques are;

a) Prior antibiotic use may decrease the sensitivity and accuracy of these methods.
b) These techniques are based on quantitative culture and results of these cultures require 24–48 hours, and, therefore miss early cases, and also give no information about appropriate initial antibiotic therapy.

c) These invasive tests may worsen the patient's status (cardiac arrhythmias, hypoxemia, bleeding, etc.).
d) Increase the costs of caring.
e) It has not been proven that the use of these invasive techniques lead to a decrease in patients' mortality.

The spread of microorganism to blood or pleural space is <10%, so blood and pleural effusion cultures have low sensitivity and specificity. Blood cultures in patients with VAP are useful if there is suspicion of another probable infectious condition, but the isolation of a microorganism in the blood does not confirm that microorganism as the pathogen causing Ventilator Associated Pneumonia. Therefore, two sets of blood samples for culture and tapping pleural effusions >10 mm should be performed in patients suspected Ventilator Associated Pneumonia.

Microbiological testing should be always performed to decide the appropriate initial empirical antibiotic therapy. Clinicians can choose optimal diagnostic test for specific patients in their clinical setting.[98]

MANAGEMENT OF VENTILATOR ASSOCIATED PNEUMONIA

Early recognition and appropriate management of ventilator-Associated Pneumonia reduces the incidence of complications such as acute lung injury, multiple organ dysfunction and respiratory decompensation. Empirical therapy should be started as a matter of urgency if infection is identified. Unnecessary delay in antibiotic therapy leads to adverse outcomes, particularly if the patient is septic .However, antibiotic therapy for non-infective syndromes is also

detrimental. It is important to balance the risks and benefits of treatment and this is a matter for individual clinical judgement.

Antibiotic rationale

Empirical therapy will usually take into account:

- Time of onset of illness (<5 vs. ≥5 days after admission) and therefore probable pathogens
- Previous antibiotic administration (rates of Pseudomonas aeruginosa or Acinetobacter spp. infection increase significantly in patients treated with antibiotics within 10 days before the onset of pneumonia)
- Severity and speed of progression of the illness
- Local pathogens and resistance patterns
- Other patient-related factors such as renal or hepatic impairment.

Therapy should be broad-spectrum, and have high activity against the probable pathogens. In patients previously untreated with antibiotics the predominant pathogens are Gram-positive cocci in 'early' infections and aerobic Gram-negative bacilli in 'late' infections. There are some data to suggest that monotherapy may be as effective as combination therapy in severe ventilator-associated pneumonia.[130]

However, there is considerable debate about the merits of monotherapy in these patients largely because of some limitations in the data, particularly the range of infections included in the trials, the sample sizes and the use of sub-optimal doses of Aminoglycosides. Combination therapy has the advantage of giving cover against a broader-spectrum of organisms and some combinations have a

synergistic mechanism of action which reduces the potential for resistance developing during treatment, e.g. an Aminoglycosides with a beta-lactam. Pseudomonas aeruginosa has been associated with resistance developing during the course of treatment and therefore if pseudomonas involvement is suspected, vigorous anti-pseudomonas therapy is indicated.[35]

Empirical therapy

Given that there is minimal margin for error in seriously ill patients, it would be prudent to use empirical combination therapy. Factors to be considered include:

- Previous antibiotic therapy
- Known prevalence and resistance patterns
- Patient condition.

If a satisfactory clinical response is observed with combination therapy after –4 days, monotherapy can be considered and the Aminoglycosides withdrawn. The optimal treatment duration has not been established in ventilator-associated pneumonia. Most studies report treatment durations of 7–10 days, although shorter courses may be effective.[26]

PREVENTION OF VENTILATOR ASSOCIATED PNEUMONIA

The following measures to reduce Ventilator Associated Pneumonia:

- Strict infection control policies
    - Alcohol-based hand disinfection
    - Collection of timely microbiologic surveillance data on multidrug- resistant pathogens

    - Monitoring and early removal of invasive devices
    - Programs to reduce antibiotic prescribing practices
    - Continuous aspiration of subglottic secretions
    - Detection of pneumonia and deescalation of drug treatment
- Use of oral rather than nasal endotracheal tubes
- Maintenance of endotracheal cuff pressure > 20 cm H20
- Limited use of sedative and paralytic agents
- Positioning of the patient
    - Semi recumbent positioning (30 to 45 degrees) is recommended to reduce the risk of aspiration.
    - Proper care should be taken when turning the patient or the bed rail is raised to avoid inadvertently flushing the condensate that collects on the ventilator circuit into the lower airway or to inline medication nebulizers.
- Intensive insulin therapy to maintain normal blood glucose level
- Emphasis on bleeding prophylaxis; use of H2 antagonists or sucralfate
- Avoidance of intubation by using noninvasive ventilation wherever possible, particularly in patients with chronic obstructive pulmonary disease and cardiogenic pulmonary edema
- Avoidance of blood transfusion
- Adequate nurse-to-patient ratios
- Staff education [40]

Though various Indian and International studies on epidemiology of Ventilator Associated Pneumonia are available, no such study has been carried out in Madurai. Since Government Rajaji Hospital, (GRH) Madurai is the largest tertiary care hospital attached to Madurai Medical College catering to the needs

of lakhs of people from southern districts of Tamilnadu, the present study was carried out among patients admitted at Intensive Respiratory Care Unit, Government Rajaji Hospital in Ventilators and the data were analysed with reference to objectives.

Efforts have been made to diagnose ventilator associated pneumonia by collecting blood, bronchoscopic and non bronchoscopic sampling, from patients satisfying CDC criteria of Ventilator Associated Pneumonia and processing them by various microbiological techniques like Gram stain, isolation of microbes using quantitative culture methods and antimicrobial susceptibility testing.. In addition, fungal culture was also performed. The results were analysed sample wise and also technique wise and epidemiological factors of Ventilator Associated Pneumonia were studied.

AIM AND OBJECTIVES OF THE STUDY

The study on microbial etiology of Ventilator Associated Pneumonia was conducted on patients admitted into the Respiratory Intensive Care Unit of Government Rajaji Hospital and was put on mechanical Ventilation. The objectives of the study were

1. To understand the prevalence of various pathogens causing Ventilator Associated Pneumonias.
2. To assess the usefulness of clinical samples obtained from patients with suspected Ventilator Associated Pneumonias in the diagnosis.
3. To find if clinically relevant correlations exist between age, sex and underlying clinical conditions.
4. Having identified pathogens, antibiotic susceptibility patterns would be studied.
5. To understand prognosis of patients with Ventilator Associated Pneumonias.
6. To be able to develop strategies that would bring down Ventilator Associated Pneumonia in patients on mechanical ventilation.

REVIEW OF LITERATURE

DEFINITION

Johanson WG Jr, Pierce AK, Sanford JP, Thomas GD et al[86] defined ventilator associated pneumonia as nosocomial pneumonia in a patient on mechanical ventilatory support by endotracheal tube or tracheotomy for more than 48 hours. For many years ventilator associated pneumonia has been diagnosed by the clinical criteria published by the **Johansson et al**[87] in 1972 which include the appearance of new or progressive pulmonary in filtrate, fever, leukocytosis, and purulent tracheobronchial secretion.(1972).

Kollef MH et al[92] showed the onset of ventilator associated pneumonia into 2 types; early and late. Early onset ventilator associated pneumonia occurs 48 hours to 96 hours after intubation and is associated with antibiotic susceptible organisms. Late onset ventilator associated pneumonia occurs more than 96 hours after intubation and is associated with antibiotic resistant organisms. Interventions to prevent ventilator associated pneumonia should begin at the time of or if possible, before intubation. (1995).

PREVALENCE AND GEOGRAPHICAL DISTRIBUTION

Bowton DL et al[30] showed in their study that the incidence of nosocomial pneumonia in mechanically ventilated patients ranges from 9% to 68% and mortality rates ranges from 33% to 71%. (2006).

Thongpiyapoom S, Narong MN, Petdachai W. et al[144] in their article stated that incidence of VAP varying from 3.5 to 46 per 1000 ventilator days. A recent

study from Thailand found incidences of VAP of 10.8 per 1000ventilator days in an adult ICU and 70.3 per 1000 ventilator days in newborn patients. An Indian study of 51 critical care unit patients found an incidence of VAP of 46 per 1000 ventilator days (33% early onset and 67% late onset). From Hong Kong, surveillance data collected in 2004–2005 from a large tertiary care hospital represented in the panel found an incidence of VAP of 10.6 per 1000 ventilator days. (2004)

Rakshit P, Nagar VS, Deshpande AK et al[120] mentioned that VAP accounts for 2.9% of all nosocomial infections. One Chinese study reported that 41.2% of intubated patients developed VAP, with an incidence of 1 per 1000 ventilator days. (2005)

Thanamee N, Sujaritjan N, Techasena W[139]in their study observed that pneumonia on mechanical ventilation in the ICU found a overall rate of 7.5% (17.5% in the paediatric ICU, 6.5% in the medical ICU, and 2.5% in the surgical ICU). In a recent Indian study of 328 patients in the ICU, the overall rate of HAP was reported to be 53.9%, and that of VAP was 81.7%. (1995)

Saenghirunvattana S, Charoenpan P, Kiatboonsri S, Aeursudkij B[137] in their article described that the mortality for HAP (including VAP) ranged from 25% to 54%. In China, several epidemiologic studies have been published recently, but the data are generally of poor quality. One limitation to such studies is that much data are drawn from major metropolitan medical centres, such as Shanghai and Beijing, with little date from the relatively under developed areas of China. A Chinese study of 372 patients with HAP found an

overall mortality rate of 25.3%. Mortality rates associated with Pseudomonas spp and Staphylococcus aureus infection were higher, 70.6% and 66.7%, respectively.(1994)

