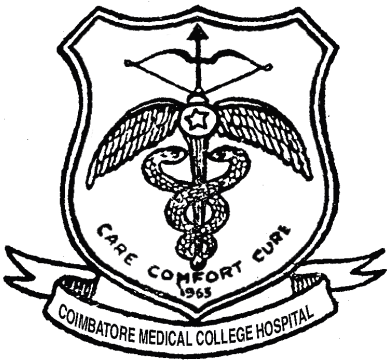
Epidemiological and Bacteriological profile of Hospital Acquired Pneumonia with special reference to Klebsiella pneumoniae and its characterization by Antibiogram and Klebocin typing



Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

in

MICROBIOLOGY – BRANCH IV

The Tamil Nadu

Dr. M.G.R. Medical University

Chennai

March -2009

CERTIFICATE

This is to certify that the dissertation, entitled **"Epidemiological and Bacteriological profile of Hospital Acquired Pneumonia with special reference to Klebsiella pneumoniae and its characterization by Antibiogram and Klebocin typing"** submitted to The TN Dr. M.G.R. Medical University, in partial fulfillment of regulations required for the award of M.D. Degree in Microbiology - Branch IV is a record of original research work done by Dr.V.Vasuki at the Department of Microbiology, Coimbatore Medical College Hospital during the period from May 2007 to April 2008 under my guidance and supervision and the conclusions reached in this study are her own.

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Dissertation Topic : Epidemiological And Bacteriological Profile Of Hospital Acquired Pneumonia With Special Reference To Klebsiella Pneumoniae And Its Characterisation By Antibiogram And Klebocin Typing

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation is accepted / ~~Not accepted~~ and you are permitted / ~~Not Permitted~~ to proceed with the above Study.

Coimbatore - 14.

Date : 8.10.2007

Secretary
Ethics Committee

DECLARATION

I, Dr.V.Vasuki solemnly declare that the dissertation, entitled **"Epidemiological and Bacteriological profile of Hospital Acquired Pneumonia with special reference to Klebsiella pneumoniae and its characterization by Antibiogram and Klebocin typing"** submitted to The TN Dr. M.G.R. Medical University, in partial fulfillment of regulations required for the award of M.D. Degree in Microbiology –Branch IV, was done by me at Coimbatore Medical College Hospital during the period from May 2007 to April 2008 under the guidance and supervision of **Dr.Anbu.N.Aravazhi, M.D.,** Professor and Head of the Department of Microbiology. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place:

Date: Dr.V.VASUKI

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LIST OF ABBREVIATIONS

APACHE	Acute Physiology, Age and Chronic Health Evaluation
BAL	Broncho alveolar lavage
CFU	Colony forming unit
CLSI	Clinical Laboratory standards Institute
COPD	Chronic Obstructive Pulmonary Disease
DM	Diabetes Mellitus
EPIC	European Prevalence of infection in Intensive Care
ESBL	Extended Spectrum Beta Lactamase
ETA	Endo tracheal aspirate
HAI	Hospital Acquired Infection
HAP	Hospital Acquired Pneumonia
ICO	Intra Cellular Organism
ID	Inhibitory Dilution
IMCU	Intensive Medical Care Unit
LPF	Low power field
MRSA	Methicillin- Resistant Staphylococcus aureus
MSSA	Methicillin- Sensitive Staphylococcus aureus
MTCC	Microbial Type Culture Collection
NNISS	National Nosocomial Infections Surveillance System
SDS-PAGE	Sodium dodecyl sulfate- polyacrylamide gel electrophoresis
SEC	Squamous epithelial cell
VAP	Ventilator Associated Pneumonia

CONTENTS

INTRODUCTION

Hospital acquired infections continue to be an important cause of morbidity and mortality among hospitalized patients. [1, 24] The critically ill patient is at particular risk of developing ICU acquired infection, with the lungs being especially vulnerable. [2]

Hospital acquired pneumonia (HAP) is currently the second most common hospital infection accounting for 13 to 18 percent of all nosocomial infections, with estimates of associated mortality ranging from 20 to 50 percent. [2]

The majority of cases of HAP occur outside of ICUs. However the highest risk is in patients on mechanical ventilation. Estimates of incidence range from 4 to 7 episodes per 1000 hospitalizations. [3] Intubated patients may have rates of pneumonia 7 to 21- fold higher than patients without a respiratory therapy device. [5] Infection rates are twice as high in large teaching hospitals as compared with smaller institutions. [5]

HAP results in a significant increase in the cost of care of hospitalized patients. [3] Its development prolongs a patient's stay in the ICU [3, 4] and most of the extra cost is due to an increased length of hospital stay. [3, 19]

The causes of HAP are varied and differ across different patient populations and different types of ICUs. [4] Hospital acquired bacterial

pneumonia is frequently polymicrobial with gram negative bacilli predominating. [5, 22] This varied presentation underscores the need for the intensivists treating the patients with HAP to have a clear knowledge of the ambient microbiological flora in their ICU.[4]

Delayed administration of adequate antibiotic therapy is linked to an increased mortality rate. [6] Hence, the focus of initial antibiotic therapy should provide rapid antibiotic coverage for all likely pathogens. The antibiotic spectrum may then be focused or narrowed based on the results of cultures. A guideline-based approach using the local hospital or ICU antibiogram may help in appropriate and adequate initial therapy and hence reduce the overall use of antibiotics and the associated selection pressure for multi drug resistant organisms.[6]

Bacteria belonging to the genus Klebsiella frequently cause human nosocomial infections. In particular, the medically most important Klebsiella species K.pneumoniae, accounts for a significant proportion of HAP. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. Hospital outbreaks of Multi Drug Resistant Klebsiella species are often caused by newer type strains, the so called Extended Spectrum Beta lactamase (ESBL) producers. The incidence of ESBL producing strains among clinical Klebsiella isolates has been steadily increasing over the past years. [7]

Epidemiological typing is useful in determining the extent of an outbreak and in elucidating the sources and spread of infection. [8] Combination of Klebocin typing and antibiogram suggest an important tool for epidemiological studies. [9]

Though HAP is widely analyzed by many researchers, not much is known about the incidence and bacteriological profile i.e., only few studies are being published by them. This study is conducted prospectively to evaluate the clinical and bacteriological profile of HAP in ICU patients. It may increase the awareness of clinicians about the need to reduce the morbidity and mortality by coming to know about the various pathogens causing HAP and its sensitivity and/or resistance to various antibiotics. The clinicians need to establish a suitable antibiotic policy by working out local ICU antibiogram charts. This study is also done to find out the prevalence of ESBL producers in HAP. Klebocin typing in association with antibiogram may help to find out the source of Hospital Acquired Infections.

