CHAPTER: I

INTRODUCTION

A tracheostomy is surgical procedure that opens wind-pipe and is performed if enough is not getting into lungs. Air may not enter into the lungs due to various reasons for example blockage of wind pipe by swelling by severe injury to the nose, neck, mouth or paralysis of throat muscle or by tumor. The patients may be in coma and require a ventilator to pump air for a period of time (Fagan et al, 1997).

The tracheostomized patients are severely ill and generally immunocompromised so they are highly susceptible to colonization and infection by different bacteria, either endogenous or exogenous route of colonization. The frequency of exogenous colonization is 33% in pediatric unit. The potentially pathogenic includes hospital bacteria such as *Pseudomonas*, *Serratia, Acinetobacter* and mecithillin resistant *Stapylococcus aureus* and Enterobacteria. Apart from these endogenous bacteria like *Streptococcus pneumoniae*, *Bramnehella catarrhalis* and *H. influenzae* also been aspirated and colonize the lower respiratory tract. The external sources may be both in-animated, animate reservoir, ventilator equipment, nebulizer and humidifiers have been implicated in exogenous infection whereas other long-stay patients are in general the animate source of potentially pathogenic microorganisms responsible for the infection of exogenous pathogenesis. Among these organisms the exogenous pathogens are considered to be highly potentially pathogen (Morar et al, 2002).

These colonizing bacteria either causes nosocomial tracheobronchitis (Bacterial count more than 10^6/ ml, CRP more than 15µg/ ml, leucocytosis and fever more than 38^oC) and the broncho pneumonia (above criteria plus abnormal chest x-ray) (Van saene et al, 1996). To ascertain these bacteria, lower respiratory tract secretion (tracheal aspirates) applying is collected applying the negative pressure.

Tracheostomy is one of the various risk factors associated with the ventilator associated pneumonia (Geogres et al, 2001). Tracheostomy accounts for 25% of the total VAP and also increases the mortality rate which is otherwise 30% (Torres et al, 2001).Pneumonia is the sixth leading cause of death in USA and risk increases in patients with immunocompromised patients. As the patients are unconscious, the post tracheostomy and have impaired immune system, infection is really problematic. The infections are mostly super infection, which halts the recovery and increases the hospital stay (Gotsman and Whitby, 1964). This also increases the opportunity of over treating the patients and may lead to emergence of multi-drug resistant bacteria. So understanding of the etiological agents of post tracheostomy infection will give us an advanced knowledge of relevant pathogens which in turn improve the patient care and boost the therapy.

Today, antibiotics remain the front –line therapy for conquering bacterial infections. Antibiotic strategies should encompass the most like causative organism, prevent emergence of resistance and control costs are needed. Unfortunately antimicrobial resistance has escalated dramatically within the past decade and has created obstacles to effective antibiotic choice. These trends are most problematic in intensive care unit. Awareness of the relevant pathogen is critical to successful design of empiric and pathogen directed antibiotic therapies for hospital acquired pathogen and to understand silent risk factor for hospital acquired pathogen via tracheostomy and strategies to decrease mortality and morbidity due to it.

National Institute of Neurological and Allied Sciences was constructed to treat traumatic and severely ill neurological patients and has become successful in saving life of hundreds of such patients. The facilities available at the hospital have contributed to decrease the expenses of abroad treatment. The hospital has a good facility of tracheostomy and well equipped laboratory. Since there are limited data suggesting pathogen colonizing/ infecting in tracheostomy patients and study was carried out to know the burden of organisms and problem associated with tracheostomy.

CHAPTER: II

OBJECTIVE

2.1 General objectives:

To determine etiological agents causing post tracheostomy infection and antibiotic susceptibility pattern of the isolates.

2.2 Specific objectives:

1. To isolate and characterize the bacterial pathogens in post tracheostomy infection.
2. To determine the antibiotic susceptibility pattern of the above isolates.
3. To estimate the burden of the Multi-drug resistant bacteria.

CHAPTER: III

3.0 LITERATURE REVIEW

A tracheotomy is a surgical procedure that opens up the windpipe (trachea). It is performed in emergency situations, in the operating room, or at bedside of critically ill patients. The term tracheostomy is sometimes used interchangeably with tracheotomy. Strictly speaking, however, tracheostomy usually refers to the opening itself while a tracheotomy is the actual operation.

A tracheotomy is performed if enough air is not getting to the lungs, if the person cannot breathe without help, or is having problems with mucus and other secretions getting into the windpipe because of difficulty swallowing. There are many reasons why air cannot get to the lungs. The windpipe may be blocked by a swelling; by a severe injury to the neck, nose, or mouth; by a large foreign object; by paralysis of the throat muscles; or by a tumor. The patient may be in a coma, or need a ventilator to pump air into the lungs for a long period of time (Fagan et al, 1997)

The infection associated with the tracheostomy patients are:

a) Tracheobronchitis

b) Pneumonia

The following definitions were used, in accordance with van Saene et al, 1996.

Infection of the lower airways was defined as a microbiologically proved diagnosis of systemic inflammation. The diagnostic sample obtained from the lower airways yielded greater than or equal to 10^6 CFU/mL of sample, and there were many leukocytes in the lower airway secretions.

Tracheobronchitis was defined as follows:

(a) Purulent endotracheal aspirate (white blood cells +++),

(b) Fever (temperature, ≥38.5°C),

(c) Leukocytosis (white blood cell count, $>12x10^3/\mu L$) or leucopenia (white blood cell count, $<4x10^3/\mu L$),

(d) 10^6 CFU/mL or more of tracheal aspirate, and

(e) Elevated C-reactive protein level more than 15 μg/mL.

Bronchopneumonia was diagnosed by means of the same 5 criteria as above, combined with the presence of a new or progressive pulmonary infiltrate on chest radiograph.

3.1 Anatomy of the parts involved

3.1.1 Trachea

The lower respiratory tract starts at the vocal cords. Inferior to the vocal cords, the rigid cricoid cartilage encases a 1.5–2.0-cm region known as the subglottic space. Access to this space is possible via the cricothyroid ligament, a membrane that runs from the thyroid cartilage inferiorly to the cricoid cartilage. Inferior to cricoid is the trachea, a cylindrical tube that extends inferiorly and slightly posteriorly. The trachea is made up of 18–22 C-shaped rings consisting of rigid cartilage anteriorly and laterally, and a membranous posterior portion. In the average adult, the distance from cricoid to carina is approximately 11 cm in length, with a range of 10–13 cm. On average, the trachea is 2.3 cm in width and 1.8 cm from posterior membrane to the anterior cartilaginous aspect.

The trachea is wider in men than in women. In examining the landmarks of the neck, it is evident that the trachea is protected by strap muscles (sternohyoid, sternothyroid,

sternocleidomastoid) and bony structures (manubrium and sternum). Furthermore, the trachea is positioned posterior to a number of blood vessels and the thyroid isthmus. Branches of the bronchial, inferior thyroid, innominate, and subclavian arteries provide the blood supply to the trachea. Knowledge of neck and tracheal anatomy is essential for understanding the various approaches to establishing a tracheostomy, as an example, surgical tracheostomy tubes are typically placed in the region of the 2nd to 4th tracheal rings and may entail removal of tracheal cartilage or the creation of a cartilaginous flap. Percutaneous tracheostomy tubes are typically placed between the 1st and 2nd or between the 2nd and 3rd tracheal cartilages. The technique takes advantage of the Seldinger method, followed by progressive dilation of the space between tracheal rings to provide access to the trachea. Another approach is to place a tube through the cricothyroid membrane (cricothyroidotomy). Though still used extensively in some centers, in other centers it has been replaced by percutaneous tracheostomy. Cricothyroidotomy can be used to gain emergency access to the airway, but its association with numerous complications has led some to advocate replacing this tube within 48–72 hours with a standard tracheostomy. The procedure is carried out by a transverse incision through the skin and the membrane and then spreading the incision vertically to allow placement of the tube. Because the cricothyroid membrane is bounded by 2 rigid structures (thyroid cartilage and cricoid cartilage) that are not easily dilated, the height of this membrane limits the size of the tube that can be placed. In addition, the curve of a standard adult tracheostomy tube and the close soft tissue distance between anterior skin and trachea (at the level of the cricothyroid membrane) causeschronic inflammatory changes.6 Lack of adequate humidification also leads to desiccation of the tracheal mucosa and reduced ciliary function. Indeed, by these actions and by diminishing effective cough and increasing secretions, tracheostomy tubes predispose to respiratory-tract infection. Furthermore, these tubes also hamper effective swallowing, thereby predisposing to aspiration. (Esptein, 2005)

3.1.2 Bronchi and bronchioles

The trachea has divide for 23 generations to reach an alveolus. It first divides into two primary bronchi – one for each lung. Right principal bronchus is shorter (2.5cm), wider and vertical than left one. Therefore inhaled particles tend to pass more frequently to the right lung, with the result that aspiration pneumonia is more common in the right lung. The left principal bronchus is longer (5cm), narrower and more horizontal than the left one. The bronchus has the same basic structure as the trachea.

Each principal bronchus enters the lung through the hilum, and divides into secondary (lobar) bronchi one for each lobe of the lungs (three on the right and two on the left). Each lobar bronchus divides into tertiary (segmental) bronchi, one for each bronchopulmonary segment (ten on the right side eight on the left side). The segmental bronchi divide repeatedly to form very small branches called terminal bronchioles. Still smaller branches are called respiratory bronchioles (17th generation). Each respiratory bronchiole aerates a smaller part of lung know as pulmonary unit. Respiratory bronchiole ends in microscopic passages which are termed (in the order)

a) Alveolar ducts
b) Atria
c) Air saccules
d) Pulmonary alveoli

The respiratory passage up to 16th generation (i.e. terminal bronchi) does not take part in gaseous exchange but they conduct environmental gas to the site where gaseous exchange takes place. Therefore they are termed as conductive zone of respiratory system. Total capacity of respiratory zone is 150 ml and it constitutes anatomical dead space.