Zhang Y[150] in his article reported that a mortality rate was 47% in immunocompetent patients who acquired nosocomial pneumonia, compared with 54% for immunocompromised patients. A Taiwanese study conducted over a 5-year period reported overall mortality of 42.6% in patients with respiratory tract infections and 61.5% for patients in the surgical ICU. These findings are comparable to those from a study in India on HAP that found an over all crude mortality of 67.4% in ICU patients with pneumonia, with 40% of the mortality in these patients attributable to infection alone. In the Philippines, the crude mortality rate for hospitalized patients with pneumonia was 42.4%, with a mortality rate attributable to infection of 30.1%. In the Philippines, local data presented by the panel demonstrated a mortality rate of 42% for HAP. Very little data are available on mortality associated specifically with VAP. A prospective study in Singapore reported a mortality rate of 73% for VAP. A Chinese study of 120 patients with VAP reported a 14% mortality rate directly related to this infection.23 A Thai study of mechanically ventilated patients reported that 22.5% (9/40) of patients died of VAP. A study from India evaluated 51 patients in the critical care unit and found a mortality rate of 37% attributable to VAP, which also correlated very well with higher APACHE III scores; 33% of the cases were early onset, and 67% were late onset. In Thailand, a study of newborn ICU patients on a ventilator found a mortality

rate of 29.4% in infants with VAP versus 30.6% in newborn infants in the ICU without VAP. (1991)

PAHOGENESIS

Livingston DH et al[98] showed the pathophysiology of ventilator associated pneumonia involves 2 main processes. Colonization of respiratory and digestive tracts and micro aspiration of secretions of the upper and lower parts of the airway. (2000)

Bonten MJM, Gaillard CA, de Leeuw PW, Stobberingh EE et al[29] demonstrated nosocomial pneumonia is often endogenous in origin, the contribution of exogenous microorganisms from other sites, may have been under estimated, infection can arise from microorganisms in the ventilator circuit. It has been breakdown of a single step in the procedure for decontaminating ventilation equipment can be responsible for infectious episodes. (1995)

Johhanson et al[87] established that there is a link between the colonization of the patients airways, and the development of nosocomial pneumonia, and also he showed that the colonization of airway is connected to the contamination of the breathing circuit. (1988).

Kunis KA, Puntillo KA et al[93] studied the colonization of bacteria refers to the presence of bacteria without an active host response. Bacterial colonization of the lungs can be due to spread of organisms from many different sources, including the oropharynx, sinus cavities, nares, dental plaques, gastrointestinal tract, patient to patient contact and ventilation circuit. Inhalation of colonized

bacteria from any of these sources can cause an active host response and ultimately ventilator associated pneumonia. (2003)

Olson ME, Harmon BG, Kollef MH, More head RS, Pinto SJ et al[108] demonstrated the presence of an endotracheal tube provides a direct route for colonized bacteria to enter the lower respiratory tract, upper air way and oral secretions can pool above the cuff of an endotracheal tube and line the tube, forming a biofilm. Starting as early as 12 hours after intubation, the biofilm contains large amount of bacteria that can be disseminated into the lungs by ventilator induced breaths. (2002)

De Rosa FG, Craven DE et al[57] showed that the upper air way is by passed, a decreased occurrence in the body's ability to filter and humidify air, in addition, the cough reflex is often eliminated and or decreased by the presence of an endotracheal tube and mucociliary clearance can be improved because of mucosal injury during intubation. An endotracheal tube provides a place for bacteria to bind in the trachea, a situation that further increases production of secretion of mucus. The impairment of these natural host defense mechanisms increases the likelihood of bacterial colonization and subsequent aspiration of the colonized organisms. (2003).

Cook D, De Jonghe B, Brochard L, Brun- Bruisson C et al[47] studied the pathogenesis of ventilator associated pneumonia is related to host and treatment related colonization factors. Aspiration of oropharyngeal pathogens and the leakage of secretions containing bacteria around the endotracheal tube are the principal factors for the development of ventilator associated pneumonia. The

progression from colonization to tracheobronchitis to ventilator associated pneumonia is a dynamic equilibrium. (1998)

Ferror R, Artigas A et al[70] showed that aspiration of gastric contents is another potential cause of ventilator associated pneumonia, because the stomach serves as a reservoir for bacteria, most patients receiving mechanical ventilation have a naso gastric or an orogastric tube in place for Enteral feeding and a administration of medication or for gastric decompression. The presence of nasogastric or an orogastric tube interrupts the gastro esophageal sphincter, leading to increased gastrointestinal reflux and providing a route for bacteria to translocate to the oropharynx and colonize the upper airway. Enteral feedings increase both gastric ph and gastric volume, increasing the risk of both bacterial colonization and aspiration. (2001).

RISK FACTORS

Torres A, de la Bellacasa JP, Rodriguez RR, Jimenez M, Agusti VA et al[143] studied the risk factors for ventilator associated pneumonia can be divided into 3 categories; host related, device related and personnel related. Host related risk factors include preexisting conditions such as immunosuppression, chronic obstructive pulmonary disease and acute respiratory distress syndrome. Others include patient's body positioning, level of consciousness, number of intubation and medications, including sedative agents and antibiotics. In one study, bacterial contamination of endotracheal secretions was higher in patients in the supine position than in patients in semi recumbent position. Whether due to a pathophysiological process, medication in injury, decreased level of

consciousness resulting in the loss of cough and gag reflexes contributes to the risk of aspiration and therefore increases the risk for ventilator associated pneumonia. Re-intubation and subsequent aspiration can increases the likelihood of ventilator associated pneumonia 6 fold. (1988)

Kollef MH et al[92] studied Improper hand washing resulting in the cross-contamination of patients is the biggest personnel-related risk factor for VAP. Patients who are intubated and receiving mechanical ventilation often need interventions such as suctioning or manipulation of the ventilator circuit. These interventions increase the likelihood of cross-contamination between patients if healthcare staffs do not use proper hand-washing techniques. Failure to wash hands and change gloves between contaminated patients has been associated with an increased incidence of VAP In addition, failure to wear proper personal protect-resistant organisms have been identified increases the risk of cross-contamination between patients. (1995)

Bergmans DC, Bonten MJ, Gaillard CA, **et al**[26] demonstrated retention of liquid borne contamination is particularly important in mechanical ventilation. Potentially contaminated patient fluids, such as tracheal secretions, saliva and blood can be present in the expired air and can provide a source of contamination into the breathing system. Hygroscopic devices are unable to provide against ventilator associated pneumonia. (2001)

Lorente L, Lecuona M, Jimenez A, Mora ML, Sierra A et al[97] suggests that using heat and moisture exchangers instead of heated humidifiers, may increase the incidence of ventilator associated pneumonia. (2006)

Niederman MS, Ferranti RD, Zeigler A, Merrill WW, Reynolds HY et al[106] showed Risk factors for tracheobronchial colonization with GNB appear to be the same as those that favor pneumonia and include more severe illness, longer hospitalization, prior or concomitant use of antibiotics, malnutrition, intubation, azotemia, and underlying pulmonary disease. Experimental investigations have linked some of these risk factors to changes in adherence of GNB to respiratory epithelial cells. Although formerly attributed to losses of cell surface fibronectin, these changes in adherence more likely reflect alterations of cell surface carbohydrates. Bacterial adhesins and prior antimicrobial therapy appear to facilitate the process. Interestingly, Enterobacteriaceae usually appear in the oropharynx first, whereas P. aeruginosa more often appears first in the trachea (1994)

ETIOLOGICAL AGENTS

Fagon JY, Chastre J, Hance AJ, Montravers P, Novara A, Gibert C Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter P et al[69] showed Bacterial agents causing nosocomial pneumonia in patients from ATS Group 1 are defined as "core organisms" and include: enteric Gram-negative bacilli (Enterobacter sps, Escherichia coli, Klebsiella sps, Proteus sps, Serratia marcescens), Haemophilus influenzae, Streptococcus pneumoniae and methicillin sensitive Staphylococcus aureus. Organisms related to ATS Group 2 pneumonia include those of ATS Group 1 but also anaerobes, Leigeonella pneumophilia, methicillin resistant S. aureus (MRSA) and P. aeruginosa. In

ATS Group 3 pneumonia, "core organisms" are often isolated but additional pathogens like P. aeruginosa, Acinetobacter spp. and methicillin resistant S. aureus are also frequent. Patients belonging to this category are at risk of being infected with potentially multiresistant organisms. In mechanically ventilated patients, VAP is polymicrobial in 40% of cases. (2000).

Fagon JY, Chastre J, Domart Y, Trouillet JL, Pierre J, Darne C, Gibert C et al[68] studied gram negative bacteria are the most common pathogens cause ventilator associated pneumonia.(1993)

Richards MJ , Edwards JR,Culver DH, Gaynes RP et al[118] studied the most common gram negative species were pseudomonas aeruginosa (15.6%), Enterobacter species (10.9%), and Klebsiella pneumoniae (7%). (1999)

Chastre J,Trouillet JL, Vuagnat A, Joiy-Guillou ML, Clavier H, Dombret MC, Gibert C et al[51] studied polymicrobial infection rate is usually high in ventilator associated pneumonia. (1989).

Gayness R, Edwards JR et al[75] showed data from the US based National Nosocomial Infections Surveillance (NNIS) System from 2003 found that Staphylococcus aureus (27.8%) was the most common pathogen associated with ventilator associated pneumonia, followed by pseudomonas aeruginosa (18.1%), Enterobacter species (10%), Klebsiella pneumoniae (7.2%), and Acinetobacter species (6.9%). (2005).

Kollef MH, Shorr A, Tabak YP, Gupta V, Lui LZ, Johannes RS et al[91] showed another recent US multicentre survey found staphylococcus aureus and

pseudomonas aeruginosa to be the most common causes of nosocomial pneumonia. (2005).

Jiménez P et al[90] studied Antibiotic resistant microorganisms are commonly found in VAP. Staphylococcus aureus, Pseudomonas aeruginosa, and Haemophilus influenzae were the most common causes of nosocomial pneumonia in the late 1990s. The presence of methicillin-resistant strains of S. aureus, vancomycin-resistant Enterobacter species, and b-lactam-resistant streptococci also increased significantly during this period and are now commonly associated with VAP. The presence of resistant P. aeruginosa is significantly associated with mortality. Antibiotic resistance and improper antimicrobial therapy contribute to mortality in patients with VAP. (1989).