THE OVERALL AIM OF THE STUDY:

To analyze the incidence, epidemiology, antimicrobial susceptibility pattern of isolates from HAP patients in IMCU and klebocin typing of Klebsiella pneumoniae isolated in HAP.

OBJECTIVES:

1. To study the incidence of Hospital Acquired Pneumonia (HAP) among patients admitted in IMCU.
2. To evaluate the Clinical and Bacteriological profile of HAP.
3. To determine the antibiotic susceptibility pattern of the all bacteria isolated.
4. To find out the prevalence of ESBL producers in HAP.
5. To evaluate the role of Klebocin typing in epidemiological typing of K.pneumoniae strains.
6. To detect the source of infection in IMCU using antibiogram and klebocin typing.

REVIEW OF LITERATURE

Hospital Acquired Pneumonia (HAP) or Nosocomial pneumonia is defined as pneumonia that occurs at least 48 hours after a patient has been admitted to the hospital and that was not incubating at the time of admission. Ventilator- Associated Pneumonia (VAP) is a type of HAP in patients receiving mechanical ventilation that arises at least 48 hours after endo tracheal intubation. [11]

Epidemiology:

Accurate information concerning the epidemiology of HAP is lacking, as there is no universally accepted criteria for its diagnosis. [1] HAP is the second most frequent nosocomial infection. [2] In the year 2006 Glasgow et al reported that in United Kingdom, HAP accounted for approximately 25% of hospital infections and was as common as that of the UTI. [12] In various other studies conducted by different authors in United States HAP accounted for approximately 15% of all Hospital- related infections and had been reported as the second most common HAI next to UTI. [2, 10, 30, 31] In the year 2007 Muhammad et al reported that HAP accounted for 21% of all HAI in two medical ICUs of a public tertiary care hospital, Karachi and showed that HAP was the third most common HAI next to UTI (44.6%) and blood stream infection (27%). [22]

The incidence of HAP differs in different Intensive Care Units. [22] Factors like patient population, criteria used for diagnosis, length of ICU stay and prior exposure to antibiotics may influence the incidence of HAP.[1,18] According to European Prevalence of Infection in Intensive Care study (EPIC) by Vincent et al involving over 4500 patients, the incidence of HAP in ICU was 46.9%. [25] Chevret et al from France reported in their multicenter prospective study on 996 patients admitted in ICUs that the incidence of HAP was 8.9%. [29] Alp et al from Netherlands reported that the incidence was 6.8% in ICU patients in the year 2004. [32] In US the incidence of HAP in ICU patient ranged between 7.8 to 68 percent. [2, 10, 31] Sopena et al from Spain reported that the incidence of HAP was 36.4% during the year 2005. [30] Berba et al from Philadelphia reported that incidence was 28.2% among ICU patients. [33]

The incidence of HAP is age dependent. HAP is most common in elderly patient aged >60 years. However patients of any age may be affected. No racial and sexual predilection exists. [12, 20] Muhammad et al from Karachi in their study divided the patients admitted in ICU into six age groups and reported that the highest incidence was seen in patients of 41 to 60 years of age. [22] Berba et al from Philadelphia reported that 30% of the patients were more than 60 years of age. [33]

HAP is the leading cause of death from infections that are acquired in the hospital. [2] Heyland et al from France showed that the patients with HAP

stayed in the ICU for 4.3 days longer and had a trend toward an increase in risk of death. [26] Mortality rate for HAP ranges between 24 and 50%. [4, 28] Celis et al from Spain reported that the mortality rate was 36.6% among HAP patients in a 1000- bed teaching hospital. [27] Sopena et al from Spain in the year 2005 reported that the mortality rate was 26% among HAP patients. [30] Alp et al from Netherlands reported that the mortality rate was 65% in ICU patients with HAP in the year 2004. [32]

Time is an important epidemiological variable and risk factor for specific pathogens & outcomes in patient with HAP & VAP. [11] Early-Onset HAP is defined as pneumonia occurring within the first 4 days of hospitalization, usually having a better prognosis. Late-Onset HAP is defined as pneumonia occurring after five or more days of hospitalization, which are more likely to be caused by MDR pathogens and are associated with increased patient mortality & morbidity. [11]

Indian scenario:

Merchant et al from Mumbai in year 1998 reported that the incidence of HAP among IMCU patients was 16.7% and the mortality rate was 40% in HAP. [19] Trivedi et al from Mumbai in the year 2000 reported that the incidence of HAP in IMCU was 9.38% with the mortality rate of 21.3%; in the same year another study from Mumbai by Tullu et al in Pediatric Intensive Care Unit showed the incidence of HAP as 27.54% with the mortality rate of 47.37%. [16, 17]

Mukhopadhyay et al from Lucknow in the year 2003 reported the incidence of HAP in ICU patients as 53.9% and the mortality rate as 47.3%. [18] Pawar et al from New Delhi in the year 2003 reported the incidence of HAP as 2.6% among ICU patients with the mortality rate of 16%. [14] Rakshit et al from Mumbai in the year 2005 reported the incidence as 47% and the mortality rate as 37%. [13] Dey et al from Manipal in the year 2007 reported that the incidence of HAP being 45.4%.[15]