The respiratory passage from 17th generation (i.e. alveolar sac) to 23rd (i.e. alveolar sac) takes part in exchange of gases. Therefore they are termed as respiratory zone of respiratory system. Cartilages are absent in respiratory bronchioles and flattened (simple squamous) at the level of alveoli (Waugh and Grant, 2001).

3.1.3 Lungs

Lungs are a pair of conical organs situated in thoracic cavity. Each lung invaginates the corresponding pleural cavity. Right and left lungs are separated by mediastinum. Lungs are spongy in texture. In the young, lungs are brown or grey in colors. Right lungs weigh 70g. and left lungs weight 600 gm. Each lungs has

a) An apex which is blunt and extends to root to neck
b) A base which rests on the diaphragm.

The right lung is divided into 3 lobes by two fissures-oblique and horizontal.

a) Upper lobe: It has 3 lobules or segments.
b) Middle lobe: It has 2 lobules or segments.
c) Lower lobe: It has 5 lobules or segments.

The left has only 2 lobes (upper and lower) divided by an oblique fissure.

a) Upper lobe: It has cardiac and a tongue shaped projection below it known as lingula, which corresponds to middle lobe of right lung. It has 4 lobules.
b) Lower lobe: It also has 4 lobules or segments (Waugh and Grant, 2001)

3.1.4 Bronchopulmonary segment

Each bronchopulmonary segment is structurally and functionally independent unit of lung. It is aerated by segmental bronchus and supplied by segmental artery which runs together. Pulmonary vein from peripheral venous plexus; therefore one bronchopulmonary Segment contain more than one vein. Surgeon has to work along pulmonary veins while going for segmental resection. There are 10 bronchopulmonary segments in right lung and 8 bronchopulmonary segments in the left side (Waugh and Grant, 2001)

3.1.5 Blood supply: the lungs have double blood supply.

a) The pulmonary circulation: It is for gas exchange with the alveoli. Each pulmonary artery brings deoxygenated to lung and resultant oxygenated blood is returned back to heart by two pulmonary veins from each lung (total pulmonary veins are four). The pulmonary circulation is a low pressure (25/10mmHg), low resistance system with a capacity to accommodate a substantial increase in blood flowing through it without a major increase in pressure. Vascular distention and recruitment of unperfused capillaries achieve this. The main stimulus which produces a marked increase in pulmonary vascular resistance is hypoxia.

b) The bronchial circulation: it is for supplying the parenchyma of the lung itself (mainly conductive zone). Most of the blood from the bronchial circulation drains into the left side of the heart via the pulmonary veins and this deoxygenated blood makes up part of the normal physiological shunt present in the body (Waugh and Grant, 2001)

3.1.6 Pleura

The pleura are a double layered serous membrane surrounding the lungs. The inner visceral pleura envelope the lung itself and the outer parietal pleura line the thoracic cavity. The

visceral pleura are pain insensitive while parietal pleura are pain sensitive. It is lined with simple squamous epithelium. It is a mesothelium. Under normal circumstances the interpleural space between these layers contains only a tiny amount (about 2ml) of lubricating called pleural fluid which facilitates its smooth sliding between the two layers (Waugh and Grant, 2001).

3.1.7 Respiratory epithelium

It is the characteristic epithelium of the upper respiratory tract, trachea and large bronchi. It is typified by pseudostratified columnar, ciliated epithelium with mucus secreting goblet cells. As brochus progressively decrease in size, there is a gradual transition to ciliated simple columnar and finally cuboidal epithelium. There is also a gradual decrease in number of goblet cells (Waugh and Grant, 2009)

3.2 Physiological changes after tracheostomy

By passing the nasal airway, disturb the normal humidification and warming of inspired air therefore air must be humidified using heated humidifiers or heat and moisture exchanger. In the absence of adequate humidification, the trachea develops sqamous metaplasia and chromic inflammatory changes. Lack of adequate humidification also leads to desiccation of tracheal mucosa and reduced ciliary function. In deed by this and by diminishing effective cough and increasing secretions tracheostomy tubes predisposes to respiratory tract infection. Furthermore, these tubes also hamper effective swallowing thereby tracheostomy tubes predisposes to aspiration.

Similarly tracheostomy also affects the airflow resistance. Air flow resistance of the normal airway is substantial, constituting upto 80% of total airway resistance during nose breathing and 50% during mouth breathing. Theoretically, tracheostomy tubes should decrease airflow resistance but in fact this does not occur because of the radius (inner diameter 7-

8mm) of the tubes. Tracheostomy tubes may reduce dead space by upto 100ml when compare to spontaneous breathing.This occurs because the tubes are small and bypass the subglottic and supraglottic space. (Epstein, 2005)

3.3 Host Defences

The study under our consideration excludes the upper respiratory tract. The lower respiratory tract is generally sterile; however, the continuous suctioning and other related procedure facilitate the entrance of potential pathogens. The different mechanisms to prevent the establishment of infection are;

3.3.1 Mucocilliary escalator

The airways are protected by humidification all the way to the alveoli with a layer, which prevents dehydration of the epithelium and surrounds the epithelial cilia. The airway mucous consists of polysaccharides from goblet cells and from mucous gland in the bronchial wall.

Each ciliated cell contains about 200 cilia that beat in coordinated waves 100 times per minutes, with a fast forward stroke and a slower backward recovery. Ciliary motion is also coordinated between adjacent cells so that each wave is propagated toward the oropharynx. The cilia are covered by a liquid film that is about 5 to 10 micrometer thick and is composed of two layers. The outer, or gel layer is viscous and traps deposited particles. The cilia beat in the less viscous inner of sol layer. During the forward stroke, the tips of the cilia just touch the viscous gel and propel it toward the oropharynsx. During recovery, the cilia move entirely within the lower resistance sol layer. The cilia continuously move the gelatinous blanket with inhaled particles on the top upward towards the pharynx, where they are swallowed.

Clearance of the respiratory bronchioles may take days, whereas clearance of the main bronchi is typically accomplished within hour (Read and Sinch, 1998)

3.3.2 Non specific antimicrobial agents

Mucus contains many factors that enhance the clearance of micro-organisms from the airway: most of these are plasma proteins and include α1 antitrypsin, a low molecular chymotrypsin inhibitor; Neutral protease and elastase are secreted by polymorphonuclear leucocytes and alveolar macrophages. Alpha 1 Antitrypsin deficiency is associated with chronic pulmonary disease, probably because of failure to inhibit the neutrophil products during recurrent inflammatory response to lung infection.

Lysozyme is secreted by neutrophils and found in most mucosal secretion, where it is directly active against most bacteria. Lactoferrin is secreted by mucosal epithelium and neutrophils. Like transferring it has affinity for iron and inhibits the bacterial replication within the airway by restricting iron availability.

Surfactant is secreted by pneumocytes within alveoli and has the vital function of reducing surface tension in gas exchange areas and keeping this patent. It also contains non immune opsonin, notably fibrinolectin, produced locally by alveolar macrophages it binds to some bacteria which are then recognized by receptors on the macrophages surface (Read and Sinch, 1998).

3.3.3 **Immunoglobulins**

Immunogolbins IgA, IgG, IgM and IgE including specific antibody can be recovered from respiratory tract secretion (Lipscomb and Bice, 1995). IgM is found only in lung in only very low concentration. Secretory IgA is synthesized by lymphoid cells beneath the basement membrane, predominantly in the upper and middle part of respiratory tract.

Whereas IgA is the predominant immunoglobulin in the upper airways, lower airways secretions more resembled serum in that the IgG to IgA ration is much higher. The major function of sIgA is complement –independent neutralization of respiratory viruses. It also opsonize organism for phagocytosis by macrophages but does hot contribute to polymorph opsonization; nor it activates complements.secretory IgA inreracts with lysozyme and complement to augment their bacrericidal activity, mediates bacterial adherence to mucus and inhibits bacterial adherence to epithelial surfaces. Once challenged by a given agent, the host produces sIgA in reponse to homologous challenge. However, the period of immune protection is relatively short in contrast to serum IgA and booster response is variable after later challenge. Certain bacteria, notably *streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitides*, secrete IgA protease capable of inactivating mucosal IgA. IgA gains access to the airways by transduction from the circulation but it can be produced locally in the lung. It effectively agglutinates bacteria, mediates bacterial opsonization for macrophages and neutrophils, neutralizes bacterial exotoxins and activates complement. After a primary challenge, specific antibody is released into blood from lung associated lymph nodes and transudes into the airway. Subsequently, memory B cell traffic to the lung and produce specific IgG in response to further specific challenge (Bice et al, 1991).

3.3.4 Recruited defences

If the combined effect of mucocilliary clearance complement and macrophages phagocyrosis fails to clear an inoculum of bacteria, or if the infecting organism is particularly virulent, for example the type 3 pneumococcus, polymorphonuclear neutrophils and lymphocytes are recruited to augment the host response. Neutrophils migrate from the vascular compartment into the alveolus by chemotaxis. The stimulus for chemotaxis originates within the alveolus and is due to direct generation of chemotactic factors by micro-organisms entering the alveolus and is due to direct entering the alveoli, and the

releasing of chemotactic factors from alveolar macrophages after phagocytosis. Leukotriene B4 an important chemotactic factor, that also alters pulmonary capillary permeability. This together with the secretion of TNF-α by macrophages, promotes the accumulation of neutrophils and fluid and other humoral substances in alveoli. Neutrophils kill ingested bacteria very much faster than macrophages with a combination of oxidative metavolites. This process, together with injury due to proteolytic enzymes neutrophils elastase, results in consolidation of the lung.