Antonelli M, Moro ML, Capelli O, De Blasi RA, D'Errico RR, Conti G, Bufi M, Gasparetto A et al[8] studied The prognosis for aerobic, gram-negative bacilli (GNB) VAP is considerably worse than that for infection with gram-positive pathogens, when these organisms are fully susceptible to antibiotics. Death rates associated with Pseudomonas pneumonia are particularly high, ranging from 70 to more than 80% in several studies. According to one study, mortality associated with Pseudomonas or Acinetobacter pneumonia was 87% compared with 55% for pneumonias due to other organisms. Similarly, Kollef and coworkers demonstrated that patients with VAP due to high-risk pathogens (Pseudomonas aeruginosa, Acinetobacter spp., and Stenotrophomonas maltophilia) had a significantly higher hospital mortality rate (65%) than patients with late-onset VAP due to other microbes

(31%) or patients without late-onset pneumonia (37%) (65). Concerning gram-positive pathogens, in a study comparing VAP due to methicillin-resistant Staphylococcus aureus (MRSA) or methicillin-sensitive S. aureus (MSSA), mortality was found to be directly attributable to pneumonia for 86% of the former cases versus 12% of the latter, with a relative risk of death equal to 20.7 for MRSA pneumonia (1998).

el-Ebiary M, Torres A, Fabregas N, de la Bellacasa JP, Gonzalez J, Ramirez J, del Bano D, Hernandez C, Jimenez de Anta MT et al[64] studied Isolation of fungi, most frequently Candida species, at significant concentrations poses interpretative problems. Invasive disease has been reported in VAP but, more frequently, yeasts are isolated from respiratory tract specimens in the apparent absence of disease. One prospective study examined the relevance of isolating Candida spp. from 25 non-neutropenic patients who had been mechanically ventilated for at least 72 hours. Just after death, multiple culture and biopsy specimens were obtained by bronchoscopic techniques. Although 10 patients had at least one biopsy specimen positive for Candida spp., only two had evidence of invasive pneumonia as demonstrated by histological examination. Many of the endotracheal aspirates, PSB specimens, and BAL specimens also yielded positive cultures for Candida spp., sometimes in high concentrations, but they did not contribute to diagnosing invasive disease. On the basis of these data, the use of the commonly available respiratory sampling methods (bronchoscopic or nonbronchoscopic) in mechanically ventilated patients appears insufficient for the diagnosis of

Candida pneumonia. At present, the only sure method to establish that Candida is the primary lung pathogen is to demonstrate yeast or pseudohyphae in a lung biopsy. However, the significance of Candida isolation from the respiratory samples of mechanically ventilated patients merits being investigated in greater depth (1993).

DIAGNOSTIC STRATEGIES

Clinical Strategy

Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM et al[113] showed body temperature, white blood cell count, volume and appearance of tracheal secretion, oxygenation (PAo2/Fio2), chest X ray and tracheal aspirate cultures into a clinical pulmonary infection score as a diagnostic tool for pneumonia. They found that clinical pulmonary infection score of more than six was associated with sensitivity 93% and a specificity of 100%. (1991)

Singh N, Rogers P, Atwood CW, Wagener MM, Yu VL et al[136] used modified clinical pulmonary infection score with in a clinical management algorithm in an attempt to reduce unnecessary antibiotic use in patients in whom ventilator associated pneumonia was suspected. In this series of patients, modified score remaining less than six at 3 days safely allowed stopping antibiotics. However, the diagnostic value of the clinical pulmonary infection score has yet be confirmed. In addition, clinical utility of such a score would be higher if it helped clinicians in their decision to initiate or with hold antibiotic therapy in patients clinically suspected of ventilator associated pneumonia rather than

only to confirm or exclude pneumonia after 2-3 days when tracheal aspirate culture results are available. (2000).

Bacteriologic Strategy

Ioanas A,Ferrer R, Angrill J, Ferrer M, Torres et al[84] demonstrated the use of endotracheal aspirate in ventilator associated pneumonia management is increasing, there are few data regarding the usefulness of quantitative as opposed to qualitative cultures. Some studies suggested that quantitative cultures should be used in order to avoid false positive results, but little is known about the sensitivity and specificity of quantitative culture findings in severely ill patients who have previously received broad spectrum antibiotics. (2001).

Chastre J, Fagon JY et al[31] studied that bronchoalveolar lavage can also facilitate accurate modification of initial antibiotic treatment regimens for ventilator associated pneumonia. The airway of the mechanically ventilated patient is commonly colonized with potentially pathogenic bacteria. Consequently obtained from endotracheal or tracheostomy tube, cannot consequently differentiate between upper airway colonization and lower respiratory infection. The use of bronchoalveolar lavage for the microbiological diagnosis of ventilator associated pneumonia appears to be associated with greater confidence amongst clinicians that the culture results actually reflect the presence or absence of ventilator associated pneumonia, together with its etiology. (2002).

Ibrahim EH, Ward S, Sherman G, Schaiff R, Fraser VJ, Kolllef MH et al[85] studied sampling methods that minimize contamination from the upper airway (such as bronchoscopic or catheter bronchoalveolar lavage and brushing) help to establish a move precise microbiological diagnosis of ventilator associated pneumonia to guide subsequent antimicrobial changes. (2001).

Medurai GU[98]**, Mauldin GL, Wunderink RG, Leeper Jr KV, Heyland DK**[82]**, Cook DJ et al**[34] showed the bronchoscopic methods bronchoalveolar lavage and protected specimen brush are well standarised and widely accepted invasive diagnostic techniques for identifying the etiological pathogen of ventilator associated pneumonia. In recent study, Heyland et al found that invasive diagnostic testing might increase the confidence of physicians in the diagnosis and management of ventilator associated pneumonia as well as decreased antibiotics usage and lower mortality. (1999).

Rello J, Gallego M, Mariscal D, et al[126] showed The results of Microbiological tests of sputum specimens obtained by either invasive or Noninvasive methods are not sufficient for the diagnosis of VAP, but culture and sensitivity results can be helpful for choosing an antibiotic. (1997)

Comparing Diagnostic Strategy

Lambert R, Vereen L, George R,Sanchez- Nieto JM, Garcia et al[96] studied Quantitative Culture of endotracheal aspirate may avoid false positive results, but also provide controversial results, depending on the bacterial load, duration of ventilation and prior antibiotic treatment. The sensitivity ranges 38- 100%, while specificity ranges 14- 100% using a threshold 10^5- 10^6 cfu/ml, the sensitivity appears to have narrow range 50- 70% as well as specificity 70-

85%. Comparing outcome of patients, 2 studies came to the conclusion that there are no differences between endotracheal aspirate and invasive bronchoscopic methods in terms of mortality, ICU stay, and duration of mechanical ventilation. (1989).

Dr Daren Heyland et al[60] report the results of a Canadian multicentre randomized trial comparing bronchoalveolar lavage and endotracheal aspirate for the diagnosis of ventilator associated pneumonia. The study also addressed whether empiric antimicrobial monotherapy was equivalent to combination therapy. They concluded that utilization of bronchoalveolar lavage and endotracheal aspirate is associated with similar clinical outcomes and overall antibiotic utilization. (1999).

Blasi F, Gallego M, Mariscal D, Sonora R, Valles J, Brayan C S, Reynolds K L et al[15] demonstrated the rate of positive blood culture in ventilator associated pneumonia ranges 8- 20% in critically ill patients, bactereamia is not always related to a pulmonary infection and up to 50% of the patients with positive culture may have an additional source of infection. Luna et al pointed out that blood culture in patients with ventilator associated pneumonia are useful to suspect and identify another simultaneous infection when the microorganisms isolated in blood does not coincide with the microorganisms isolated respiratory secretion. (1997)

Luna CM, Videla A, Mattera J, Vay C, Famiglietti A, Vujacich P, Niederman MS et al[95] studied The spread of microorganism to blood or pleural space is <10%, so blood and pleural effusion cultures have low

sensitivity and specificity. Luna and colleagues demonstrated that the positive predictive value of blood cultures to detect the etiologic microorganism was 73% and the sensitivity of blood cultures was only 26%. [1997].

TREATMENT

American Thoracic Society[2] has stated that to guide empirical antibiotic choices. These guidelines are divided into those for patients at risk for VAP caused by multidrug-resistant organisms and those for patients without such risk. In the absence of risk factors for multidrug-resistant bacteria, the clinician should choose empirical therapy for Streptococcus pneumoniae, Haemophilus influenzae, methicillin-sensitive Staphylococcus aureus, and antibiotic-sensitive gram-negative enteric organisms. Antibiotic choices include Ceftriaxone, quinolones (levofloxacin, moxifloxacin, or ciprofloxacin), Ampicillin/sulbactam, or ertapenem .When risk factors for multidrug-resistant organisms are present, the clinician must consider not only the organisms listed above but also Pseudomonas aeruginosa, Klebsiella, Enterobacter, Serratia, Acinetobacter, Stenotrophomonas maltophilia, Burkholderia cepacia, and methicillin-resistant S. aureus. Empirical therapy is broadened to include (i) either an antipseudomonal cephalosporin (Cefipime or ceftazadime), an antipseudomonal carbepenem (imipenem or meropenem), or a β-lactam/β-lactamase inhibitor (Piperacillin-tazobactam) plus (ii) an antipseudomonal fluoroquinolone (ciprofloxacin or levofloxacin) or an Aminoglycosides (Amikacin, Gentamicin, or tobramycin) plus linezolid or vancomycin. (2005)

Adair CG, Gorman SP, Byers LM, Jones DS, Feron B, Crowe M, Webb HC, McCarthy GJ, Milligan KR et al[4] proposed that high concentrations of antibiotic on the endotracheal luminal surface, achieved either by nebulizer or endotracheal surface modification, would be expected to prevent biofilm formation on the endotracheal tube and may have a role in reducing the incidence of VAP, also minimising patient exposure to systemic antibiotics. (1995)

Kress JP, Pohlman AS, O'Connor MF, Hall JB et al[94] To reduce the aspiration of oropharyngeal contents, over use of sedatives should be avoided. Kress et al reported that for reducing over use of sedatives, daily interruption of sedative-drug infusions until the patients were awake decreased the duration of mechanical ventilation and the length of stay in the ICU. (2000).