Risk factors for the development of HAP:

There are several risk factors for the development of HAP like Mechanical ventilation, Reintubation, Age > 60 years, Severity of illness, Acute or chronic lung disease, Excessive sedation, Enteral nutrition, Supine body positions, Glasgow coma scale < 9, Use of Muscle relaxants, Cigarette smoking, Administration of antacids or histamine type 2 antagonists, Emergency surgery, Prior Antibiotic use, Duration of ICU or Hospital stay. [1, 21, 37]

Intubation and mechanical ventilation increase the risk of HAP from 6- to 21- fold and up to 28% of patients receiving mechanical ventilation will develop this complication. [1] Many studies on HAP showed that the occurrence of HAP was high in the ICUs mainly because of high utilization of invasive procedures like mechanical ventilation. [36] Rakshit et al reported that the significant risk factors for development of HAP were prolonged duration of mechanical ventilation, higher APACHE III scores on

admission signifying severe illness and reintubation. [13] Mukhopadhyay et al from Lucknow in the year 2003 reported unplanned or failed extubation followed by re-intubation as a significant risk factor for the development of pneumonia. It is probable that aspiration of infected upper airway secretions occurs at the time of reintubation. Dey et al from Manipal in the year 2007 reported that reintubation was a definitive risk factor for the development of HAP. [15]

Pawar et al reported that the risk factors for HAP were COPD, emergency surgery, reintubation, coma, steroid treatment, enteral feedings and prior antibiotics in their study. [14] Stress ulcer prophylaxis is routinely used in the critically ill. The use of H_2 blockers is associated with a change in the acidity of the gastric juices that favors bacterial colonization with Gram negative bacteria. [33]

Celis et al reported that factors significantly predisposing to HAP were tracheal intubations, depressed level of consciousness, underlying chronic lung diseases, thoracic or upper abdominal surgery and age older than 70 years. [27] Enteral feeding via a nasogastric tube promotes gastro-esophageal reflux and is also associated with an increase in gastric pH and colonization of the stomach with aerobic Gram negative bacilli. [33] Mukhopadhyay et al reported in their study that the rate of acquisition of HAP increased along with the duration of stay in the ICU. [18]

Pathogenesis of HAP:

For HAP to occur, the delicate balance between host defenses and microbial propensity for colonization and invasion must shift in favor of the ability of the pathogens to persist and invade the lower respiratory tract.

Sources of pathogens for HAP include healthcare devices, the environment (Air, water, equipment, and fomites), and can occur with transfer of microorganisms between the patient and staff or other patient. A number of host- and treatment- related colonization factors, such as the severity of the patient's underlying disease, prior surgery, exposure to antibiotics, other medication and exposure to invasive respiratory devices and equipment are important in the pathogenesis of HAP and VAP. [11]

Inhalation, aspiration and hematogenous spread are the three main mechanisms by which pathogens reach the lungs. Inhalation or direct inoculation of pathogens into the lower airway, hematogenous spread from infected intravenous catheters, and bacterial translocation from the GIT lumen are uncommon pathogenic mechanisms. Aspiration of oropharyngeal pathogens or leakage of secretions containing bacteria around the endotracheal tube cuff is the primary routes of bacterial entry into the LRT. [20]

Infected biofilm in the endotracheal tube, with subsequent embolization to distal airways, may be important in the pathogenesis of VAP. The stomach and sinuses may be potential reservoirs of nosocomial pathogens that contribute to bacterial colonization of the oropharynx, but their contribution is controversial which may vary by the population at risk, and may be decreasing with the changing natural history and management of HAP. [11, 20]

Microbiology of HAP:

HAP is caused by a wide spectrum of bacterial pathogens, may be polymicrobial and rarely due to viral or fungal pathogens. Common pathogens include aerobic gram-negative bacilli such as P.aeruginosa, Klebsiella pneumoniae, Escherichia coli, Acinetobacter species, Streptococcus pneumoniae and Haemophilus influenzae. Serratia, Legionella pneumophila , Stenotrophomonas maltophilia and Burkholderia cepacia are uncommon bacteria causing HAP.

Fungal pathogens isolated from HAP are Candida species and Aspergillus fumigatus. Influenza, Para influenza, Adenovirus, Measles and Respiratory Syncytial Virus are viral pathogens causing HAP. [11, 20] The bacteriology of nonventilated patients was similar to that of ventilated patients, including infection with pathogens such as methicillin- resistant Staphylococcus aureus (MRSA), P.aeruginosa, Acinetobacter species and K.pneumoniae.[11]

Chastre et al from Paris in the year 2002 reported that the predominant organisms responsible for HAP being Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacteriaceae. [28]

Leroy et al from France in the year 2002 reported that the main organisms were P.aeruginosa (31.2%), Enterobacteriaceae (20.8%), Staphylococcus aureus (18.8%) with 33% Methicillin- resistant strain, Haemophilus influenzae (6.5%), Streptococcus pneumoniae (5.8%), Acinetobacter spp. (5.8%) and Stenotrophomonas maltophilia (5.2%). [35]

Alp et al from Netherlands in the year 2004 studied that the most commonly isolated pathogens were Acinetobacter baumanii (29.6%), P. aeruginosa (20.6%) and Klebsiella pneumoniae (14.4%) among HAP patients. [32]

Tullu et al from Mumbai in the year 2000 studied the bacteriology of HAP in Pediatric Intensive Care Unit and reported that the organisms commonly isolated being E. coli (34.4%), Klebsiella pneumoniae (30.2%), Pseudomonas (11.5%), Proteus (11.5%) and Acinetobacter (5.2%). [17]