Bronchoalveolar lavage fluid from the normal resting human lung contains predominantly macrophages but also lymphocytes (20%) including CD4 (helper), CD8 (suppressor) and a few B lymphocytes (Reynolds, 1994). There is a complex interplay between lymphocytes, macrophages and neutrophils, in the management and termination of inflammation. Lymphocytes can regulate the activation of macrophages and subsequently co-ordinate the inflammatory response in a given infection.

Macrophages infected with organisms capable of intracellular survival, such as *Mycobacterium tuberculosis, Pneumocystis carinii*, *Legionella pneumophila*, requires cell mediated immunity to eradicate the infection

At the time of primary challenge, antigen is transport to lung associated lymph nodes either contained within alveolar and dendritic macrophages or recruited neutrophil, or free in the afferent lymphatic fluid (Lipscomb and Bice, 1995). In the presence of lung inflammation, this process is accelerated. Within lymph nodes, antigen is reprocessed by antigen presenting cells. Specific T and B cell clones expand and differentiate, and effector T cells and B lymphoblast migrates out (maximally by 10-14 days), the latter to provide specific antibody locally in lung tissue. Subsequently responses to the same infecting organism are produced by immune memory B cells lodged within the lung parenchyma (Read and Sinch, 1998).

3.4 Pathogenesis

For respiratory infection to occur, at least one of three conditions must be present: host defenses must be impaired, innoculum organisms of sufficient number must reach the patient's lower respiratory tract and overwhelm the host's defenses, or a highly virulent organism must be present.

3.4.1 Routes of Bacterial Entry

Entry into the lungs may occur by various routes, including microaspiration of oropharyngeal secretions colonized with pathogenic bacteria, aspiration of esophageal/gastric contents, inhalation of an infected aerosol, hematogenous spread from a distant site of infection, exogenous penetration from an infected site (in, pleural space), direct inoculation into the airways of intubated patients from ICU personnel or, questionably, translocation from the gastrointestinal tract. Not all routes are equally effective means of entry for any given pathogen. Of the potential routes of entry into the lower respiratory tract, microaspiration of a small volume of oropharyngeal secretions previously colonized with pathogenic bacteria is the most common (Craven and Dricks, 1987). In one study, oropharyngeal colonization by enteric gram-negative bacilli (EGNB) was relatively rare (< 10%) or of short duration among healthy, nonhospitalized individuals, but as patients developed more severe systemic illness, the incidence of oropharyngeal colonization by EGNB increased to 35% in moderately ill patients and to 75% among critically ill patients (Johanson et al, 1969).So not surprisingly, upper airway colonization and pneumonia frequently coexist. Gross aspiration of large volumes of material as a cause of HAP is less common, but when it occurs it can include both oropharyngeal and esophageal/gastric contents. The incidence of aspiration increases when the gag reflex is impaired, if there is an alteration in the patient's level of consciousness,

when certain devices such as nasogastric or endotracheal tubes are used, or if esophageal disease is present (Huxley et al, 1978).

.Among mechanically ventilated patients, additional routes of entry exist. The endotracheal tube bypasses host defenses above the vocal cords and impairs lower respiratory tract defenses such as cough and mucociliary clearance. Contaminated secretions can pool above the inflated endotracheal tube cuff and are not easily removed by suctioning. These secretions can leak around the endotracheal tube cuff and directly enter the lower respiratory tract when there are changes in airway caliber during swallowing and breathing. In addition, if medical staff or respiratory therapy equipment harbor pathogenic flora, bacteria can be directly inoculated into the tracheobronchial tree. For example, *Pseudomonas* species are known to colonize the tracheobronchial tree without first appearing in the oropharyngeal secretions of intubated patients, presumably entering the lung via direct inoculation (Niederman et al, 1989). These factors increase the incidence of HAP in mechanically ventilated individuals and can account for differences in the spectrum of potential pathogens (especially the prominence of *P.aeruginosa* and *Acinetobacter* spp.) in this population when compared with other patients who

3.4.2 How Specific Risk Factors Lead to Pneumonia

The risk factors for respiratory tract colonization and HAP have considerable overlap and include patient-related conditions, infection control-related problems, and intervention-related alterations in host defenses or bacterial exposure.

3.4.2.1 Infection control-related factors

Hospitalized patients commonly are exposed to potentially large inocula of bacteria from a number of sources. Poor infection control practices can lead to the transmission of hospital-acquired pathogens by the hands of medical personnel. This can occur by either not

washing hands or not changing gloves between patients, or through the use of contaminated respiratory therapy devices and equipment (Maki et al, 1978). Respiratory therapy devices can deliver large numbers of bacteria to the lung if contaminated condensate in the mechanical ventilator tubing washes back to the patient. The gastrointestinal tract also may be a source of large quantities of EGNB that can enter the tracheobronchial tree. Although investigators disagree whether gastric overgrowth leads to respiratory tract colonization and pneumonia, colonization at one of the two sites often predicts nearly simultaneous colonization at the other site (Craven et al, 1991).

3.4.2.2 Intervention-related factors

A number of procedures and therapies can lead to both host defense impairment and increased exposure to large inocula of bacteria. Certain therapeutic agents, particularly sedatives, can suppress CNS function and lead to an increased incidence of aspiration. Corticosteriods and cytotoxic agents impair a number of vital host defensive functions. Prolonged or complicated surgery, especially thoraco-abdominal procedures, is associated with a number of changes in mucociliary function and cellular host defenses that lead to increased rates of oropharyngeal colonization and pneumonia. Similarly, endotracheal tubes can impair mucociliary and mechanical clearance from the lower airways, as well as injure the epithelial surface and predispose to increased bacterial binding to the surface of the lower respiratory tract. Many therapeutic interventions increase the exposure of hospitalized patients to large bacterial inocula. For example, prolonged and inappropriate use of antibiotics may increase colonization by antibiotic-resistant bacteria, including potentially virulent gram-negative bacilli (Weinstein et al; 1991). Antacids and histamine type 2 (H-2) blockers are commonly used for prophylaxis against stress gastritis and ulceration but may increase the frequency of gastric colonization by EGNB, and possibly the incidence of pneumonia (Craven et al, 1991). Also, enteral feedings via a nasogastric tube can result in increased gastric volume, reflux, and gram-negative bacterial overgrowth

in the stomach (Jacobs et al, 1990). Nasogastric tubes themselves probably impair the function of the lower esophageal sphincter, thereby promoting aspiration and bacterial contamination of the tracheobronchial tree. The effect of all these manipulations is augmented if patients are maintained in the supine position, because this position increases the rate of reflux of gastric contents into the lung (Torres et al, 1992). The endotracheal tube not only interferes with host defenses but also can become encrusted with a bacterial biofilm that may embolize to the lung (Inglis et al, 1989). Also, contaminated secretions can pool above the inflated endotracheal tube cuff and may leak around the endotracheal cuff, directly entering the lower respiratory tract develop HAP.

3.4.3 Adherence to respiratory surface

In studies of oropharyngeal and tracheobronchial colonization investigator have found that the epithelium cells of colonized patient allow pathogenic Gram negative bacteria to bind more avidly than epithelial cells than non colonizing do.(Niedermam et al,1984)

Bacterial adherence involves the interaction between adhesion between adhesion on a bacterial surface and receptors on epithelial surface. In the tracheobronchial tree receptors for Gram negative bacteria and *Haemophlilus influenzae* are present on the epithelial cells and in respiratory mucus.

Using non mucoid *Pseudomonas* as bacterial prove, it has been demonstrated that tracheal cells are able to bind to the organism more avidly than buccal cells can(defining a tropism, or preference of *Pseudomonas aeruginosa* for the lower respiratory tract) and that the tracheal cells from tracheostomized patient bind more bacteria from normal individuals do (Niederman et al,1984).

In addition to species and strain specific variability, in adhesion composition other variable can affect adherence, including the presence of pili or alginate and ability of bacteria to

produce exopolysaccharide, that interferes the mucociliary clearance or injure the epithelial surface itself.

Mechanical injury to the tracheal surface (as could occur from endotracheal intubation and suctioning) may expose binding site for *Pseudomonas aeruginosa* (Yamaguchi and Yamada, 1991) and the bio-film inside the endotracheal tube itself may be a surface to which bacteria bind avidly (Inglis et al, 1989).

Once the tracheobronchial surface is injured, the epithelium can repair itself and in doing so novel tracheobronchial cell surface carbohydrate may be produced. This reparative change allowed for enhanced binding of binding of *Pseudomonas aeruginosa* (Plotkowski et al, 1991).

3.5 Invasion and Inflammation

One of the two mechanisms by which bacteria cause disease is invasion of tissue followed by inflammation. Several enzymes secreted by invasive bacteria play a role in pathogenesis.

- Collagenase and Hyaluronidase: They degrade collagen and hyaluronic acid repectibely therby allowing the bacteria to spread through subcutaneous tissue and helping in rapid dissemination: e.g. *S. pyogenes.*
- Coagulase: It is produced by *S. aureus* and accelerates the formation of fibrin clot from its precursors, fibrinogen.
- Immunoglobulin A protease: It degrades IgA, allowing the organism to adhere to mucous membrane and is produced chiefly by *Haemophilus influenzae* and *S. pyogenes.*

- Leucocidins: These are the substances secreted by several bacteria which can destroy both leukocytes and macrophages (Collee et al, 1997)

In addition to these enzymes, **several virulence factors** contribute to invasiveness by limiting the ability of the host defence menhanism, especially phagocytosis to operate effectively.