GENERAL PROPHYLAXIS

Johanson WG Jr, Pierce AK, Sanford JP, Mc-Clain RE ,Combes P, Fauvage B, Oleyer C et al [86] Suctioning the secretions in the trachea is another approach to VAP prevention. Two types of tracheal suction catheters are used on ventilated patients; the open, single-use catheters and the closed, multiple-use catheters. In single-use system, HCWs have to use sterile solutions during rinsing these catheters and have to care aseptic technique when suctioning endotracheal secretions. In closed suctioning systems, secretions can be suctioned without removal of mechanical ventilation support. This may cause less hypoxia, hypotension and arrhythmias, and also less

environmental contamination .However similar VAP rates with closed and open system were suggested in the earlier trials ,Combes and colleagues reported a 3.5 times greater risk of VAP in open suctioning system than closed suctioning system in a recent study. Indeed, closed suction catheter is an extension of the ventilator circuit, daily change of this catheter is not necessary for infection control, and in one study no significant difference in VAP rate was reported when daily changes were compared with no routine changes, that may decrease the costs .The use of closed suction system is recommended as part of a VAP prevention program. (1972)

Craven DE, Steger KA et al[49] showed The devices used on the respiratory tract come into contact with mucous membranes, therefore cleaning and high-level disinfection (at 75°C for 30 minutes) of reusable equipments are required. Resuscitation bags, spirometers, and oxygen analyzers must be cleaned and disinfected between patients to avoid cross-transmission [1984].

Harris AD, Samore MH, Nafziger R, DiRosario K, Roghmann MC, Carmeli Y,Albert RK, Condie F et al[81] showed Basic hygiene principles of infection control (hand washing/disinfection just before and after each patient contact, the use of glove and sterile equipment) remain important for the prevention of VAP. Healthcare workers (HCW) can spread microorganisms from patient to patient by their hands easily. Although HCWs realize the importance of hand washing/disinfection, their compliance is still low (25–40%). Especially their compliance rate is lowest in activities that carried higher risk for transmission and in ICU. High workload decreases their compliance

.wrist watches, bangles, and other jewellery act as reservoirs for organisms, and inhibits effective hand cleaning. Therefore, staff has to take off wrist watch and jewellery to achieve effective hand cleaning. They have to use gowns and gloves when appropriate and must change and wash/disinfect their hands between patients. Bedside hand antiseptics (alcohol-based hand rub solution), easier access to sinks and availability of washing equipment, decrease in workload, communication and education tools (posters) and feedback improve compliance and decrease the cross-transmission of nosocomial infection [2000].

Drakulovic et al[62]. found that the simple elevation of the head of bed to 45° results in dramatic reductions in VAP incidence and a trend toward reduced mortality. Nonetheless, a recent survey by the University Hospital Consortium revealed that compliance with the simple and no-cost intervention of elevating the head is woefully low, and a study by Heyland et al. revealed that the head of bed is on average elevated to 29° and not 45°. Kinetic bed therapy has also led to a reduction in the incidence of VAP (1999).

MATERIALS AND METHODS

This is a prospective study where One hundred patients admitted into the Respiratory intensive care unit of Government Rajaji Hospital attached to Madurai Medical College between May 2008 and December 2008 (7 months period) were studied. As per inclusion criteria those patients who were under mechanical ventilation for more than 48 hours by endotracheal tube or tracheostomy were evaluated for the development of ventilator associated pneumonia. The Respiratory intensive care unit is equipped with 6 ventilators and central oxygen. Each bed has multiparameter monitor for continous hemodynamic and respiratory monitoring. Nurse on duty maintain vital sign and intake- output record on daily basis.

The study was approved by the local ethical committee and separate informed consent was obtained from each participant. For each patient, a Proforma was filled out and bronchoalveolar lavage endotracheal aspirate samples and blood culture were collected and taken immediately for processing to the microbiology laboratory.

INCLUSION CRITERIA

1. A new and persistent infiltration in the chest X-ray in patients mechanically ventilated for more than 48 hrs.

2. Body temperature above 38.5 or below 36°C.

3. White cell count above 12,000/µl or below 4000/µl.

4. Purulent tracheobronchial secretion (TBS).

5. Impairment of pulmonary function as defined by the PaO2/FiO2 ratio > 240.

6. Respiratory tract infection with absence of alternative sources of infection such as urinary tract infection or peritonitis.

EXCLUSION CRITERIA:

- Poor oxygenation (PaO2/FiO2 < 100mmHg)
- Unstable hemodynamic condition.
- Human Immunodeficiency Virus infection.
- Cytotoxic chemotherapy induced neutropenia.
- Organ transplantation.
- Solid and hematological malignancy.
- Patients with tracheal aspirates < 1 ml

DATA COLLECTION:-

Clinically diagnosed Ventilator Associated Pneumonia were observed and data such as age, gender, date of admission into and discharge from the Respiratory intensive care unit, risk factors involved, underlying diseases, date of intubation/ tracheostomy, duration of mechanical ventilation etc. (copy of Proforma enclosed) were obtained. Time period of Respiratory intensive care unit stay prior to initiation of ventilation, duration of Respiratory intensive care unit and hospital stays, were also recorded. Days of antibiotic therapy and a

short description of radiological findings were recorded. Patients were monitored from the time of inclusion in the study to the date of discharge from Respiratory intensive care unit.

Resolution of the disease in the patients was defined as clinical improvement accompanied by normal temperature, decreased volume and transparency of tracheobronchial secretion and radiologically confirmed elimination of the infiltrate.

Once clinical suspicion was established, empiric antibiotic therapy was initiated. The antibiotics were changed after the quantitative culture results of Bronchoalveolar lavage and endotracheal aspirate.

SPECIMEN COLLECTION

With each episode of clinically suspected Ventilator Associated Pneumonia, the patient was subjected to three different sampling techniques within 12 hours of clinical diagnosis of Ventilator Associated Pneumonia with an average of an hour between each procedure. A set sequence of sampling was followed in each case with the Endotracheal aspirate first, blood sample and Bronchoalveolar lavage at the last. All patients were premedicated prior to performing these sampling procedures unless they were already sedated and paralyzed.

First, Endotracheal aspirates were obtained with sterile precaution from 100 patients using a 22-inch, No. 14 Fr suction catheter and collected in a mucus collector. A length of approximately 24 cm of the catheter was passed through

the endotracheal tube, and secretions were aspirated without instilling saline. After the catheter was withdrawn, approximately 2-5 mL of saline was injected into it with a sterile syringe to flush the exudate into a sterile container for collection. Chest vibration or percussion for 10 min was used to increase the retrieved volume (1 mL) in case the patient produced very little secretions. These samples were retrieved for quantitative microbial cultures. Then without interrupting mechanical ventilation, through the endotracheal tube and using a special adaptor, the fiber optic bronchoscope was introduced 10 min later without bronchial suctioning after adequate sedation and curarization for 90 patients, and adjusting ventilator settings to a fraction of inspired oxygen of 100% with proper rate and tidal volume, a bronchoscope was passed through the endotracheal tube via a specific adaptor without local anesthesia. No endobronchial suction was attempted during the advance of the bronchoscope. Bronchoalveolar lavage sampling was obtained from the orifice of a lung segment with the most radiographic abnormality or new infiltrates, the bronchoscope was then introduced and wedged into the segmental bronchial orifice. Seven aliquots of sterile saline (20ml each) were instilled and aspirated gently.

The first two aliquots were discarded, and the last five aliquots were pooled for analysis. Generally 5 mL of the retrieved BAL fluid were adequate for microbiological examination. Before the protocol procedures, blood samples were taken for culture from 55 patients with septicaemia.

Blood culture

The vein from which blood is to be drawn was selected; skin site was disinfected with 70% isopropyl alcohol. About 5ml of blood was collected from 55 patients and inoculated into 50ml glucose broth (1in 10 dilution). Culture bottles were incubated at 35°C for 18 hours. After 18 hrs incubation subculture was made aseptically into a nutrient agar, macconkey agar and blood agar. The plate was incubated at 35°C for 48 hours. Culture negative bottles were then reincubated for 5-7 days.

The following thresholds of bacterial cultures were used to distinguish colonization from true infection: ETA, 10^5 CFU/ml; BAL, 10^4 CFU/ml.

MICROBIOLOGICAL METHODS

All the samples were transported to the laboratory within 15 minutes and cultured within an hour of collection. After receipt in the laboratory, the samples were first vortexed for 60 seconds after which, direct examination of gram stained preparations were performed and studied for the presence of squamous cells, polymorphonuclear cells and to differentiate Gram positive and Gram negative bacteria and also yeast cells and their morphology.

Simultaneously, quantitative cultures using the calibrated loop method were performed on common media such as nutrient agar, blood agar, and Macconkey's agar using standard techniques. The results of the Gram stain

were obtained within the first 24 hours and quantitative cultures were obtained within the following 48 to 72hours. In patients with repeated incidence of VAP symptoms, a repeat culture was performed.

Microbiological examination for unusual organisms such as leigeonella, Mycoplasma, Chlamydia, Pneumocystis carinii, anaerobes and viruses did not form a part in this study.

MICROBIOLOGICAL PROCESSING

AEROBIC CULTURE

ETA and BAL samples were mechanically homogenized using glass Beads and were vortexed for 1 min.. The samples were then serially diluted in 0.9% sterile saline solution with final dilutions of 10^{-2}, 10^{-3} and 10^{-4} for ETA and 10^{-1}, 10^{-2} and 10^{-3} for BAL. ETA and BAL were mixed 1:1 with sterile normal saline. Thereafter, 100 micro liters were inoculated into the following agar media: Nutrient agar, 5% sheep blood, and Macconkey agar. All cultures were incubated at 37° C under aerobic atmosphere.

Cultures were evaluated for growth 24 hrs and 48 hrs later and discarded, if negative, 5 days after. The next day, aerobic cultures were examined for the growth of organisms. The plates which showed growth were studied by colony morphology, gram reaction and motility (hanging drop).

BIOCHEMICAL REACTIONS

They were then subjected to biochemical tests for identification. Catalase, Coagulase and oxidase tests were performed. A single colony was inoculated into peptone water which was used as inoculum for the following biochemical test

- Indole
- TSI
- Christensen's urease medium
- Simmon's citrate utilization test
- MR
- VP
- Nitrate reduction test

All microorganisms isolated were identified by standard laboratory methods. Results are expressed as colony forming units per milliliter (CFU/ ml = number of colonies × dilution factor × inoculation factor).

After initial characterisation of the isolates by colony morphology and Gram stain, species identification and susceptibility testing were done

ANTIBIOTIC SUSCEPTIBILITY TEST

It was performed by Kirby-bauer standard disc diffusion method on Muller-Hinton agar for all isolates. The organism inoculated into peptone water was

incubated for half an hour and Muller-Hinton agar was seeded by pour plate method. The excess was pipetted off, the plate allowed to dry and antibiotic discs (commercially available) were placed and incubated for 18- 24 hrs. Following over night incubation the plates were examined for the zone of inhibition around the drug disc is measured with a scale and the sensitivity pattern of the isolates were studied. The following were the commercial antibiotic discs employed.