Mukhopadhyay et al from Sanjay Gandhi Post Graduate Institute Medical Sciences, Lucknow, in the year 2002 reported that Gram negative organisms were isolated more frequently than Gram positive cocci in patients with HAP; Pseudomonas aeruginosa was the most frequent isolate followed by E.coli and Klebsiella pneumoniae. [18]

Pawar et al in the year 2003 from New Delhi reported that the most common pathogens isolated from HAP cases being Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae, Staphylococcus species and Acinetobacter species in that order. [14]

Rakshit et al from Mumbai reported that most common offending organism isolated in cases with VAP was P.aeruginosa followed by Klebsiella and E. coli in the year 2005. [13]

Rajasekhar et al from Nizam's Institute of Medical Sciences, Hyderabad, in the year 2006 reported that Acinetobacter baumanii and Klebsiella pneumoniae were commonest isolates among HAP patients. [23]

Dey and Bairy in the year 2007 from Manipal reported that Acinetobacter species (48.94%) was the commonest organism followed by Pseudomonas aeruginosa (25.53%), Klebsiella pneumoniae (12.77%), E. coli (10.64%) and Serratia marcescens (2.13%).[15]

Klebsiella pneumoniae – An important pathogen in HAP:

The Genus Klebsiella is defined as containing gram-negative, non-motile, usually encapsulated rod-shaped, lactose fermenting bacteria of the family Enterobacteriaceae which produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test.

Epidemiology:

Klebsiella species are ubiquitous in nature. The two common habitats of Klebsiella are the environment such as surface water, sewage, soil & plants and the mucosal surfaces of mammals such as humans, horses or swine. In humans Klebsiella pneumoniae is present as a commensal in the nasopharynx with carrier rate ranging between 1 and 6% and in the intestinal tract with the detection rate in the stool samples ranging from 5 to 38%. It is rarely found on the human skin. [7]

In the hospital environment colonization rates increase in direct proportion to the length of stay. Tulsi et al reported that colonization of intestine, umbilical stump, throat, skin and external ear with Klebsiella pneumoniae increased from 10 percent on admission to 26 percent on day 3 and 39 percent on day 6 in a Neonatal Intensive Care Unit. [69] Reported carrier rates in hospitalized patients are 77% in stool, 19% in pharynx and 42% on the hands of patients.

The significance of increased colonization was illustrated by the observation that the attack rate of Klebsiella nosocomial infection in patients having colonization of Klebsiella was four times as high as for patients without colonization. [7] Even hospital personnel have elevated rates of Klebsiella colonization. The principal reservoirs of K. pneumoniae in the hospital setting are medical equipment (contaminated due to faulty hygienic

procedures), blood products, GIT of patients and hands and pharynx of hospital personnel. [7]

Incidence of Klebsiella pneumoniae in HAI:

As an opportunistic pathogen, K.pneumoniae primarily attack immuno compromised individuals who are hospitalized and suffer from severe underlying diseases such as DM or COPD. [7] Table 1 show the percentage of Hospital acquired Infections caused by Klebsiella pneumoniae and the rank of Klebsiella pneumoniae compared to all other bacterial pathogens.

Table 1.HAI caused by K.pneumoniae: [7]

Infection	**% of infections caused by Klebsiella**	**Rank (Compared to all other bacterial pathogens)**
UTI	6 – 17	5 – 7
Pneumonia	7 – 14	2 – 4
Septicemia	4 – 15	3 – 8
Wound infections	2 – 4	6 – 11
Nosocomial infections in ICU patients	4 – 17	4 – 9
Neonatal septicemia	3 – 20	2 – 8

Lockhart S R et al from Chicago reported that during their 12-year study period from 1993 to 2004, 74,394 gram-negative bacillus isolates recovered from intensive care unit (ICU) patients in United States hospitals

and Klebsiella pneumoniae accounted for 14.2% of the total HAI. [76] Aly et al from Egypt in the year 2008 reported that the incidence of Klebsiella pneumoniae in nosocomial infections in a medical – surgical Intensive Care Unit was 11%. [77]

Gaynes et al and the National Nosocomial Infections Surveillance System (NNISS), Centers for Disease Control and Prevention, Atlanta reported in the year 2005 that the incidence of Klebsiella pneumoniae in nosocomial pneumonia was 8.4%, in urinary tract infection 4.6%, in blood stream infection 4.5% and in surgical site infection 2.7%. [78] Muhammad in the year 2007 from Karachi reported that the incidence Klebsiella pneumoniae in nosocomial infections in Intensive Care Unit was 17.4%. [22] Pal R B et al from Mumbai reported that Klebsiella pneumoniae accounted for 26.66% of nosocomial infections in Intensive Care Unit. [9]

Incidence of Klebsiella pneumoniae in HAP:

In most of the studies, K.pneumoniae was the second most common pathogen causing HAP next to P.aeruginosa. Merchant et al from Mumbai in year 1998 reported that the incidence of Klebsiella pneumoniae in HAP among IMCU patients was 34% and it was the second most common pathogen next to P. aeruginosa (44%). [19]

Tullu et al from Mumbai in the year 2000 in their study of bacteriology of HAP in Pediatric Intensive Care Unit reported the incidence of Klebsiella pneumoniae as 30.2%. [17]

Mukhopadhyay et al Sanjay Gandhi Post Graduate Institute Medical Sciences, Lucknow, in the year 2002 reported that Klebsiella pneumoniae had been isolated as a pathogen from 16.8% of HAP patients.[18]

Pawar et al in the year 2003 from New Delhi reported that the incidence of Klebsiella pneumoniae was 9.5% among HAP patients. [14]

Rakshit et al from Mumbai in the year 2005 reported that the incidence of Klebsiella pneumoniae in VAP was 21.8% and it was the second most common pathogen next to P.aeruginosa. [13]

Dey and Bairy in the year 2007 from Manipal reported that 12.77% of HAP was caused by Klebsiella pneumoniae. [15]

Rajasekhar et al from Nizam's Institute of Medical Sciences, Hyderabad, in the year 2006 reported Klebsiella pneumoniae accounted 18.18% of total isolates among HAP patients. [23]

Pathogenicity Factors of Klebsiella: [7]

1. Capsular Antigens:

The capsular repeating subunits consisting of four to six sugars and very often uronic acids (as negatively charged components) can be classified into 77 serological types. The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers. This protects the bacterium from phagocytosis by polymorphonuclear granulocytes and prevents killing of the bacteria by bactericidal serum factors. The molecular mechanism presumably consists of inhibiting the activation or uptake of complement components, especially C3b.