- Capsule production*: S. pneumoniae, H. influenzae, B. antracis, K. pneumoniae*.
- Replication inside cells: Viruses and *Chlamydia* spp are obligate intracellular parasite that replicate inside the cells of the lung avoiding the phagocyte.
- Being too large to phagocytose; Parasites and fungi are often too large for phagocyte to engulf.
- Yops: Outer membranes proteins are produced by several *Yersinia* spp are important virulence factors. The most important effects of the Yops proteins are to inhibit phagocytosis by neutrolphil and macrophages and to inhibit the cytokine production e.g. Tumor necrosis factor by macrophages.
- M protein: It is present in cell wall of Group A Streptococci (*S pyogenes*).
- Mimicry: Some parasites produce surface proteins which are very similar to host protein or acquired host proteins and appear to the phagocyte as self. Some bacteria produce proteins that cause host proteins to bind to their surfaces (e.g. protein A of *Staphylcoccus aureus* binds to IgG and prevents the activation of complement).
- Some other bacteria also produce toxins: Cytotoxins and Leucocidins are produced by *S. aureus*. Similarly *Pseudomonas aeruginosa* produces exotoxin A. These exotoxins are secreted by the bacterium into the extracellular space, bur others are transferred by type III secretion sys tem from the bacterium directly into the adjacent human cell. This secretion system is mediated by transport pumps in the bacterial cell membrane and provides the major advantage of allowing the toxins to

avoid neutralizing antibodies located in the extra-cellular space. The importance of this system is illustrated by the finding that the strains of *Pseudomonas aeruginosa* that have this secretion system are significantly more virulent than those that does not (Forbes et al, 2007)

3.6 Disease development

Once a microorganism arrives in the alveoli, it can be opsonized by IgG in the fluid lining the alveoli. These organisms will then be ingested by the macrophages. If no specific antibody is present to the organism, the macrophages may still be able to phagocytose the invader, however, at a slower rate. Once the microorganism is phagocytosed, the microphages will destroy the organism if it can, and present microbial antigens on the surface to awaiting B and T cells. Once activated the B and T cells can produce more antibody and/or activate the macrophage. Meanwhile the macrophages are also releasing factors that help bring in polymorphonuclear (PMN) cells from the blood stream and initiate an inflammatory response. Along with the PMNs, more antibodies and complement components also come that are useful in destroying the invader. The invaders can also leave the lung and get into the general circulation.

Deleterious effects on the host fall into two categories:

Systemic effects such as fever shock (particularly associated with gram negative bacteria) and wasting (chronic tuberculosis).

Inference with the ability of the lungs to carry out air exchange; This can be due to thickening of the membrane that separates erythrocytes from inspired air in the alveoli, and inflammation in the alveoli resulting in regional mismatch in the ventilation and perfusion of the lungs. The consolidated alveoli are perfused but not ventilated because they are filled with inflammatory exudates.

3.7 Infection followed by tracheostomy

Little attention has been paid to respiratory infection after tracheostomy. Nelson and Bowers, (1957) reviewed 310 cases of tracheostomy but did not mention pulmonary infection as a complication. Other large series mention infection, but the incidence and bacteriological characteristics of the infection received no attention. Atkins, (1960) reviewed 526 cases, and a table in his paper revealed that 129 of these cases came to necropsy. Pneumonia was found in 99 (76%) but there is no comment upon this in the text. Head, (1961) in another exhaustive series of patients from the Massachussets General Hospital, described infection as a late complication in 80 out of 462 cases; the nature of the infection was not classified and any manifestations from superficial sepsis to pulmonary infection were included in these figures. The July number of Anesthesiology 1962 is devoted to a symposium on inhalation therapy, but while tracheostomy was discussed in detail, infectious complications were not considered.

By contrast Lepper et al, (1954) described the bacteriological findings in 72 poliomyelitis cases where respiratory paralysis was treated with tracheostomy. Pulmonary infection occurred in every case and the causative organisms were wide but included *Stretococcus. pyogenes, Pseudomonas* sp., and *Proteus* sp. It is not clear whether the infected cases represented all the poliomyelitis cases with tracheostomy in one hospital, or only those who developed respiratory infection, but the evidence for repeated infection with change in the organism responsible is formidable and illustrates the ease with which infection occurs in these patients. In two other small but intensively studied series (Davidson 1959; Nisbet and Wilson 1958) the bacteriology of infection is discussed since respiratory infection was the indication for tracheostomy; the incidence of post-operative respiratory infection is difficult to assess. Smythe and Bull (1961); Cole, (1959) found that some patients who had tetanus and who were treated by tracheostomy developed a fatal pneumonia: but they give no bacteriological data.

In an account of the management of tracheostomy, Frew, (1961) made the point that tracheostomy patients are readily cross-infected with organisms in the hospital environment and that nursing of tracheostomy patients in individual cubicles should lead to a reduction in the incidence of infection. He gives no illustrative cases and no specific information on the causal organisms.

Acute bronchitis or tracheitis or bronchopneumonia is regarded respiratory infection followed by tracheostomy (Gotsman and Whitby, 1964).

3.7.1 Pneumonia

Definition: Inflammatory exudative consolidation of the lung parenchyma and involving the airways distal to terminal bronchioles caused by various microorganism including bacterial species, Mycoplasma, Chlamydiae, Rickettsiae, Viruses, Fungi and parasites. Pneumonitis is occasionally used as a synonym for pneumonia, particularly when an inflammation of the lung has resulted from a non-infectious cause, such as chemical or radiation injury (Crofton and Douglas, 2000).

Bacterial pneumonia has gross pattern of anatomic distribution: The lobarbroncho pneumonia and lobar pneumonia. Patchy consolidation of the lung is the dominant characteristic of lobarbroncho pneumonia. Lobar pneumonia is an acute bacterial infection resulting in a lobe or entire lobe.These anatomic but still classical categorization is often difficult to apply in individual case because patterns overlap. The patchy involvement may became confluent, producing constantly total lobar consolidation, in contrast effective antibiotic therapy for any form of pneumonia may limit involvement to a substantial consolidation. Moreover the same organism may produce bronchopneumonia in one patient whereas in more vulnerable individual, a full blown lobar involvement develops (Robbin, 1997).

Classification of pneumonia: In the past a distinction was made between typical and atypical pneumonia (typical being those caused by common pathogens and atypical referring to those caused by *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, or *Legionella pneumophila*). It was thought that the clinical presentations were different and could help identify the etiological organism but such a classification has not been proven to be useful.

No categorization of pneumonia is satisfactory but for descriptive purpose the classification both anatomical and causal. As the setting in which a pneumonia develops has such major implications for the likely organisms and hence dictates the immediate choice of antibiotics, pneumonia are now classified as

a) Community acquired pneumonia
b) Aspiration pneumonia
c) Pneumonia occurring in immunocompromised host
d) Hospital acquired pneumonia

3.7.1.1Community acquired pneumonia

Definition: Community acquired pneumonia refers to pneumonia acquired outside of hospitals or extended-care facilities. The community acquired pneumonia is mainly caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Community acquired pathogens are less likely to establish the infections in patients with tracheostomy.

3.7.1.2 .Aspiration pneumonia

Aspiration pneumonia occurs in markedly debilitated patients or those who aspirate gastric contents either while unconscious (e.g. after stroke) or during repeated vomiting. These

patients have abnormal gag and swallowing reflexes that predisposes to aspiration. The resultant pneumonia is partly chemical owing to the extremely irritating effects of the gastric acid and partly bacteria (from the oral flora) .Typically more than one organisms recovered on culture, aerobes being more common than anaerobes. This type is often necrotizing type, a fulminate clinical course and is a frequent cause of death. In those who survive lung abscess formation is common complication (Robbin, 1997).

3.7.1.3 Hospital acquired pneumonia

Definition: A consensus statement published by American Thoracic society in 1995 defines hospital acquired pneumonia (HAP) as a pneumonia that is not incubating at the time of hospital admission and begins more than 48 hours after admission.

Epidemiological factors and Microbial pathogens; It is the second most common nosocomial infection after urinary tract infections and has the highest fatality rate amongst nosocomial infection. Hospital acquired pneumonia (HAP) accounts for 15%of all nosocomial infections and affecrs 0.5-2.0% of hospitalized patients (Campbell et al, 1996). The highest rates are seen in the intensive care units (ICU) where the rate are 15% -20%, particularly in intubated patients on mechanical ventilation (Vincent and Bihari, 1995)

3.8 Samples for microbiological testing

Tracheal swab, the excretion from tracheobronchial tree is the sample for the diagonosis of infection. This sample is almost similar to the sputum.

3.9 Microbiological examination of samples

The importance of sputum examination was first documented by Hippocrates in the fifth century B.C. His observation of the sputum sample included, color, taste and smell for

diagnostic criteria in the treatment of patients. Today, good agreement has been noted between macroscopic type and cell counts, providing a scientific basis for the subjective assessment.

In pneumococcal pneumonia, the character of the sputum varies with the stage of the disease. In the early stage of typical lobar pneumonia, the sputum is scanty and transparent, with occasional blood flakes. As the disease progress to the red hepatization stage, the sputum becomes rust red, very tenacious and mucopurulent.

Staphylococcus pneumonias, yellow, purulent, voluminous sputum is present. On Gram staining, a large numbers of Staphylococcus in grapelike clusters and neutrophils are present.