Ampicillin (10 µg), inj. Gentamicin (10 µg), trimethoprim / sulphamethazole (1.2 µg / 23.8 µg), ciprofloxacin (1 µg), Cephalexin (30 µg), Cefotaxime (10 µg), Ceftriaxone (10 µg), Amikacin (30 µg), Doxycycline (30 µg), erythromycin (5 µg), Piperacillin/ tazobactam (100 µg/10 µg), Carbencillin (100 µg), tobramycin (10 µg), Ofloxacin (5 µg), Gatifloxacin (5 µg), Cloxacillin(1 µg), Cefipime(30 µg), oxacillin (5 µg).

FUNGAL CULTURE

The standard media for primary isolation of fungus namely Sabouraud's dextrose agar, containing Gentamicin and cycloheximide was used. ETA and BAL samples were inoculated onto Sabouraud's dextrose agar. The inoculated slant was incubated at 37° C. The cultures were maintained for 30 days before discarding them as negative.

When growth became evident on the primary isolation media, fungi were identified macroscopically on the basis of colony appearance, pigmentation, consistency and microscopically by the appearance of conidia.

For observing the microscopic appearance, using teasing needle, mounts from the culture were made in Lacto phenol cotton blue and gram staining.

INTERPRETATION OF QUANTITATIVE CULTURE RESULTS

The diagnostic thresholds for ETA and BAL were taken as 10^5cfu/ml and 10^4cfu/ml respectively. Growth below the threshold was assumed to be due to colonization or contamination. For Gram stain results the thresholds for the diagnosis of VAP with the ETA and BAL samples were as follows: > 10 polymorphonuclear neutrophils (PMN) / high-power field (HPF), ≥ 1 bacteria / oil immersion field (OIF), presence of intracellular bacterial inclusions.

PATIENT FOLLOW-UP

All patients with positive quantitative cultures of ETA / BAL were treated as per the antibiogram reports obtained. After the modification of antibiotic drug regimen, these patients were followed up to exclude other diagnoses. VAP was considered to have occurred in those patients when there was a correlation between the positive culture result and the clinical outcome.

RESULTS

In the study period of seven months, there were a total of 215 Respiratory intensive care unit admissions at Government Rajaji Hospital Madurai where the study was made.176 (81.8%) patients were mechanically ventilated and 152 patients among them (70.7%) were ventilated for more than48 hours. One Hundred patients among 152(46.5%) were with clinical pulmonary infection score more than six. Only these patients who were mechanically ventilated for > 48 hrs with CPIS score > 6, considered as Ventilator Associated Pneumonia were included in this study.

From these 100 patients, two hundred and forty five samples were collected. The samples included one hundred endotracheal aspirates, ninety broncho alveolar lavages and fifty-five blood cultures. Sample wise distribution is given in table no.1

TABLE 1

SAMPLE WISE DISTRIBUTION

Total no. of samples (n)	Sample wise distribution	Percentage (n) %
245	Endotracheal aspirate	100(40.8%)
	Bronchoalveolar lavage	90(36.7%)
	Blood culture	55(22.4%)

The Sex and age distribution of these cases were studied and it was found that, out of One hundred patients, **68 (68%)** patients were males and 32 (32%) were females. Out of 68 male patients, 2(2.9%) were in the age group

0-20 years, 50 **(73.5%)** were 21**- 40 years**, 14 (20.6%) were 41- 60 years and 2 (2.9%) were > 60 years. Out of 32 female patients, 4 (12.5%) were 0-20 years, **16 (50%) were 21- 40 years**, 10 (31.3%) were 41- 60 years and 2(6.3%) were > 60 years**. It was found that infection rate in Ventilator Associated Pneumonia was more common in males than in females and the Predominant age group was 21- 40 years, in both males and females**. **Age and sex wise distribution of cases are shown in table no.2**

TABLE 2

DEMOGRAPHIC DATA OF PATIENTS INVESTIGATED

Sl.No	**Age wise distribution**	**Male (68) n=68**	**Female (32) n =32**
1.	0- 20 years	2 (2.9%)	4 (12.5%)
2.	21- 40 years	**50 (73.5%)**	**16 (50%)**
3.	41- 60 years	14 (20.6%)	10 (31.3%)
4.	> 60 years	2 (2.9%)	2 (6.3%)

All One hundred patients were further analysed according to the clinical conditions and it was found that 44 (44%) were poisoning cases, 24 (24%)with abdominal surgeries, 12(12%) were suffering from guillian barre syndrome, 8 (8%) had history of snake bite, 4 (4%) had head trauma, multiple injuries and diabetic Ketoacidosis. **It was observed that incidence of Ventilator Associated Pneumonia was more in poisoning cases followed by abdominal surgeries. This is given in** table **no.3**

TABLE 3

DISTRIBUTION OF CASES Vs CLINICAL CONDITIONS

Sl.NO	Clinical conditions	Number of patients
1.	**Poisoning**	**44 (44%)**
2.	**Abdominal surgery**	**24 (24%)**
3.	Gullian barre syndrome	12 (12%)
4.	Snake bite	8 (8%)
5.	Diabetic Ketoacidosis	4(4%)
6.	Head trauma	4 (4%)
7.	Multiple injuries	4 (4%)

Endotracheal aspirate: - Out of 100 endotracheal aspirate samples collected from clinically suspected Ventilator Associated Pneumonia cases, 90 (90%) samples yielded growth on culture. There were 112 isolates totally as some cultures yielded more than one isolates. These 112 isolates were subjected to gram staining and it was found that out of 112 isolates 80 (71.4%) were Gram negative bacteria, 18 (16.1%) were Gram positive bacteria and 14 (12.5%) were fungi. **Thus it was observed that more cases of ventilator associated pneumonia were caused by Gram negative organisms , than Gram positive organisms. This is given in table no.4**

TABLE 4

GRAM REACTION OF ISOLATES OF

ENDOTRACHEAL ASPIRATES

Total no of isolates	Etiological agents	Percentage (n) %
112	**Gram negative bacteria**	**80(71.4%)**
	Gram positive bacteria	18(16.1%)
	Fungus	14(12.5%)

The 112 isolates were further subjected to genus identification and it was found that out of 112 isolates, 32(28.6%) were **Pseudomonas aeruginosa,21(18.8%) were Klebsiella pneumoniae**, 9(8%) were Klebsiella oxytoca, **14(12.5%) were Staphylococcus aureus**, 4(3.6%) were Coagulase negative Staphylococci, Acinetobacter and Enterobacter, 2(1.8%) were Citrobacter freundi and koseri, 3(2.6%) were Proteus mirabilis and Escherichia coli, 12(10.7%) were Candida species and 1(0.8%) was Aspergillus fumigatus and Aspergillus niger. **Thus it was proved that among gram negatives Pseudomonas aeruginosa and Klebsiella pneumoniae were common and Among the Gram positives Staphylococcus aureus was common. Among fungi Candida species was common. This is given in figure no.3**

Quantitative analysis of 112 positive isolates showed that, colony counts of $>10^5$ cfu/ml were present in 85 (75.9%) isolates, colony count between 10^4 and 10^5 were seen in 16 (14.3%) isolates and colony count between 103 and 10^4 cfu/ml were seen in 11(9.8%) isolates. **It was observed that 75.9% of positive isolates showed colony count $>10^5$ cfu/ml, which is a diagnostic threshold for endotracheal aspirates. This is given in table no. 5**

TABLE 5

QUANTITATION OF ENDOTRACHEAL ASPIRATES

Sl.No	Quantitation	No of isolates	Percentage (%)
2.	**$>10^5$ cfu/ml**	**85**	**75.9%**
3.	10^4 - 10^5 cfu/ml	16	14.3%
4.	10^3 - 10^4 cfu/ml	11	9.8%

Quantitation of culture was analysed organism wise and it was found that, Out of 85 isolates with colony counts of $>10^5$ cfu/ml , 35(41.2%) were Pseudomonas aeruginosa isolates, 21(24.7%) were Klebsiella Pneumoniae isolates, 16(18.8%) were Staphylococcus aureus isolates, 11(12.9%) were Klebsiella oxytoca isolates and 2(2.4%) were Enterobacter isolates. Out of 16 isolates with colony count between 10^4 and 10^5, 4(25%) each were Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis. Out of 11 isolates with colony count between10^3 and 104 cfu/ml, 5(45.5%) were Coagulase negative staphylococci isolates and 3(18.2%) each were Citrobacter Sps and Acinetobacter Sps. **Quantitation revealed that**

Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus showed colony counts of diagnostic threshold. This is given in table no.6

TABLE 6
QUANTITATION Vs ORGANISMS IN ENDOTRACHEAL ASPIRATE

Quantitation	No of positive isolates	Isolates	% Percentage
$>10^5$ cfu/ml	85	Pseudomonas aeruginosa Klebsiella pneumoniae Staphylococcus aureus Kl.oxytoca Enterobacter	35 (41.2%) 21 (24.7%) 16 (18.8%) 11 (12.9%) 2 (2.4%)
10^4 - 10^5 cfu/ml	16	Pseudomonas aeruginosa Klebsiella pneumoniae Escherichia coli Proteus mirabilis	4 (25%) 4 (25%) 4 (25%) 4 (25%)
10^3 - 10^4 cfu/ml	11	CONS Citrobacter Sps Acinetobacter Sps	5 (45.5%) 3 (18.2%) 3 (18.2%)

Bronchoalveolar lavage:- :- Out of 90 Bronchoalveolar lavage samples collected clinically suspected Ventilator Associated Pneumonia cases, 81(90%) samples yielded Growth on culture. There were 98 isolates totally as some cultures yielded more than one organisms. These 98 isolates were subjected to Gram staining and it was found that out of 98 isolates, 69(70.4%) were Gram negative bacteria**,** 15(15.3%) were Gram positive bacteria and 14(14.3%) were fungi. **Thus it was found that there was more number of Gram negative bacteria in bronchoalveolar lavage, causing ventilator associated pneumonia than Gram positive bacteria. This is given in table no.7**