2. Pili (Fimbriae):

These are nonflagellar, filamentous projections on the bacterial surface which are up to 10μm long and have a diameter of 1 to11 nm. They consist of polymeric globular protein subunits (pilin) with a molecular mass of 15 to 26 kDa.

Type1 (common) pili:

This adhesin is designated as mannose-sensitive hemagglutinin (MSHA), located on the fimbrial shaft. The role of these pili is mainly for binding of the bacteria to mucus or to epithelial cells of the urogenital,

respiratory and intestinal tracts. Adherence of bacteria to cells of the repiratory tract leads to impairment of colonization resistance in the upper airways with a subsequent proliferation of facultative pathogenic bacteria. This impairment may result in the development of pneumonia especially in patients undergoing long-term mechanical ventilation.

Type 3 pili: mannose-resistant, Klebsiella-like hemagglutinin (MR/K-HA).Strains of K.pneumoniae expressing type 3 pili adhere to endothelial cells, epithelia of the respiratory tract and uroepithelieal cells.

Other types of pili are KPF-28 Fimbria which has been found in the majority of K.pneumoniae strains producing CAZ-5/SHV-4 type ESBL, CF29K (Non Fimbrial) adhesin belonging to the K88 adhesin family and Aggregative adhesin composed of capsule-like extra cellular material.

3. Serum Resistance and Lipopolysaccharide:

Capsular polysaccharides (CPS) may cover and mask the underlying LPS and exhibit a surface structure that does not activate complement. The O side chains of the LPS may reach through the capsule layer and be exposed to the exterior milieu. C 3b is subsequently deposited onto LPS molecules. Since it is fixed preferentially to the longest O-polysaccharide side chains, C3b is far away from the bacterial cell membrane. Thus the formation of the lytic membrane attack complex (C5b-C9) is prevented and subsequent membrane damage and cell death do not take place.

4. Siderophores:

These are high-affinity, low-molecular-weight iron chelators that are capable of competitively taking up iron bound to host proteins. Two different chemical groups of siderophores are Phenolate-type siderophores: Enterobactin (also known as enterochelin) and Hydroxamate-type siderophores: Aerobactin.

Although the production of cytotoxins, enterotoxins and hemolysin has been described, these features probably play a rather minor role in Klebsiella.

Epidemiological typing of Klebsiella:

From an epidemiological point of view it is often necessary to determine the clonality of the strains. A useful and effective typing system should be (1) standardized, (2) reproducible, (3) sensitive, (4) stable, (5) available, (6) inexpensive and (7) field tested in conjunction with epidemiologic investigation. This is very useful in endemic and epidemic nosocomial outbreaks of Klebsiella infections to improve the management of such outbreaks. Klebsiella marker systems include determination of antibiotic susceptibility patterns, biotype, serotype, bacteriophage susceptibility, Bacteriocin susceptibility, and plasmid content, size and endonuclease fragment size. [47, 48, 50]

Biotyping:

Biotyping based on an extended panel of biochemical and culture test is certainly the most practicable method of typing for laboratories that are epidemiologically not optimally equipped. Biotyping can be carried out by using macro tube tests alone or by combining a commercially available miniaturized system such as the API 20E system with additional macro tube tests. The drawbacks of this typing method are the large number of reactions to be tested, the often long cultivation times, poor reproducibility and poor sensitivity. Therefore biotyping of Klebsiella species is not very suitable as an epidemiological tool. [7, 47]

Serotyping:

Of 82 capsule antigens described, 77 types form the basis for an internationally recognized capsule antigen scheme. Capsule typing shows good reproducibility and is capable of differentiating most clinical isolates. [7] The drawbacks of this method are the large number of serological cross-reactions that occur among the 77 capsule types, the serotyping procedure is cumbersome because of the time needed to perform the test, it is susceptible to subjective interpretations because of weak reactions that are not always easy to interpret, since anti-capsule antisera are not commercially available, this technique is practiced mostly in specialized laboratories. [7, 49]

Although 12 different O-antigen types of Klebsiella have also been described, they are difficult to classify because their determination is hampered by the heat-stable capsules. [7]

Phage Typing:

The phage reaction is easily read and the reproducibility of the method is acceptable. This technique shows a relatively poor typing rate of 19 to 67% and as a single typing method phage typing is not very sensitive. [7, 47]

Bacteriocin typing:

Bacteriocins are bactericidal substances usually proteins produced by bacteria to inhibit the growth of other bacteria usually members of the same species. [63] In year 1988 James from UK reported that klebocin production was encoded by a 5.5 kb plasmid and SDS- PAGE klebocin gave rise to two polypeptides, one of 85 kDa and the other of 11 kDa. [56] An isolate can be characterized either by its ability to inhibit specific indicator strains or by its sensitivity to bacteriocins synthesized by a set of producer strains. Since the synthesis of bacteriocins is not frequent enough in Klebsiella, the latter technique has become the method of choice for Bacteriocin typing of organisms belonging to this genus. This method has proven superior for typing of clinical and environmental Klebsiella strains as well as of nosocomial outbreaks of Klebsiella. [45, 57]