3.10 Direct examination of stained samples

Examination of the sputum remains the mainstay of the evalution of a patient with acute bacterial pneumonia. Unlike the expectorated sputum the tracheal aspirates are less likely to be contaminated with the bacteria that colonize the upper respiratory tract but may contains mixture of many colonizing bacteria. This reduces the diagnostic specificity of any suctioned material; however, as the trachea is sterile and colonizing bacteria are the potential pathogens so detection of each and every bacterium is equally important (Forbes et al, 2007)

3.11 Detection of antigen

Detection of antigen is another direct test which gives rapid results. Pneumococcal antigen can be detected by either latex agglutination or counter-counter immunoelectrophoresis. It has been suggested that the presence of pneumococcal antigen in sputum, as detected by

latex agglutination in patients with pneumonia, is highly predictive of a pneumococcal aetiology.

Legionella antigen testing of urine is both sensitive and specific for legionellosis. However, at present the test only detects cases caused by serogroup I, the most common pathogen. Cryptococcal antigen testing of serum has high sensitivity and specificity for Cryptococcal disease and therefore is a useful test for investigating possible pulmonary cryptococcosis (Vandepitte et al, 2003).

3.12 Culture

As the trachea, lower respiratory tract is sterile and suctioning of sample through the trachea is less likely to be contaminated by upper respiratory tract, isolation and identification of each and every organism is very important. After initial processing and microscopic examination the specimen is cultured in chocolate agar, blood agar, and Macconkey agar.

When culturing the specimen for the possible pathogen, two methods can be used. The first and most popular is the classic technique of streaking on an agar plate, In our laboratory, each specimen is routinely plated in chocolate agar, blood agar, and Macconkey agar.

Choclate agar plate is added with the 10U Bacitracin and 5 mcg Optochin in primary and secondary inoculums respectively. Then plates are incubated at $37^{o}C$ overnight with the chocolate agar plates in a 5% CO_2 atmosphere.

The hypothesis underlying the streaking method is that the pathogenic organism will be present in greater numbers than any other superficial contaminating organisms. Specific identification of all pathogen is performed by standard methods. The culture should be correlated with the previous Gram stain. If many organisms are seen on Gram stain, but no

growth or scanty growth was obtained on culture, then either the culture method was inadequate or the flora was inadequate or the flora was suppressed by antibiotics. Moreover, anaerobes require more special attention.

3.13 Identification of isolated organism

Standard biochemical tests are performed for proper identification of causal organism. In cases of with difficult diagnosis, serological tests can be performed.

3.14 Serological tests

Pulmonary infection by a range of respiratory pathogens can be predicted late in infection or retrospective by a range of serological tests. The presence of IgM antibodies is predictive of recent or active infection. This test has been useful in testing for *Mycoplasma* cytomegalovirus, *Legionella* and Chlymydail infection. The usual approach to making a serological diagnosis is the measurement of a fourfold rise in antibody levels between and acute and convalescent serum sample. Spp. Serological tests for a number for a number of infections, such as histoplamosis, coccidiodimycosis infection have been used. In these infections, the presence of antibody suggests the presence of active infection by these organisms (Vandepitte et al, 2003)

3.15 Detection of specific DNA sequences

The amplication of species-specific DNA sequences using the polymerase chain reaction offers an attractive new approach to detecting the presence of a pathogen. Therefore when used as a test of respiratory infection, its potential is limited to infections caused by organisms which are not associated with colonization of the upper respiratory tract. Also, slow growing or non cultivable organisms may be detected more rapidly with the use of this test. Many studies are under way evaluation this technique in the diagnosis of

tuberculosis and pneumonia caused by *Pneumocystis carinii, Mycoplasma pneumonia*, and *Legionella pneumophila*. Many often respiratory pathogens are suitable for detection using this approach. However, it may be many years before the polymerase chain reaction or other amplication technique are routinely and widely used in the diagnosis of respiratory infectious disease (Carroll, 2002).

3.16 Antibiotic sensitivity test (Modified Kirby-Bauer Disk Diffusion Method)

The disk diffusion method (Kirby-Bauer) is more suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics. An agar plate is uniformly inoculated with the test organism and a paper disk impregnated with a fixed concentration of an antibiotic is placed on the agar surface. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentration. The diameter of the zone is a function of the amount of drug in the disk and susceptibility of the microorganism. This test must be rigorously standardized since zone is also dependent on size of inoculum, composition of medium, temperature of incubation, degree of moisture and thickness of the agar. If these conditions are uniform, reproducible tests can be obtained and zone diameter is only a function of the susceptibility of the test organism.

Zone diameter can be correlated with susceptibility as measured by the dilution method. Further correlations using zone diameter allow the destination of an organism as “susceptible”, “intermediate” or “resistance” to concentration of an antibiotic which can be attained in the blood or other body fluids of patients requiring chemotherapy (Bauer et al, 1966).

3.17 Antibiotic resistance

3.17.1 Multi Drug Resistance (MDR)

The emergence of resistance to antimicrobial agents is a global public health problem. During succeeding decades, the introduction of numerous antimicrobial agents was followed, after varying intervals, by the emergences of resistance among many bacterial species. Resistance was usually noted first isolates from patients in the hospital, an environment characterized by heavy antimicrobial use and proximity of patients that favors cross contamination. Resistance to antibiotic may develop by means or chromosomal mutation, by the acquisition of a transferable resistance plasmid or transposom, by the capture bay an integron of antibiotic- resistance genes that are parts of discrete mobile cassettes, or possibly by inter-specific genetic transformation, as when the DNA of closely related Streptococcal species generated the mosaic penicillin-binding protein genes were found in nature before the introduction of antibiotics. Extensive use of antimicrobial agents favours the resistant strains or species by eliminating more susceptible competitors. Antibacterial drugs are powerful weapons when used reasonably against infection targets, but when they are imprudently prescribed for nonspecific symptoms or infection that is probably viral; their use may only contribute to bacterial resistance. Defining criterion for MDR in our study was resistance to ≥ 3 of the antimicrobial agents.

CHAPTER: IV

MATERIALS AND METHODS

4.1 Materials

Equipments

1. Microscope Binocular (Olympus, Japan)
2. Autoclave
3. Hot air oven (Chitarash, India)
4. Incubator (Associated Scientific Technologies, India)

Reagents and Media

1. Media (MA, BA, CA, NA, NB, MHA and biochemical media: Simmon citrate agar, Triple sugar iron agar, Sulphide Indole Motility Media, Urea agar)
2. Gram' staining reagents
3. 3% H_2O_2

(Details in Appendix II)

4.2 Methodology

A descriptive study was done to determine the bacterial etiology of Tracheal aspirates. This study was carried out in bacteriology laboratory, Institute of National Neurological and Allied Sciences during April 2008 to February 2009.

4.2.1 Specimen collection

The samples were collected in mucus trapper by concerned physician, applying a negative pressure through the automated machine. For most examination early morning samples were collected in sterile, disposable, impermeable container of 25ml capacity fitted with tight cap. Each sample was clearly labeled with patient's name, age, sex and sample type and in the mean while patient's name, age, sex, bed number, time of collection was noted in investigation form received together with sample.

4.2.2 Inclusion criteria

All the tracheal aspirates collected in mucus trapper and properly labeled were included in study. Patients of any age and sex, but tracheostomized were included in this study.

4.2.3 Exclusion criteria

Samples that were not collected in mucus trapper, not properly labeled were excluded

4.2.4 Transport

After receiving the sample in the sample collection site, it was immediately transported to bacteriology laboratory for further processing.

4.3 Processing of specimen

4.3.1 Macroscopic Examination

All received specimens were visually observed for color, consistency, presence of blood and pus.

4.3.2 Microscopic examination of tracheal aspirates (swab)

Gram's staining of all received samples were prepared and observed under microscope for bacterial morphology to distinguish between gram positive and gram negative bacteria as well as presence of pus cell (Forbes et al,2007).

4.3.3 Culture of specimen

The specimen was inoculated in Macconkey agar, blood agar and chocolate agar plate. In chocolate agar plate 10U Bacitracin disk and 5mcg Optochin disk were placed in primary and secondary zone respectively to screen out *Haemophilus Influenzae* and *Streptococcous pneumoniae* respectively. Blood and chocolate agar plates were incubated at 37^{o}C for 24 hours in candle jar. Macconkey agar plates on the other hand were incubated in aerobic condition for 24 hours at 37^{o}C.

4.3.4 Identification of isolated organisms

After 24 hours of incubation, visual growth was observed for colony morphology and the isolated colonies were identified on the basis of morphology and biochemical tests: Oxidase test, catalase test, Indole test, Citrate utilization test, Urea hydrolysis tests and TSI agar tests, coagulase test along with selective screening with antibiotic 10U bacitracin and

5 mcg optochin, Satellitism test and Bile solubility test (Forbes et al, 2007; Collee et al, 1996). (Details in Appindix III)

4.3.5 Antibiotics Susceptibility testing

The antibiotic susceptibility testing was done by following NCCLS (National committee for Laboratory standard) recommended modified Kirby-Bauer disc diffusion method. Sensitivity testing was done for all the isolates (Bauer et al, 1966).(Detail in appendix II B).

4.4 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical test was pure culture and also to see whether the biochemical tests were preceded in an aseptic condition or not. So while performing biochemical tests, the same inoculum was sub-cultured in respective medium and incubated. Pure growth of organisms both in pre inoculation and post inoculation portion of the medium was considered as the indication of aseptic condition.

4.5 Quality control for test

Quality of reagents, chemicals, and media and protocols were maintained and recorded as per standard norms (Vandepitte et al, 2003)

CHAPTER: V

RESULT

5.1 Distribution of sample and patients

Samples from 50 different patients were received during April 2008 to February 2009.