TABLE 7

GRAM REACTION OF ISOLATES OF BRONCHOALVEOLAR LAVAGES

Total no of isolates	Etiological agents	Percentage (n) %
98	**Gram negative bacteria**	**69(70.4%)**
	Gram positive bacteria	15(15.3%)
	Fungus	14(14.3%)

The 98 isolates were further subjected to genus identification and it was found that out of 98 isolates, **28(28.6%) were Pseudomonas aeruginosa,15(15.3%) were Klebsiella pneumoniae**, 10(10.2%) were Klebsiella oxytoca, **11(11.2%) were Staphylococcus aureus**, 4(4.1%) were Coagulase negative staphylococci, Acinetobacter Species and Enterobacter, 2(2%) were Proteus mirabilis, Citrobacter freundi, Citrobacter koseri and Escherichia coli, 12(12.2%) were Candida species and 1(1%) was Aspergillus fumigatus and Aspergillus niger. **Thus it was observed that among gram negatives, Pseudomonas aeruginosa and Klebsiella pneumoniae was the common and among gram positives, Staphylococcus aureus was common organism isolated. Among fungi, Candida species was common. This is given in figure no.4**

Quantitative analysis of 98 positive isolates showed that, colony count of >105 cfu/ml were present in 7(7.1%) isolates, colony count between 104 and

10^5cfu/ml were seen in 83(84.7%) isolates and Colony count of between 103 – 10^4 cfu/ml were seen in 8(8.2%) isolates**. It was observed that 84.7% of positive isolates showed colony count between 104 - 10^5 cfu/ml which is a diagnostic yield for bronchoalveolar lavage. This is given in table no. 8**

TABLE 8

QUANTITATION OF BRONCHOALVEOLAR LAVAGES

Sl.No	Quantitation	No of isolates	Percentage (%)
1.	>105 cfu/ml	7	7.1%
2.	**10^4 - 10^5 cfu/ml**	**83**	**84.7%**
3.	103 - 104 cfu/ml	8	8.2%

Quantitation of culture was analysed organisms wise and it was found that, out of 83 isolates with colony count between 10^4 - 10^5 cfu/ml, 30(36.1%)were Pseudomonas aeruginosa isolates, 17(20.5%) were Klebsiella pneumoniae isolates, 13(15.7%) were Staphylococcus aureus isolates, 12 (14.5%) were Klebsiella oxytoca isolates, 4(4.8%) were Enterobacter and Escherichia coli isolates, 2(2.4%) were proteus mirabilis and 1(1.2%) was Acinetobacter Sps. Out of 8 isolates with Colony count between 10^3 and 10^4, 4(50%) were Coagulase negative staphylococci, 2(25%) each were seen in Citrobacter Sps and Acinetobacter Sps. Out of 7 isolates with Colony count of >105 cfu/ml**,** 4(57.1%) were Pseudomonas aeruginosa isolates, 2(28.6%) were Klebsiella pneumoniae isolates and 1(14.3%) was Staphylococcus aureus isolates. **It was observed that Pseudomonas aeruginosa, Klebsiella**

pneumonia and Staphylococcus aureus showed colony counts of diagnostic threshold. This is given in table no.9

TABLE 9
QUANTITATION Vs ORGANISMS IN BRONCHOALVEOLAR LAVAGE

Quantitation	No of positive isolates	Isolates	% Percentage
10^3 - 10^4 cfu/ml	8	CONS Citrobacter Sps Acinetobacter Sps	4 (50%) 2 (25%) 2 (25%)
10^4 - 10^5 cfu/ml	**83**	Pseudomonas aeruginosa Klebsiella pneumoniae Staphylococcus aureus Kl.oxytoca Escherichia coli Proteus mirabilis Enterobacter Acinetobacter Sps	30(36.1%) 17(20.5%) 13(15.7%) 12(14.5%) 4(4.8%) 2(2.4%) 4(4.8%) 1(1.2%)
>10^5 cfu/ml	**7**	Pseudomonas aeruginosa Klebsiella pneumoniae Staphylococcus aureus	4(57.1%) 2(28.6%) 1(14.3%)

Blood culture: A total of fifty five blood samples were collected from patients of clinically suspected Ventilator Associated Pneumonia with septicaemia for non enteric blood culture. Out of 55 samples, 16 cultures yielded growth.

There were 20 isolates from these 16 cultures as some cultures yielded more than one organisms. These isolates were subjected to gram staining and it was found that, out of 20 isolates 13(65%) were Gram negative bacteria, 5 (25%) were Gram positive bacteria and 2 (10%) were fungus. **It was observed that**

there were more number of Gram negative isolates in blood culture, causing ventilator associated pneumonia than Gram positive isolates. This is given in tableno.10

TABLE 10

GRAM REACTION OF ISOLATES IN BLOOD CULTURE

Total no of isolates	Etiological agents	Percentage (n) %
20	**Gram negative bacteria**	**13(65%)**
	Gram positive bacteria	5(25%)
	Fungus	2(10%)

The 20 isolates were analysed for varied organisms and it was found that, Out of 20 isolates, **6(30%) were Pseudomonas aeruginosa**, 5(25%) were Staphylococcus aureus, 4(20%) were Klebsiella pneumoniae, 2(10%) were Escherichia coli, 1(5%) were Klebsiella oxytoca and 2(10%) were Candida species**. It was found that the most common organism isolated among gram negative was Pseudomonas aeruginosa (30%) among gram positive was staphylococcus aureus and among fungi Candida species. This is given in table no.11**.

TABLE 11

PATHOGENS ISOLATED FROM BLOOD CULTURE

Total No of isolates	Organisms isolated	No .of each isolates	Percentage %
20	**Pseudomonas aeruginosa**	6	30
	Staphylococcus aureus	**5**	**25**
	Klebsiella pneumoniae	4	20
	Escherichia coli	2	10
	Klebsiella oxytoca	1	5
	Candida species	2	10

Correlation of lab reports with clinical condition

In patients with Poisoning, Pseudomonas aeruginosa 13(29.5%) was predominantly isolated. In Abdominal surgeries, Klebsiella pneumonia 12(50%) and Pseudomonas aeruginosa 9 (37.5%) were predominantly isolated. In Guillian barre syndrome, Pseudomonas aeruginosa 7(66.6%) was predominantly isolated. In Snake bite, Staphylococcus aureus 2(25%) and Coagulase Negative Staphylococcus2 (25%) were predominantly isolated. In Diabetic Ketoacidosis, Pseudomonas aeruginosa 2 (50%) was predominantly isolated. In Head trauma, Klebsiella pneumoniae and Staphylococcus aureus were isolated. In Multiple injuries, Staphylococcus aureus 3(75%) was predominantly isolated. **Pseudomonas aeruginosa was the common organism causing Ventilator Associated Pneumonia and was isolated from Poisoning, Guillian barre syndrome and Diabetic Ketoacidosis cases and in this study was isolated more frequently (66.6%) from patients with Guillian barre syndrome. This is shown in table no 12.**

TABLE 12

ORGANISMS ISOLATED Vs ETIOLOGY

Isolates	Poisoning (44)		Abdominal surgery(24)		Gullian barre syndrome (12)		Snake bite(8)		Diabetic Ketoacidosis (4)		Head trauma (4)		Multiple injuries (4)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ps.aeruginosa	13	29.5	9	37.5	7	66.6	0	0	2	50	0	0	0	0
Kl.pneumoniae	4	9.1	12	50	2	16.7	0	0	1	25	1	25	1	25
Kl.oxytoca	3	6.8	5	20.8	1	8.3	0	0	0	0	0	0	0	0
S.aureus	4	9.1	3	12.5	1	8.3	2	25	0	0	1	25	3	75
CONS	2	4.5	0	0	0	0	2	25	0	0	0	0	0	0
Acinetobacter	2	4.5	0	0	1	8.3	0	0	1	25	0	0	0	0
Citrobacter	2	4.5	1	4.2	0	0	1	12.5	0	0	0	0	0	0
Proteus mirabilis	0	0	3	12.5	0	0	0	0	0	0	0	0	0	0
Escherichia coli	0	0	2	8.3	0	0	0	0	0	0	1	25	0	0
Candida sps	6	13.6	3	12.5	2	16.7	0	0	0	0	0	0	1	25
Aspergillus sps	0	0	0	0	2	16.7	0	0	0	0	0	0	0	0

Comparing all the three techniques of diagnosing infectious agent in ventilator associated pneumonias, it was found that endotracheal aspirate and bronchoalveolar lavage yielded more isolation (90% in each) than blood culture (29%). This is given in table no. 13

TABLE 13

COMPARISION OF DIFFERENT TECHNIQUES

Investigation	Performed	No of culture positive cases	% Positivity
Endotracheal aspirate	100	90	90
Bronchoalveolar lavage	90	81	90
Blood culture	55	16	29

There was total agreement between culture results of endotracheal aspirate and bronchoalveolar lavage techniques whereas blood culture showed less positivity rate.

Antimicrobial Susceptibility:-

Drug sensitivity pattern for the various bacterial isolates were analysed.

Pseudomonas aeruginosa showed resistant to Gentamicin (61.9 %), Ceftriaxone (79.7%), Ciprofloxacin (56.2%), Ofloxacin (62.5%), Amikacin (20%), Piperacillin tazobactam (62.5%), Cefipime (68.7%) and Carbencillin (76.6%) and 100% resistant to Ampicillin, Cephalexin, Cefotaxime, Cotrimoxazole and Doxycycline.

Staphylococcus aureus were resistant to Gentamicin (75.9%), Ceftriaxone (86.2%), Ciprofloxacin (72.5%), Ofloxacin (79.4%), and 100% resistant to Ampicillin, Cephalexin, Cefotaxime, Cloxacillin, Cotrimoxazole, Doxycycline, Erythromycin and Oxacillin.

Klebsiella pneumoniae showed resistant to Gentamicin (47.4%), Ceftriaxone (60.5%), Ciprofloxacin (47.4%), Ofloxacin (52.6%), Piperacillin tazobactam (52.6%), Cefipime (57.9%) and Carbencillin (68.5%) and 100% resistant to Ampicillin, Cephalexin, Cefotaxime, Cotrimoxazole and Doxycycline.

Escherichia coli showed 100 % resistant to Ampicillin, Cephalexin, Cefotaxime, Cotrimoxazole, Doxycycline, Gentamicin, Ceftriaxone, Carbencillin, and Cefipime.