Bacteriocin susceptibility typing is easier and cheaper. Depending on the producer strains used, between 67% and 96% of strains are typable. [47] Israil in year 1981 reported that klebocin typing was a quite simple, reliable method and did not imply any special requirements. The number of strains typable was higher than of those typable by phages and the typability was 85% in his study. [46] The same author in year 1980 reported that the typability was 88.9%. [52] Podschun and Ullman in the year 1996 studied that 96% of strains were typable by klebocin typing. [58] They developed the modification of the scrape and point method for klebocin typing. [53]

Hall used a set of 10 klebocin producing strains to type 630 clinical isolates of Klebsiella. The typability was 77% by streak and point method. A major problem in his study was the lack of reproducibility among large numbers of weak reactions encountered. [59] Bauerfeind et al showed that the typability was 96.3% using streak and point method. [62] Buffenmyer et al in the year 1976 developed standard procedures for the klebocin production, storage, klebocin titration and the utilization of klebocins for typing and reported that the typability was 67% in their study. [61]

In the year 1997 Pal et al from Mumbai reported that the typability was 71.62% using six producer strains by spot inoculation method. In view of the high induction of klebocins by mitomycin C, storage stability at -20° C and the simplicity of the technique, they stated spot inoculation method as more appropriate typing method. [9]

Chhibber et al from Panjab University, Chandigarh in the year 1998 reported that by using six klebocin producer strains the typability was 72.8%. [60] They studied the effect of various inducing agents on klebocin production by Klebsiella pneumoniae. A significant level of klebocin was detected only after induction. The highest level of klebocin was achieved with mitomycin C followed by rifampicin and polymyxin B. [54] They also purified and characterized the klebocin and reported that chemical analysis of the purified preparation showed it to be a protein and was sensitive to digestion by various proteolytic enzymes. [55]

Malik et al from Aligarh Muslim University in the year 2003 reported that the typability of klebocin typing was 83.3% and reproducibility was 73.3%. [43] Aggarwal et al in year 2003 from Government Medical College, Amritsar reported that klebocin typability in their study was 73.5%. [44]

Molecular typing methods:

The procedures like plasmid profiles, ribotyping, multilocus enzyme analysis by polyacrylamide gel electrophoresis and pulsed-field gel electrophoresis which are technically more complicated, vary from laboratory to laboratory and lack standardization making it difficult to compare them. [7, 47]

ESBL producing Klebsiella pneumoniae:

Especially feared are epidemic hospital infections caused by multi drug resistant strains. In the 1970s, these strains were chiefly amino glycoside-resistant Klebsiella strains. Since 1982 strains that produce ESBLs which render them resistant to extended-spectrum cephalosporins have evolved. [7] Today ESBL producing Klebsiella strains are emerging and spreading in the community as well as hospital settings. [41] In Europe the β-lactamases of ceftazidime-resistant Klebsiella strains are commonly of the SHV-type whereas TEM-10 and TEM-12 are more prevalent in the United States. [7]

Feizabadi et al reported that production of ESBL was detected in 72.8% of nosocomial Klebsiella pneumoniae isolates during their study from 2006 to 2007 in Tehran hospitals. [74]

Gonlugur et al in the year 2004 Turkish University Hospital reported that the incidence of ESBL producing Klebsiella pneumoniae among respiratory isolates was 12.2%. [39]

Hosoglu et al in the year 2007 reported that 68.3% of Klebsiella pneumoniae isolates in Turkish hospitals were detected as ESBL producers. [72]

Asian HAP working group in 2008 reported the overall incidence of ESBL producing Klebsiella pneumoniae as 0.9 to 40% of cases of HAP. [80]

Verhamme et al in the year 2007 from Netherlands reported that the

incidence of ESBL producing Klebsiella pneumoniae was highest in Late-onset HAP (100%). [79] In the year 2007 Vitkauskiene et al reported that the incidence of ESBL producing Klebsiella pneumoniae among HAP patients was 88.9%. [84]

Indian studies on ESBL producing Klebsiella pneumoniae:

In India, ESBL producing strains of K.pneumoniae have emerged as a challenge in hospitalized as well as community based patients. [65] High prevalence of ESBL producing Klebsiella strains has been reported by various groups. In 1997, from Nagpur 17 out of 66 Klebsiella isolates showed ESBL production. [86] In the year 2002, 68 percent of Gram negative bacteria were found to ESBL producers in a study from New Delhi in which 80 percent of Klebsiella were ESBL producers. [87]

In 2004 two other studies from Delhi showed 70.6 and 12.6 percent Klebsiella isolates to be ESBL producers respectively. [88, 89]

In Lucknow the ESBL production were seen in 58% of Klebsiella isolates from neonatal septicemia during 2004-2005. [64] Dey and Bairy in the year 2007 from Manipal reported that 100 percent of K. pneumoniae isolates from VAP cases were ESBL producers. [15]

Source detection in Hospital Acquired Klebsiella infections:

In the year 1997 Pal et al from Mumbai reported that by combination of klebocin types and antibiogram of clinical isolates, the source of infection could be traced in 86.84% cases. The sources of infection were ICU sites and staff in 60.52% cases, 6.31% cases as endogenous flora and rest was unknown. [9]

Malik et al in the year 2003 reported that Klebocin typing and antibiogram of the environmental isolates of Klebsiella pneumoniae showed the same pattern as was observed in patient isolates and concluded these isolates as the source of infection. [43] Cooke et al reported that the sources of Klebsiella infections in hospital patients could be found out using Serotyping and bacteriocin typing. [51] Tulsi et al detected the source of Klebsiella pneumoniae from ward environments using klebocin typing. [69]

Clinical outcome of HAP:

Rakshit et al from Mumbai in the year 2005 reported that 37% of HAP patients succumbed while being treated in Medical Critical Care Unit. Five patients were more than 60 years old and none of them could survive. The mortality was significantly higher in older age group and in patients with co morbid illnesses. Significantly higher survival rates were found in cases that had early and planned tracheostomy, as compared with those who needed reintubations. [13] Trivedi et al from Mumbai in the year 2000

reported that high mortality was associated with habits like smoking (33.3%), age group over 60 years (27.3%), presence of comorbid illness like DM and COPD (38.5%), complications like ARDS (61.3%) or sepsis with end organ failure (73.7%) and need of intubation (36.2%) or mechanical ventilation (40.55). [16]

Rajasekhar et al from Hyderabad in the year 2006 reported that the clinical condition of 87% cases in Early onset HAP and 33% cases in Late onset HAP improved significantly with the modification of antibiotic therapy made after the results of cultures of ETA samples. The over all mortality in their study was 18%. [23] Berba R et al in their study showed that the mean duration of hospitalization was almost doubled when HAP occurred. The mortality rates were more than three times higher among HAP patients than among non -HAP patients. 35% of deaths among HAP patients occurred within 48 hours from the time the HAP diagnosis was made. [33]

MATERIALS & METHODS

This prospective study was conducted over a period of one year among patients admitted in Intensive Medical Care Unit (IMCU) of Coimbatore Medical College & Hospital, Tamil Nadu. The study population comprised all patients admitted to the IMCU from May 1, 2007 to April 30, 2008. Approval was obtained from the Ethical Committee prior to conducting the study and informed consent from all patients under study was also obtained.

HAP was diagnosed based on standard diagnostic criteria adapted by the Centers for Disease Control and Prevention for the diagnosis of pneumonia if signs of pneumonia occurred after 48 hours following IMCU admission. [34] HAP was considered when new or progressive chest radiographical infiltrates occurred ≥48 hours after hospital admission in conjunction with the following clinical criteria:

At least <u>one</u> of the following:

1. Fever (> 38° C/ 100.4° F) with no other recognized cause
2. Leukocytosis (≥ 12,000 WBC/mm^3) or leucopenia (< 4,000 WBC/ mm^3)
3. For adults ≥ 70 years old, altered mental status with no other recognized cause

And

At least <u>two</u> of the following:

1. New onset of purulent sputum / change in the character of the sputum / increased respiratory secretions / increased suctioning requirements.
2. New Onset or worsening cough / dyspnea / tachypnea (Respiratory Rate > 25 breaths / min)
3. Rales or bronchial breath sounds
4. Worsening gas exchange : O_2 desaturations [$P_a O_2 / F_i O_2 \leq 240$] / increased O_2 requirements / increased ventilation demand

The following cases were excluded from the study:

1. Patients who died within 48 hours from the time of admission to the IMCU
2. Patients discharged or went home against medical advice within 48 hours of admission
3. Patients who were diagnosed to have pneumonia during the time of or within 48 hours of admission (Pneumonia in these cases were presumed to have developed from a previous hospital admission or community)

Data collection:

Data collection began from the time of admission to the IMCU and continued until the occurrence of HAP, death or discharge from IMCU whichever occurred first.

On IMCU admission name, age, sex, address, date of admission, diagnosis on admission, underlying illness, presence of immuno compromised state (DM, Malignancies and AIDS), history of smoking and alcoholism were recorded. A thorough general & systemic examination of the patient was also done.

When HAP occurred, the time of onset from hospital admission, temperature, chest radio graphical involvement and leukocyte count were recorded. Intervention – related variables including need for supplemented O_2 & device used, need for mechanical ventilation, suctioning devices used, naso gastric tube placement, stress ulcer prophylaxis, steroids, sedatives and antibiotics actually given for at least 48 hrs were also recorded.

The data on the hospitalization outcome including length of hospital stay and discharged versus mortality was also determined.

Specimens collected:

Sputum

Bronchoscopic aspirate (BAL)

Endotracheal aspirate (ETA)

Blood

For source detection:

Swabs from the patient's environment (i.e. IMCU sites such as wall, floor, trolley, suction tubing, bed & curtains) as well as swabs from the IMCU staff members (i.e. skin, nasal & throat swabs) were collected monthly.

Specimen collection & Transport:

Collection of Sputum:

The patient was instructed to rinse mouth with water before collecting sputum sample. Early morning sputum samples were collected in sterile wide mouthed containers fitted with screw capped lids.

Collection of BAL: [1, 35]

A trained respiratory therapist collected the specimen every time. The tip of the flexible Fibre Optic Bronchoscope (FOB) was positioned close to the segmental area corresponding to radiographic infiltrates. Three aliquots of 50 ml sterile 0.9% Normal saline were instilled. After the injection of each aliquot, gentle aspiration was done through the suction channel. The aspirates pooled in a sterile container were submitted to the laboratory immediately for microscopy & microbiological analysis.

Collection of ETA: [15]

The ETA was collected using a 22-inch Ramson's 12F suction catheter with a mucous extractor, which was gently introduced through the ET tube for a distance of approximately 25-26 cm. Gentle aspiration was

then performed without instilling saline and the catheter was withdrawn from the ET tube. After the catheter was withdrawn, 2ml of sterile 0.9% Normal saline was injected into it with a sterile syringe to flush the exudates into a sterile container for collection. ETA samples were immediately taken to the laboratory for processing.

Collection of Blood sample:

After cleansing the site for venepuncture with Betadine and 70% Alcohol, about 5ml of Blood was collected and added to 50ml of sterile Brain Heart Infusion (BHI) broth in Blood culture bottles. All the specimens were sent to the laboratory immediately after collection.

Collection of swabs from IMCU sites:

Swabs were collected during the time of infection from IMCU sites and staff. Moistened cotton wool swabs were used for collecting samples from the wall, floor and trolley. The swabs were placed in Nutrient Broth and incubated at 37° C overnight. After sub culturing on plates, the isolates were identified by standard laboratory techniques. The samples from bed clothes and curtains were collected by sweep plate method. The Petri dish containing culture medium was removed from its lid and rubbed over the fabrics. The colonies were identified after incubation by standard laboratory techniques.