Table: 5.1 Demographic distributions of the cases

1.Age at the time of the tracheostomy		2. Sex		3 Diagnostic No.	
Median	40			Trauma (RTA)	34
Mean(SD)	40.48 (17.88)	Male	42	Gullian barre syndrome	1
95% confidence level	35.40-45.56			Meningitis	2
Minimum	7	Female	8	Post operative complication	3
Maximum	76			Others	10

5.2 Result pattern

Among the 50 cases, 45 cases showed significant growth and 5 cases showed no growth.

Table: 5.2 Pattern of result

Specimen	Significant growth		No growth	
	No	%	No	%
Tracheal aspirates	45	90	5	10

5.3 Growth pattern

From the 45 positive cases, 67 bacterial isolates were identified some of the sample showed infection involving multiple organisms. Out of 45 cases multiple sample were obtained from 20 cases.

Table: 5.3 distributions of the growth patterns

Type of growth	Single sample %	Multiple sample	Total
Multiple growth of bacteria	0 (0.00%)	20(80%)	20
Single growth of bacteria	20(100%)	5(20%)	25
Total	20	25	45

5.4 Staining pattern of isolates

The Gram's staining showed more Gram negative rods than any other type of bacteria.

Table 5.4 Gram's staining pattern of isolates

Bacteria	Rods	Cocci	Total
Gram positive	0	9	9
Gram negative	56	2	58
Total	56	11	67

5.5 Pattern of microbial isolates isolated from tracheal aspirates

Among the 67 isolates, *P. aeruginosa* was found to be most predominant (40.30%), followed by *K. pneumonia* (16.42%), *E. coli* (13.43%). Other common bacteria are *S. aureus* (10.45%), *K. oxytoca* (8.95%), *Streptococcus viridans* (2.98%), *A. calcoaceticus*, *Enterobacter cloacae*, *M. morganii*, *M. catarrahalis*, *P. maltophila* each (1.49%) Table: 5.4.

Table: 5.5 Distribution of microbial isolates from tracheal aspirates

Bacteria	Number	In single growth of isolates		In multiple growth of isolates		%	Total %
Gram positive		No	%	No	%		
S. aureus	7	2	28.57	5	71.42	77.77	10.45
S. viridans	2			2	100	22.22	2.98
Total	9	2				100	13.34
Gram negative							
P.aeruginosa	27	13	48.41	14	51.48	46.55	40.3
K. pneumoniae	11	3	27.27	8	72.72	18.96	16.42
E. coli	9	1	11.11	8	88.88	15.51	13.43
K. oxytoca	6	3	50	3	50	10.34	8.95
E. cloacae	1			1	100	1.72	1.49
M. morgani	1			1	100	1.72	1.49
M.catarrhalis	1	1	100			1.72	1.49
P. maltophila	1	1	100			1.72	1.49
A. calcoaceticus	1	1	100			1.72	1.49
Total	58	23		35		100	100

5.6 Antibiotic susceptibility pattern

5.6.1 Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Of the 27 isolates, *Pseudomonas aeruginosa* were most sensitive to Amikacin and least to cephotaxime. Table 5.6.1

Table: 5.6.1 Antibiotics susceptibility of *Pseudomonas aeruginosa*

Antibiotics	Sensitive	Intermediate	Resistance	Sensitive percentage
Amikacin	22	1	4	81.48
Ciprofloxacin	19	2	6	70.37
Cotrimoxazole	1		26	3.7
Cephotaxime	0		27	0
Gentamicin	18		9	66.66
Carbenicillin	3	2	22	11.11

5.6.2 Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Of the 11isolates**,** *K. pneumoniae* were most sensitive to Chloramphenicol, followed by Amikacin, ciprofloxacin, Cotrimoxazole and totally resistance to Ampicillin and Cephalexin. Table 5.6.2

Table: 5.6.2 Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Antibiotics	Sensitive	Intermediate	Resistance	Sensitive percentage
Ampicillin			11	0.00.
Amikacin	7	1	3	63.63
Ciprofloxacin	7	1	3	63.63
Cotrimoxazole	7		4	63.63
Cephalexin	0		11	0.00
Gentamicin	4	1	6	36.36
Chloramphenicol	10		1	90.90

5.6.3 Antibiotic susceptibility pattern of *E. coli*

E.coli (9 isolates) was most sensitive to Amikacin and least to Ampicillin and cephalexin.

Table: 5.6.3 Antibiotic susceptibility pattern of *E.coli*

Antibiotics	Sensitive	Intermediate	Resistance	Sensitive percentage
Ampicillin			9	0.00.
Amikacin	7		2	77.77
Ciprofloxacin	1	1	7	11.11
Cotrimoxazole	4		5	44.44
Cephalexin	0	2	7	0.00
Gentamicin	5	1	4	55.55
Chloramphenicol	4		5	44.44

5.6.4 Antibiotic susceptibility pattern of *Klebsiella oxytoca*

Klebsiella oxytoca (6 isolates) was most sensitive to Amikacin and Gentamicin and least to Ampicillin and Cephalexin: Table 5.7.4

Table: 5.6.4 Antibiotic susceptibility of *Klebsiella oxytoca*

Antibiotics	Sensitive	Intermediate	Resistance	Sensitive percentage
Ampicillin			6	0.00.
Amikacin	5		1	83.33
Ciprofloxacin	1		5	16.66
Cotrimoxazole	1	1	4	16.66
Cephalexin	0		6	0.00
Gentamicin	5		1	83.33
Chloramphenicol	6		1	100.00

5.7.5 Antibiotic susceptibility pattern of *Staphylococcus aureus*

S. aureus (7 isolates) were most sensitive to Vancomycin and least to Amoxycillin, Ciprofloxacin and Erythromicin: Table 5.7.5

Table: 5.7.5 Antibiotic susceptibility patterns of *S. aureus*

Antibiotics	Sensitive	Intermediate	Resistance	Sensitive percentage
Amoxycillin	2		5	28.57
Amikacin	4		3	57.14
Ciprofloxacin	2		5	28.57
Cloxacillin	3		4	42.85
Erytromicin	2		5	28.57
Methicillin	3		4	42.85
Vancomicin	7			100.00

5.6.6 Antibiotic susceptibility pattern of viridans *Streptococci*

The 2 isolates of *Streptococcus* viridans were resistance to none of the antibiotics used. These were sensitive to Ampicillin, Ciprofloxacin, Cotrimoxazole, cephotaxime, Erythomicin, and Vancomicin but intermediate to Amikacin.

5.6.7 Antibiotic susceptibility pattern of *Enterobacter Clocae*

The single isolate of *Enterobacter* spp was sensitive to Amikacin and Gentamicin but resistant to Ampicillin, Ciprofloxacin, Cephotaxime, Cotrimoxazole and Chloramphenicol.

5.6.8 Antibiotic susceptibility pattern of *Morganella morganii*

The single siolate of *M. morganii* was sensitive to Amikacin, Gentamicin and Chloramphenicol. The isolate was resistant Ampicillin, Ciprofloxacin and Cephotaxime.

5.6.9 Antibiotic susceptibility pattern of *Moraxella catarrhalis*

The single isolate of *Moraxella catarrhalis* was sensitive to all antibiotics, Ampicillin, Amikacin, Ciprofloxacin, Cotrimoxazole, Cephotaxime and Gentamicin

5.6.10 Antibiotic susceptibility pattern of *Pseudomonas maltophila*

The single isolate of *P. maltophila* was sensitive to Amikacin and Chloramphenicol. It was resistant to all other antibiotics used, Ciprofloxacin, Cotrimoxazole, cephotaxime,.

5.6.11 Antibiotic susceptibility pattern of *Acinetobacter calcoaceticus*

The single isolate of *Acenetobacter calcoaceticus* was only sensitive to Amikacin and resistant to all other antibiotics used Ciprofloxacin, Cotrimoxazole, cephotaxime, chloramphenicol and Gentamicin.

5.7 Burden of Multi Drug Resistance among the total isolates

Except *Moraxella catarrhalis* and viridans *Streptococcus,* all other bacteria showed high numbers of MDR.

Table: 5.7 Pattern of Multi Drug Resistance among the total isolates

S.N.	Pathogens	No (%)	No and % of MDR
1	*Pseudomonas aeruginosa*	27(40.30%)	24(88.88%)
2	*Klebsiella pneumoniae*	11(16.42%)	6(54.55%)
3	*Escherichia coli*	9 (13.43%)	7(77.77%)
4	*Klebsiella oxytoca*	6(8.95%)	5(83.33%)
5	*Staphylococcus aureus*	7(10.45)	5(71.14%)
6	*Streptococcus* viridans	2 (2.98%)	0(0%)
7	*Enterobacter clocae*	1(1.49%)	1(100%)
8	*Morganella morganii*	1(1.49)	1(100%)
9	*Moraxella catarrhalis*	1(1.49%)	0(0%)
10	*Pseudomonas maltophila*	1(1.49%)	1(100%)
11	*Acenetobacter calcoaceticus*	1(1.49%)	1(100%)

CHAPTER: VI

6.1 DISCUSSION

This study was conducted in the patients of 7-76 years who were admitted to National Institute of Neurological and Allied Sciences, Bansbari Kathmandu to find out the etiological agents of post tracheostomy infection and the efficacy of the drug being used against them. The study also includes prospective studies concerning the incidence of MDR organisms.

A total of 50 cases were enrolled in the period during April 2008 to February 2009. Among the total cases 42 were male and 8 were female, with male to female ratio 5.25:1. The median age of patients was 40 years Sub group based on age reveled the maximum of patients were in 21-30 and 31-40 (10; 20%).