Proteus mirabilis showed 100 % resistant to Ampicillin, Cephalexin, Cefotaxime, Cotrimoxazole, Doxycycline, Gentamicin, Ofloxacin, Piperacillin tazobactam, Carbencillin, and Cefipime.

Acinetobacter showed resistant to Gentamicin (87.5%), Cotrimoxazole (75%), and 100 % resistant to Ampicillin, Cephalexin, Cefotaxime, Doxycycline, Ofloxacin, Ciprofloxacin, Ceftriaxone, Piperacillin-tazobactam Carbencillin, Cotrimoxazole and Cefipime.

Citrobacter showed resistant to Gentamicin (87.5%), Ciprofloxacin (87.5%) and 100 % resistant to Ampicillin, Cephalexin, Cefotaxime, Doxycycline, Ofloxacin, Ciprofloxacin, Ceftriaxone, Piperacillin-tazobactam, Carbencillin, Cotrimoxazole and Cefipime.

All the isolates were sensitive to Gatifloxacin, and Aminoglycosides group of drugs (80%). Resistance was more commonly observed among beta lactam group of antibiotics for all isolates. This is shown in table no.14

It was observed that 75 % of Pseudomonas aeruginosa isolated were multidrug resistant, 69% of Klebsiella pneumonia was multi drug resistant and 100% of Staphylococcus aureus isolates were resistant to methicillin (MRSA).

Mortality and morbidity:

With regard to the mechanical ventilation period, patients were artificially ventilated for 15 ± 5 days. Average duration of stay in Respiratory Intensive Care Unit was 15± 5 days.

The crude observed mortality rate was 26% (26 of 100 cases). Mortality rate was determined based on the duration of respiratory care unit stay and isolation of organisms. Out of 26 deaths, 2 (7.7%) deaths were seen in 5 days RICU stay, 8 (30.8%) were seen in 10 days stay, 13(50%) were seen in 15 days stay, 3(11.5%) were seen in 20 days stay. **It was observed that more number of deaths was seen in 15 days RICU stay. This is given in table no.15**

TABLE 15

OUTCOME IN PATIENTS WITH VENTILATOR ASSOCIATED PNEUMONIA

Total no of death	RICU Stay duration	Number of deaths	Percentage
26	5 days	2	7.7 %
	10 days	8	30.8 %
	15 days	13	50 %
	20 days	3	11.5 %

Mortality as per organism isolated were evaluated and it was found that 18 out of 26 deaths showed **Pseudomonas aeruginosa (69.2%)** in all 3 samples**,** 4 showed Klebsiella pneumoniae (15.4%), 2 showed Staphylococcus aureus (7.7%) and Aspergillus growth (7.7%). **Mortality increased in patients infected with Pseudomonas aeruginosa which invariably is multidrug resistance.** This is given in table no.16

TABLE 16

MORTALITY IN VENTILATOR

ASSOCIATED PNEUMONIA

Total no of death	Causative agent	Number of deaths	Percentage
26	**Pseudomonas aeruginosa**	**18**	**69.2%**
	Klebsiella pneumoniae	4	15.4%
	Staphylococcus aureus	2	7.7%
	Aspergillus	2	7.7%

DISCUSSION

The present study was carried out to find out the various factors involved in Ventilator Associated Pneumonia among 100 patients who were admitted in Respiratory Intensive Care Unit of Government Rajaji Hospital on mechanical Ventilation. The cases included in the study were Poisoning, Abdominal surgeries, Gullian barre syndrome, Snake bite, Diabetic Ketoacidosis, Head trauma and multiple injuries. The study period was from May 2008 to December 2008.

In the present study it was found that 68% of the cases were males and 32% were females. Thus the infection rate was found to be more common in males than in females. Similar study by **cook DJ et al** [42](62%) and **Cook and Kollef** [91](56%) also identified male gender dominance in Ventilator Associated Pneumonias. As in these studies, the male dominance seems to be with patient specific demographic characteristics in this study also.

In the present study, age group commonly involved in Ventilator Associated Pneumonia was between 21- 40 years, and the important clinical condition involved in mechanical ventilation was poisoning cases (44%) especially suicidal poisonings. Similar study **by Han SC et al**[80] revealed that 45.6% of the poisoning cases in mechanical ventilation developed Ventilator Associated Pneumonia. **Panwar et al** [111]in his study showed 34 years as the common age group involved in Ventilator Associated Pneumonias associated with poisoning

cases. These two studies are in accordance with this present study. Most of the poisoning cases were subjected to gastric lavage prior to admission. These patients had signs of severe respiratory disease and increased need for mechanical ventilation; hence increase in Ventilator Associated Pneumonias. The pulmonary symptoms might be due to aspiration as a result of induced vomiting and lavage. As most of the poisoning cases were in the age group 21-40 years, Ventilator Associated Pneumonias shown to be common in this age group.

In the present study, 71.4% Gram negative organisms were isolated in Ventilator Associated Pneumonias. Among the Gram negative isolates, 28.6% were Pseudomonas aeruginosa and 18.8% were Klebsiella pneumonia. Both these organisms had quantitatively showed diagnostic threshold. Similar study by **Rajesh chawla et al** [119]also found that 87% of patients with Ventilator Associated Pneumonia were infected with Gram negative bacilli, most commonly Pseudomonas aeruginosa (30%) and Klebsiella pneumonia (20%), which are in support of this study. It has been known for decades that the microbial flora of hospitalized and critically ill patients becomes drastically altered within days after admission. In these patients, usual low virulent mixed flora of oropharynx and anaerobic flora of the colon become overgrown by endogenous aerobic gram negative bacilli, which can then colonize the airway and lead to lung infection. This may be the reason for increased incidence of gram negative organisms in this study also.

In this study, Pseudomonas aeruginosa and Candida were commonly presented in patients with Guillian barre syndrome, poisoning, abdominal surgeries and diabetics. Guillian barre syndrome is an autoimmune disease, in which corticosteroids played a major role and patients were in mechanical ventilation for prolonged periods and these might be the main risk factors related to Pneumonia by Pseudomonas aeruginosa and Candida in these patients. The virulent factor, type 3 secretory Exotoxin secreted by nosocomial Pseudomonas could also be another factor causing Pneumonia by Pseudomonas in these patients on mechanical ventilation.

In the present study, among Gram positive organisms, Staphylococcus aureus accounted for 12.5% and all were methicillin resistant. Similar study by **Riza et al**[124] showed methicillin resistant Staphylococcus aureus (30.4%) as the most frequently isolated organism. **Kollef MH et al**[91]**, Tejada Artigas et al**[141]**, Torres A et al**[138]**, Akca O et al** [6]in their studies showed that Staphylococcus aureus was the predominant gram positive organism in Ventilator Associated Pneumonias. In this study Staphylococcus aureus was mostly seen in multiple injuries and all the isolated staphylococcus aureus were methicillin resistant. It was observed that the injured patients were administered antibiotics, on the day of admission itself even before mechanical ventilation and the samples from these patients were collected 4-6 days after mechanical ventilation. Most of these patients stayed in the hospital for more than 10 days. Prolonged usage of antibiotics and prolonged hospital stay on mechanical ventilation might be the

reasons for resistant organisms. It is presumed that most of the strains of staphylococcus aureus might be community acquired with toxin producing capacity associated with aggressive virulent skin and soft tissue infections and necrotizing pneumonia.

It has been shown in this study that 75.9% of Endotracheal aspirate samples yielded bacterial count $>10^5$ Cfu/ml. Remaining samples showed bacterial count between 10^3- 10^4 cfu/ml. Similarly 84.7% of Bronchoalveolar lavage samples yielded threshold of $>10^4$ cfu/ ml. Remaining samples showed bacterial count between 10^3- 10^4 cfu/ml. The Quantitative endotracheal aspirate and bronchoscopy including Broncho alveolar lavage achieved a very similar yield with regards to diagnostic threshold, proving that both techniques yielded equal amount of organisms for diagnosis of Ventilator Associated Pneumonia in these cases. Similar study by **Timsit et al**[140] revealed that no difference found when either invasive (BAL) or Quantitative Endotracheal Aspirate (QEA) techniques were used to diagnose Ventilator Associated Pneumonia. Most studies have concluded that the diagnostic accuracies of non bronchoscopic and bronchoscopic techniques were similar. This is because even though the technique of obtaining specimen varies (invasive, noninvasive), the ultimate specimen obtained for diagnosis was the same that is the lung secretions. Hence no variation in the yield. All the cases in this study had clinical features of pneumonia, irrespective of threshold value, proving that

the cases with threshold below the accepted value could be an early phase of infection.

The rate of positive blood culture in the present study was 29%. Similar study by **Ioanas et al** [84]showed that the rate of positive blood culture in Ventilator Associated Pneumonia was 20% and the study by **Steven et al** [130]also revealed that blood culture positivity was 25%. These studies support the findings of the present study. As most of the patients were administered antibiotics on the day of admission and the blood samples were collected after therapy, there might be low positivity for blood culture. Hence the role of blood culture is limited in the study of Ventilator Associated Pneumonia.

In this study it was also noted that organisms isolated in the three types of samples (ETA, BAL, Blood culture) were the same in all these cases, showing that all these cases were only pulmonary infection, not associated with any additional source of infection. **Luna et al**[95] showed in his study 50% of patients with Ventilator Associated Pneumonia were with some other simultaneous infection, because organism isolated in blood culture did not coincide with isolates in respiratory secretion.

In this study, it was shown that an overall rate of 75% Pseudomonas aeruginosa were multidrug resistant. Pseudomonas aeruginosa was resistant to Gentamicin (61.9%), Ceftriaxone (79.7%), Ciprofloxacin (56.2%), Ofloxacin (62.5%),

Amikacin (20%), Piperacillin tazobactam (62.5%), Cefipime (68.7%) and Carbencillin (76.6%), and 100% resistant to Ampicillin, Cephalexin, Cefotaxime, Cotrimoxazole, and Doxycycline. Similar study **Arindam et al** [9] also showed 48% of pseudomonas aeruginosa were multidrug resistant. This correlates with present study. In this study Pseudomonas aeruginosa was susceptible for Gatifloxacin and Amikacin. Increased resistance might be due to various factors like prolonged usage of antibiotics, prolonged hospital stay or by the liberation of either IMP- type metalloenzymes or carapenemases by pseudomonas.