Out of the total cases, the yield of specimen was seen in 45; 90%. Study by Niederman et al, (1984) showed more than three quarters of the sample showing positive cluture. Another study by Morar et al, (2002) showed 87% colonization after the tracheostomy. Other study by Arola Mk showed 92% of the tracheostomized patients were colonized. So our results almost match the result from other researchers. The yield might have been higher, had we done anaerobic culture. However, lack of facilities to anaerobiosis, we were unable to find the number of anaerobic bacteria. There might be several reasons behind the high rate of positive yield. As the patients were critically ill they had a weak immune system due to various factors, like; bypassing of upper airway resistance and defected mucociliary escalator The respiration in natural air after tracheostomy might also has increased the infection rate among the tracheostomized patients. Other route of infection might be leakage of contaminated secretion around the endotracheal tube, and passing of inocula by the health professional during the respiratory therapy.

Super-infection (Infection one after another) was the important finding of the work. Out of the 45 positive cases almost half of the sample had shown super infection. As all the patients were critically ill, infection after infection is obvious. A study conducted by Gotsman et al, (1964) also showed super infection in patients who were selected under discussion. The growth of multiple organisms from tracheal specimen had been mentioned by Niederman et al, (1984). In our study most portion of polymcrobial growth was occupied by Enteric gram negative bacteria along with the *Pseudomonas* spp, however, the *Pseudomonas* spp had persisted more than other Gram negative enteric bacteria. In patients from whom multiple samples were obtained, first few samples had not shown any growth or non significant growth. This evidence also suggests tracheostomy and infection association. The obtaining of multiple organisms at a time had been really problematic for ascertaining the actual pathogen so that semiquantitave method is better to apply before dispatching a report as non significant.

In the study *Pseudomonas aeruginosa* was found in majority of the cases (40.30%), followed by the *Klebsiella pneumoniae* (16.42%), E. coli (13.43%), *S. aureus* (10.45%), *Klebsiella oxytoca* (8.95%), *Streptococcus* viridans (2.98%) cases and other gram negative bacteria (7.45%). Among the total cases, Gram positive bacteria accounts for 13.43% and Gram negative bacteria the rest.

Various references we have taken in accounts, had advocated for the presence of Gram negative enteric bacteria to be the major pathogen present in tracheostomy sunction and so does ours study. Study by Niederman et al, (1984) also showed *Pseudomonas* spp as the predominant organisms; however, the frequency is lower than what we have obtained. Study by Patrizia et al, (2000) also suggests *Pseudomonas* as the most predominant pathogen of the tracheal aspirates. Morar et al, (2000) also suggested the *Pseudomonas* spp as the most important pathogen to be colonized in tracheobronchial trees Nseir et al, (2002) also had suggested the *Pseudomonas aeruginosa* as the most common aetiological agent of

tracheobronchitis. As the *Pseudomonas aeruginosa* is not the normal flora of the body, the source of the species most be the hospital environment. We had not included the oropharyngeal specimen in our study, so we can not say the type of colonization either it is endogenous or exogenous colonization, however the route of transmission might be the hospital equipments like suctioning machine, nebulizer etc. the another contributing factor might be inadequate washing technique of the instrument and also possible source was transferring of the pathogen by health professionals from one to another patients during the courses of treatment. Whenever patients were infected with the *Pseudomonas aeruinosa* the infection had persisted and was really problematic for both the patients and the physician. The persistence of *Pseudomonas aeruginosa* may be attributed to its capacity to form biofilm in the tracheostomy tube and the calcium alginate capsule around it worked for reservoir for the organism and interfered the action of antibiotics (Inglis et al, 2002)

Klebsiella pneumoniae was the second most prevalent organism after the *Pseudomonas aeruginosa*. Out of all the *Klebsiella pneunoniae* more than three quarters of the organisms were found in mixed culture along with the other organisms. Among the major pathogen described by National nosocomial infection surveillance, *Klebsiella pneunoniae* is one of the most important aetiological agents of nosocomial respiratory tract infection. One study carried out by National Nosocomial infections Surveillance systems United States showed, prevalence of *K. Pneumoniae* was 8%. The *K. Pneumoniae* is found in bowel and respiratory tract of human (Homles B and Aucken HM, 1998). So the infection might be endogenous in nature, however, it is not the authentic as we had not taken oropharyngeal swab in our study and this bacteria sometimes is also found in environment as well. When there was mixed culture of *Klebsiella pneumoniae* along with *P. aeruginosa,* the former had persisted for shorter period than the later one.

The third most predominant case in the study was *E.coli*. The bacteria are the normal intestinal flora of human. It is excreted with the faces and may survive in the environment;

however, it appears there is no independent existence outside the body. The infection of *E. coli* might be endogenous in via micro – aspiration or exogenous via contaminated equipment or hands of nurshing staff (Altwegg and Bockemuhl, 1998). The bacterium was found mixed with other bacteria in almost all cases except for a single case. Although multidrug *E. coli* were isolated during the work, their persistence was not as much as and as long as *P. aeruginosa.*

The next predominant bacteria were the *Staphylococcus aureus*. The gram positive cocci are the normal flora of anterior nares. They are also found in pharynx, respiratory tracts of the hosts (Elek, 1959). The source of infection for this organism may be both endogenous and exogenous (may transferred by carriage via medical equipment and hands of nurshing staffs). *S. aureus* isolated in our study were found almost all except for two in polymicrobial. This attributes one quarter of the total polymicrobial growth. S. aureus is the major cause of hospital acquired pneumonia and it accounts for 40-60% of total HAP due to polymicrobial growth (Joseph, 2001). This frequency is more than ours data's frequency but we have not to forget HAP is not only due to tracheostomy. Generally the *S. aureus* were isolated in the later parts of infection (more than half of the cases). Whenever there was the MDR *S. aureus,* the infection was persisted (more than half of the MDR cases). Among the total isolated *S. aureus* more than half of the organisms were found in young age group patients, this might be due to young people are more carriage of *staphylococcus* than adult ones(Noble et al, 1974).

Next to *S. aureus* was *Klebsiella oxytoca*. This species of klebsiella is differentiated from the pneumoniae on the basis of Indole production test otherwise have same colony characteristics as pneumoniae (Maslow and Breacher, 1993). Among the all *K. oxytoca* isolated, half of them were isolated in mixed culture and other in pure culture. Habitat and route of colonization of this bacteria may be same as in the case of *K. pneumoniae,* discussed above.

Other prevalent bacteria in our study were *Streptococcus* viridans. The viridans are not the species itself, however, a group of oral *Streptococci.* The hard, adherent colonies of Gram positive and catalase negative, non haemolytic and resistant to optochin 5mcg were identified as viridian streptococci. The species of *Sterptococcus* viridans are differentiated on the basis of certain biochemical tests (Collier et al, 1998). The viridans Streptococcus are normal inhabitants of the oral, respiratory and gastrointestinal tract mucosa. Most are opportunistic pathogens and have generally been thought to be of low virulence. The two isolates identified in our study were pure growth of viridans Streptococcus. Due to lack of different biochemical testing facilities we were not able to differentiate then into species. Although it has low virulence ability it was considered be potential pathogen of aspiration pneumonia so until and unless it was isolated in pure culture it was not considered as significant case. There should not be doubt about the route of infection in this case i.e. endogenous and the organism had not persisted.

The five other bacterial isolates were isolated in equal frequencies. We are discussing the bacteria serially as in the result section. *Enterobacteria clocae* is also a bacterium of family enterobactericeae. Different workers also had isolated *Enterobacter clocae* from tracheal specimen (Neiderman et al, 2002). It is also gram negative enteric bacteria (EGNB) so route of infection this case is also similar to EGNB. Other bacteria in our study were *Morganella morganii*, this also an EGNB. Researchers prior to our study had shown presence of Proteus species in tracheal aspirates (Gotsman and Whitby, 1964; Neiderman et al, 2002) but evidence suggesting *M. morganii* might be very few. Occasionally M. morganii is isolated from blood, sputum and out breaks of hospital acquired infection some of them serious and even fatal- have been reported (Tucci and Isenberg, 1981; Williams and Hawkey, 1983)

Moraxella catarrhalis was another bacterium in our study. That Gram negative coccus is the normal flora is habitant of upper respiratory tract. It is frequently associated with lower

respiratory tract infection, sinusitis, and otitis media. Pneumonia and tracheobronchitis most often occurs in patients with underlying pulmonary diseases (Digiovanni et al, 1987). The isolation of these bacteria from tracheal aspirate and its endogenous route of colonization had been dealt in (Morar et al, 2002; Grorges et al; 2000).

The other bacteria isolated in our study were *Acinetobacter calcoaceticus*. The bacterium is saprophytes and is an important cause of outbreaks of hospital infection. *Acinetobacter* spp had been identified in different researches regarding tracheal aspirates (Morar et al; 2002, Grorges et al, 2000) in fact some researches had identified it as most predominant bacteria found in tracheal aspirates. However, most studies suggested Pseudomonas as most predominant and so do ours. Since *Acinetobacter* spp infection cases at the time of study was very low at hospital and affinity of *Pseudomonas* toward tracheobronchial tree may have produced this result.

More than 50 years physicians worldwide have relied on antibiotics for rapid and effective management of many of the most common infections. Antimicrobials agents have changed the way both physicians and the public perceive bacterial infections and their treatment. For the most of the antibiotic era, antibmicrobials have been hailed as “miracle drugs” in the euphoria surrounding these “magic bullets”, little thought was given to the adverse consequences of their indiscriminate use. Rather, the drugs were embraced as a panacea, capable of eradicating infectious micro-organisms without causing much harm to the cells of the patients being treated. Because these drugs often literally saved the life, any potentially serious problems associated with them were downplayed.