In present study Klebsiella Sps also played a major role in producing resistance (69%) for many antibiotics, as Klebsiella can produce ESBL, which are typically plasmid mediated and clavulanate susceptible enzymes, that hydrolyze penicillins, expanded spectrum cephalosporins and aztreonam.

All the isolates of staphylococcus aureus were resistance for methicillin (100%), showing that MRSA was the most frequent causative agent for Ventilator Associated Pneumonia. **Arindam et al**[9] showed more isolates of MRSA in their study, and explained that these resistance pathogens always varied in different set up. Occurrence of resistance for multiple drugs in these patients might be one of the major reasons for Ventilator Associated Pneumonia.

In present study it was found that the mortality rate was 26%. Similar study by **chastre J Fagon JY et al** [31]who proved the mortality rates to be 25% is in great support of this study. It was seen that the mortality was significantly high in patients with multidrug resistant Pseudomonas. Mortality was predominately related to underlying diseases like Guillian barre syndrome and in Poisoning cases and also duration of hospital stay, patients with hospital stay more than 15 days showed high mortality. Similar study by **Panwar Rakshit et al**[111] also demonstrated that mortality was significantly high in co morbid illness colonized with Pseudomonas. Virulence factor of Pseudomonas with steroid therapy and prolonged stay in hospital might be a reason for high incidence of mortality.

SUMMARY

The analysis of 100 samples collected from Ventilator Associated Pneumonia cases admitted in respiratory care unit of Government Rajaji Hospital showed that Ventilator Associated Pneumonia was more preponderant in males, the common age group being 21- 40 years in both sexes. The clinical condition most often associated with Ventilator Associated Pneumonia was poisoning.71.4% of Gram negative and 16.1% of Gram positive organisms and 12.5% fungi were isolated. 28.6% of Gram negative organisms were Pseudomonas aeruginosa and 12.5% of Gram positive organisms were Staphylococcus aureus and 10.7% were Candida species. Pseudomonas aeruginosa and Candida species were commonly present in patients with Guillian barre syndrome and Poisoning, whereas Staphylococcus aureus were present in multiple injuries.

On the analysis of the three different types of samples collected in this study (i.e.)Endotracheal aspirate, Bronchoscopic Bronchoalveolar lavage and blood culture, the former two techniques (i.e.) Endotracheal aspirate (75.9%) and Bronchoalveolar lavage (84.7%) yielded equal positivity in the isolation, whereas blood culture yielded less positivity (29%).

The organism isolated in the three types of samples (Endotracheal aspirate, Bronchoscopic bronchoalveolar lavage and blood culture) were same in all the cases showing that all these cases were pulmonary infection, not associated with any additional source of infection.

75% of pseudomonas aeruginosa isolated were multidrug resistant, 69% of Klebsiella pneumonia was multidrug resistant and 100% of staphylococcus aureus were methicillin resistant staphylococcus aureus (MRSA). The resistance of organism to antibiotics might have played a major role in the occurrence of Ventilator Associated Pneumonia in these patients on mechanical Ventilation.

It was also noted that mortality rate in ventilator associated pneumonia was 26%. The rate was significantly high in patients with multidrug resistant Pseudomonas aeruginosa, predominately related to underlying diseases like Guillian barre syndrome and poisoning and also in patients with hospital stay more than 15 days.

CONCLUSION

A study on microbial etiology of ventilator associated pneumonia Conducted at Government Rajaji Hospital, Madurai during the period from May 2008 to December 2008, revealed the following findings

- Male predominance in the age group 21- 40 years, especially in the poisoning cases on mechanical ventilation noted.
- Pseudomonas aeruginosa among gram negative and Staphylococcus aureus among gram positive organisms and Candida among fungi were commonly isolated.
- Comparative study of the three methods of collection of samples revealed that the samples collected by endotracheal aspirate and bronchoalveolar lavage yielded organisms equally both in specificity and Quantitation. Blood culture showed less positivity.
- Pseudomonas aeruginosa, Klebsiella pneumonia and Staphylococcus aureus were highly resistant for multiple drugs.
- Mortality rate was high in patients with multidrug resistant Pseudomonas with underlying diseases like Guillian barre syndrome and Poisoning and also in patients with hospital stay more than 15 days.

OUTCOME OF STUDY

Ventilator associated pneumonia is considered, a serious infective condition related to high mortality rate, hence it needs a prompt diagnosis and proper Antibiotic treatment. In this study also there is high incidence of ventilator Associated Pneumonias and important factors which increase the vulnerability to acquire Ventilator associated pneumonia were prolonged hospital stay, implication of organisms with multidrug resistance and predisposing underlying diseases. The pathogens isolated could have possibly been present in the hospital environment. Hence strategies to bring down incidence of Ventilator Associated Pneumonia and thus prevent considerable morbidity and mortality.

1. A well monitored hospital infra surveillance system has to be in place
2. Effective implementation of sterilisation and disinfection procedures to be adopted
3. A policy on rational use of antibiotic to be implemented

However, prompt and early diagnosis of pneumonias would be the mainstay in bringing down mortality. Endotracheal aspirate samples have been found to be very useful in isolation of etiological agents and should be sent to the clinical Microbiology lab as early as possible in a patient on mechanical ventilation more than 48hours.

ANNEXURE 1

GRAM STAINING:

The gram stain was prepared as follows:

PRIMARY DYE:

Crystal violet - 10g

Ammonium oxalate - 4.25g

Absolute alcohol - 50ml

Distilled water -500ml

The methyl violet dye was dissolved in 50 ml absolute alcohol and mixed thoroughly. Then ammonium oxalate 4.25 g was dissolved in 100 ml of distilled water and this mixture was added to the violet stain and finally distilled water was added to make 500 ml. The total mixture was filtered before use.

Gram's iodine solution consists of the following

Iodine - 25g

KI - 50G

DW - 500ml

Fifty grams of KI was dissolved in 500 ml of water and then 25 grams of iodine was added to that. When iodine is dissolved, the solution was made up to 500ml with distilled water.

Counter stain used in grams stain was dilute carbol fuschin. It consists of the following:

Basic fuschin - 5g

Phenol -25g

Absolute alcohol -50 ml

The basic fuschin powder was added to alcohol at intervals until it was dissolved. Then phenol too was dissolved in distilled water. Both the solution was mixed in a separate container.

CATALASE TEST:

Done by both slide & tube methods.

Tube method:

A small amount of the culture was picked up from the nutrient agar plate with a clean, sterile glass rod and inserted into a tube of 3% hydrogen peroxide; there was no effervescence or bubble formation.

Slide method:

Pure growth of the organism from the agar was transferred to a clean slide with a sterile glass rod. Immediately 2 to 3 drops of 3% hydrogen peroxide was added to the growth, observed for the release of the bubbles.

ANNEXURE 2

MEDIA PREPARATION

1. **Peptone water:**

   Peptone 1 g

   Sodium chloride 0.5 g

   Distilled water 100 ml PH – 7.4

   Sterilise by autoclaving at 121d C for 15 minutes.

2. **Nutrient broth :**

   Peptone water 100ml

   Beef extract 1 g

   Ph 7.4

   Sterilise by autoclaving at 121dC for 15 minutes.

3. **Nutrient agar :**

   To the nutrient broth, add required amount of agar. Steam to dissolve agar, filter, and adjust ph to 7.4. Sterilise by autoclaving at 121dC for 15 min.

4. **Blood agar :**

   To the 100 ml of nutrient agar, in water bath at 50dC, add 5% (5ml) of Sheep blood.

5. **Mac conkey agar**

Peptone	20 g
Sodium chloride	5 g
Sodium taurocholate	5 g
Lactose	10g
Neutral red	10 ml
Agar	15 g
Distilled water	1000 ml

Sterilise by autoclaving at 121dC for 15 minutes.

6. **Muller Hinton media:**

Beef infusion	300 g/l
Casein acid hydrolysate	17.5 g
Starch	1.5 g
Agar	17 g
Distilled water	1000 ml

Sterilise by autoclaving at 121dC for 15 minutes.

ANNEXURE 3

PROFORMA FOR VENTILATOR ASSOCIATED PNEUMONIA

CASE HISTORY:

Name: Age:

Sex

Occupation:

Address:

Duration:

(Pt on ventilator)

Diagnosis:

Risk factors:

1. Chronic lung disease
2. Head trauma
3. Burns
4. Prior antimicrobial therapy
5. Thoracic or abdominal surgery

CLINICAL PULMONARY INFECTION SCORE

Temperature	□ 36.5 &< 38,4 □ 38.5&< 38.9 < 36& > 39	Point- 0 Point- 1 Point- 2
Blood leukocytes	>4000 & < 11000 <4000 & > 11000 >500 band forms	Point- 0 Point- 1 Point- 1
Tracheal secretion	Absence of tracheal secretion Presence of non purulent secretion Presence of purulent secretion	Point- 0 Point- 1 Point- 2
Pao2/ Fio2	>240 or ARDS <240 or ARDS	Point-0 Point- 2
Xray chest	No infiltrate Diffuse infiltrate Localised infiltrate	Point- 0 Point- 1 Point- 2
Progression of pulmonary infiltrate	No radiographic progression Radiographic progression	Point-0 Point-2
Culture& gram stain of tracheal secretion	No pathogenic bacteria Pathogenic bacteria in culture & gram stain	Point-0 Point- 1

CPIS SCORE > 6 Diagnosis of VAP

ANNEXURE 4

LABORATORY REPORT

CULTURE MATERIAL

1. ENDO TRACHEAL ASPIRATE
2. BRONCHO ALVEOLAR LAVAGE
3. BLOOD CULTURE

DAY- 1

GRAM STAIN:

DAY- 2

CULTURE:

NUTRIENT AGAR PLATE:

MAC CONKEY AGAR PLATE:

BLOOD/ CHOCOLATE AGAR PLATE:

DAY- 3

BIO CHEMICAL REACTIONS:

INDOLE-

TSI -

CITRATE-

UREASE-

OTHER SPECIAL TEST:

COAGULASE TEST-

CATALASE TEST-

OXIDASE TEST-

BILE SOLUBILITY-

ANTIBIOTIC SENSITIVITY:

SENSITIVE DRUGS

RESISTANT DRUGS

REPORT:

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