Today, the antibiotics remain the front line therapy for conquering the bacterial infections. However, their indiscrimination use is no longer viewed as benign. Treatment with these drugs is acknowledged to be a two-edged sword. As antimicrobial agents have been misused and overused, bacteria have fought back with a selection process by which certain

strains are now no longer susceptible to one or more agents. In recent years bacteria have increasingly become resistant to antibiotics, leading to a decline in the effectiveness of antibiotics in treating infectious diseases. The unforgiving rise in the frequency of multi-drug resistance among leading pathogens should cause great concern and incite a commitment to act responsibly; otherwise humanity has to pay a price in both in terms of money and lives.

In this study, antibiotic sensitivity profile showed *P. aeruginosa* was most sensitive to Amikacin, followed by Ciprofloxacin and Gentamicin. All the isolates were resistant to Cephotaxime followed by Cotrimoxazole and Carbenicillin. A study in India suggested *Pseudomonas aeruginosa* were resistant to 30.5% of Amikacin, 62.5% Gentamicin, 37.6% of Ciprofloxacin and only 66% of cephotaxime (Rajput et al, 2008). Similarly study in Jordan suggested 71% of Amikacin and Gentmicin, 80% Ciproloxacin and only 5% percentage of Cephotaxime were sensitive for the Pseudomonas aeruginosa (Hussein et al, 2007). Study of suceptibiity pattern of *Pseudomonas aeruginosa* isolated from 5 different countries showed the species was resistant to 1-11% of the Amikacin, 7-46% of the Gentamicin, 11-35% of the Ciprofloxacin (Hanberger et al, 1999). This study also gives the similar result for the susceptibitily pattern.

About 19% of *Pseudomonas aeruginosa* was resistant to Amikacin. One possibility is production of Aminoglycoside modifying enzyme N' acetyl transferase (ACC6' [I]) which hydrolyses the Amikacin, Tobramicin and Neltimicin but not the Gentamicin (Miller et al, 1999).However, our study suggests Amikacin more sensitive than Gentamicin. The reason might be high rate of consumption of Gentamicin than Amikacin in the ICU. Another antibiotic, mostly sensitive to *P. aeruginosa* mostly sensitive was Ciprofloxacin. Just more than one quarter of the *P. aeruginosa* were resistant to Ciprofloxacin. Resistance is usually due to mutations in genes for DNA gyrase, but may also follow from alterations in drug accumulation, sometimes associated with, sometimes associated with alterations in outer-

membrane proteins of gram-negative bacilli (Wolfson and Hopper, 1989). Though Cephotaxime has a good activity against the gram negative bacteria, *P aeruginosa* isolated were 100% resistant to cephotaxime so that question of its use in therapy of the bacteria is always thinkable. We have also used imipenam and Tazobactum piperacillin in 8 cases. These antibiotics sensitive almost all case except one (only Polymyxin B was sensitive). Both these antibiotics have shown effect against Amikacin resistant strains (2 cases). Fourteen of the twenty seven isolates of *P. aeruginosa* showed the same antibiotics susceptibility pattern. Considering the antiabiotics sensitivity patterns as epidemiological marker we can suggest that these may be same strains of the *P. aeruginosa* and different hospital equipments might have acted as the reservoir for the strains. These strains must have transmitted among the patients through animate and different inanimate materials. A query to their source is amendable.

In the case of Gram negative enteric bacteria (*E. coli*, *K. pneumonia K. oxytoca and Enterobacter clocae*); these bacteria were most sensitive to Chloramphenicol and Amikacin followed by Gentamicin. None of the isolates were sensitive to Ampicillin and Cephalexin. Though Chloramphenicol was found most sensitive antibiotics but its use is a most thinkable question because long term use of these antibiotics is associated with toxic effect (Mulla and Barnes, 1997). Next to Chloramphenicol, The EGNB were sensitive Amikacin and Gentamicin (74.07% and 53.85%). the decreased sensitivity of Gram negative bacteria to Amikacin in five different countries of Europe was as high as 6% and Gentamicin as high as 30% (Hanberger et al,1999). Around two third of the isolates of EGNB resisted Ciprofloxacin. Few data are available regarding the pattern ciprofloxacin resistant Gram negative bacteria here in Nepal. Resistance to fluoroquinolones varies geographically and is emerging problem in both developed and developing countries. Gram-negative bacilli had a very high rate of resistance to ciprofloxacin (50%-100% of isolates) in an intensive care unit (Singh et al, 2002). A high rate of resistance was reported among community isolates

of *E. coli* from the urinary tract of persons in Greece (36%) (Chanotaki et al, 2004) and persons in Oman (31%) (Al-Lawati et al; 2000) and among community isolates (25%) (Fadel et al, 2004) and nosocomial isolates (40%-46%) from persons in Lebanon (Tomhe et al, 2001). The increase in resistance is suspected to be due to the increased use of antimicrobial agents in both humans and nonhumans. However, one limitation of our study is that we had no available data on the use of antibiotics in our hospital to correlate antibiotic consumption with resistance rate.

In the cases of *Stapylococcus aureus* Vancomycin was the most effective antibiotics followed by the Amikacin. Ciprofloxacin, Amoxacillin and Erythromicin were less effective other antibiotics. In four of the 7 *Staphylococcus aureus* infection cases, Tazobactam/ piperacillin were also used and in all the cases except one, the isolates were sensitive to the Tazobatum/Piperacillin.

High prevalence of MDR is other finding of the study. The possibility is that the patients have a long term hospital stay, a long term antibiotic therapy. Another important aspect was the persistent of these MDR infections so we should think for amendable therapy. A consensus statement by American thoracic society suggested that in case of Gram negative enteric bacteria (*E. coli*, *Klebsiella sps*, *Enterobacter* sps), the monoantibiotics therapy is adequate but when there is infection with highly resistant Gram negative such as *P. aeruginosa*, *Acinetobacter* and others a combined drug therapy is required. During study we had encountered a high frequency of MDR and persisting Pseudomonal infection we better evaluate drug therapy. Before therapy, consideration of Pharmacological features like penetration of antibiotics to the site of infection is always important. Some antibiotics penetrate respiratory secretion than others. aminglycosides have relatively poor penetration, while fluroquinolones can achieve concentration in brochial secretion that is equal to or exceeds serum level(LaForce,.1989and Honeybourne, 1994). So that Aminoglycosides can never be use alone. So a combination of Aminoglycosides and fluroquinolones is one

option. These agents are bactericidal in concentration dependent fashion, killing more rapidly in high concentration. In addition these have a prolonged post effect have a prolonged post antibiotic effect (PAE) allowing them to suppress bacterial growth even after the antibiotic concentration is below the minimum inhibitory concentration of target organisms (Craig, 1993).

One problem with the Amikacin is its getting inactivated in the pneumonic lungs due to the acidic P^H. So alternative may be combination of Fluroquinolones and B-lactum antibiotics. Such combination may provide additive or possibly even synergistic activity against pathogens and will have the advantage of good parenchymal penetration of fluroquiolones along with a reduced potential for toxicity when compared with aminoglycosides (Stratton, 1992).

Since this study showed Amikacin and Ciprofloxacin as most effective antibiotics for the Gram negative bacteria in vitro so a combined drug therapy of these antibiotics is amendable in tracheobronchitis and combination of B-lactum and Ciprofloxacin in the bronchopneumonia. This will augment the successful therapy and can also prevent from emergence of the multidrug resistant bacteria.

6.1 Conclusion

P. aeruginosa was the most predominant pathogen encountered in tracheal aspirates followed by Gram negative enteric bacteria (K. *pneumoniae, E.coli, K. oxytoca,* and *E. clocae*), *S. aureus* and viridan *Streptococci* and other gram negative bacteria. Gram negative bacteria were most sensitive to Amikacin and Gram positive bacteria were most sensitive to Vancomycin. A high prevalence of MDR was found all groups of bacteria

VIII. SUMMARY AND RECOMMENDATION

8.1 Summary

1. Gram negative aerobic bacteria are the major cause of colonization and infection after the tracheostomy. To study the prevalence of organisms in tracheal aspirates, the study was carried out from April 2008 to February 2009 among the 50 tracheostomized patients of National Institute of Neurological and Allied Sciences Bansbari.
2. Out of the 45 positive cases, 44.44% cases were polymicrobial and total 67 isolates were identified.
3. Among the total isolates 86.57% were Gram negative bacteria and rest were Gram positive bacteria.
4. Among the isolates *Pseudomonas aeruginosa* were most predominant 40.30 %, followed by *Klebsiella pneumoniae* 16.42%, *E. coli* 13.43%, *S.aureus* 10.45%, *K. oxytoca* 8.95%, *Streptococcus* viridans 2.98% and other Gram negative bacteria 7.25%.
5. The prevalence of MDR was 88.88% in *Pseudomonas aeruginosa*, 54.55% in *K. pneumoniae*, 77.77% in *E. coli*, 83.33% in *K. oxytoca*, 71.14% in *S.aureus*, and 80% in other Gram negative bacteria.
6. Regarding antibiotics *Pseudomonas aeruginosa* were most sensitive to Amikacin(81.48%) followed by Ciproflocxacin (70.37%) and all the isolates were resistant to Cephotaxime.
7. In the cases of Enteric Gram negative bacteria isolates were most sensitive to Chloramphenicol and Amikacin (74.07%) and all the isolates were resistant to Ampicillin.
8. In the case of Gram positive bacteria, all the isolates were sensitive to the Vancomycin.

8.2 Recommendation

1. High number of MDR bacterial isolates is an alarming situation requires the close look to tackle the problem before getting worse.

2. Bacterial isolates of almost same antibiotic susceptibility pattern indicating same source requires attention of the hospital authorities for further characterization and control.

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