Safety precautions:

- All samples were considered potentially infectious and leak proof containers were used for collection and transportation of the samples.
- Biological safety cabinet class II was used for carrying out all procedures and protective wears like mask, gloves etc was used.
- Disinfection of the containers by treating with 2.5% Sodium Hypochlorite solution / autoclaving was followed.

Processing of specimens:

Direct Microscopy:

The BAL, ETA and sputum samples were subjected to Gram staining and Potassium Hydroxide (KOH) mount using standard laboratory techniques [66, 68] to assess the quality of the samples for further processing.

Culture Procedures:

The samples were mechanically liquefied and homogenized by vortexing for 1 min and then serially diluted in 0.9% sterile saline solution with final dilutions of 10^{-2}, 10^{-3} and 10^{-4}. The diluted samples were inoculated onto Blood Agar plate (BAP) with 10% sheep blood, Chocolate Agar plate with 10% sheep blood (CAP) and Mac Conkey Agar plate and Sabourauds Dextrose Agar plate (SDA) by using 4mm Nichrome wire loop (Himedia, Mumbai) which holds 0.01ml of solution for quantitative culture.

All plates were incubated overnight at 37° C and CAP at 37° C with 5% CO_2 and one SDA plate was kept at room temperature.

All plates were checked for growth overnight and then after 24 & 48 hrs of incubation. SDA plates were checked for any growth daily for the first week and twice a week up to four week. Growth of any bacterial isolate below the threshold (Table 2) was assumed to be due to colonization or contamination. All the bacterial pathogens with colony count above the diagnostic threshold were identified by colony morphology, microscopy and detailed biochemical testings using standard laboratory techniques. [42, 66]

Table2. Criteria for the assessment of a good quality respiratory sample in HAP. [35, 67]

	ETA	**BAL**	**SPUTUM**
Neutrophils	>25/ LPF	77- 82%	>25/ LPF
SEC	<10/LPF	<1%	<10/ LPF
ICO	No data	≥5%	No data
Quantitative culture threshold (cfu / ml)	$\geq 10^5$-10^6	$\geq 10^4$	$\geq 10^5$-10^6

Antimicrobial susceptibility test:

Antimicrobial susceptibility test of the bacterial isolates was performed on Mueller Hinton Agar (Hi-media, Mumbai) plates by Kirby Bauer's disc diffusion method and antibiotic sensitivity pattern studied according to Clinical Laboratory Standards Institute (CLSI).

Gram negative bacilli were tested for the following antimicrobials (Hi-media, Mumbai):

Antimicrobial agent	Disc content
Gentamicin	10 µg
Amikacin	30 µg
Amoxycillin/Clavulanic acid	20/10 µg
Cotrimoxazole	25 µg
Cephalexin	30 µg
Cefotaxime	30 µg
Ceftazidime	30 µg
Ceftriaxone	30 µg
Cefpodoxime	10 µg
Aztreonam	30 µg
Cefepime	30 µg
Ciprofloxacin	5 µg
Ofloxacin	5 µg
Gatifloxacin	5 µg
Imipenem	10 µg

Gram positive cocci were tested for the following antimicrobials (Hi-media, Mumbai):

Antimicrobial agent	Disc content
Ampicillin	10 µg
Amoxycillin/Clavulanic acid	20/10 µg
Oxacillin	1 µg
Gentamicin	10 µg
Amikacin	30 µg
Cotrimoxazole	25 µg
Cephalexin	30 µg
Cefuroxime	30 µg
Cefotaxime	30 µg
Ceftazidime	30 µg
Ciprofloxacin	5 µg
Ofloxacin	5 µg
Erythromycin	15 µg
Vancomycin	30 µg

Isolates showing inhibition zones ≤22 mm for cetazidime, ≤27 mm for cephotaxime, ≤25 mm for ceftriaxone, ≤22mm for Cefpodoxime and ≤27 mm for Aztreonam were identified as potential ESBL producers [70, 73] and they were confirmed by Double disk potentiation test and Double disk approximation test. [75]

Double disk potentiation test: [85]

Ceftazidime (30mcg, Himedia, Mumbai) and Ceftazidime -Clavulanic acid (30/10mcg, Himedia, Mumbai) discs were placed onto Muellar Hinton Agar (MHA) plates inoculated with a suspension (adjusted to 0.5 McFarland turbidity standard) made from an overnight agar plate of the test strain. Plates were then incubated at 35° C overnight. Regardless of the zone diameter, ≥5 mm increase in a zone diameter for Ceftazidime -Clavulanic acid disc than disc with Ceftazidime alone confirmed the presence of ESBL producers.

Double Disk approximation test: [40, 71]

A disc of Amoxicillin-Clavulanic acid (20/10mcg, Himedia, Mumbai) and a disc of Ceftazidime (30mcg, Himedia, Mumbai) were kept 30mm apart from center to center on Muellar Hinton Agar (MHA) plates inoculated with a suspension (adjusted to 0.5 McFarland turbidity standard) made from an overnight agar plate of the test strain. Plates were then incubated at 35° C overnight.

Enhancement of the zone of inhibition around the Ceftazidime disc towards the Clavulanic acid disc was interpreted as positive for ESBL production.

All cultures of Klebsiella pneumoniae were reconfirmed by colony morphology on MacConkey agar, reaction in TSI agar, the absence of motility, absence of Indole production, Negative Methyl Red test, Positive

Voges-Proskauer test, ability to grow on Simmon's citrate agar and the decarboxylation of lysine but not ornithine. Stock cultures of all Klebsiella pneumoniae were maintained on NA slants.

KLEBOCIN TYPING:

Media used for klebocin production:

Trypticase Soy Broth (Himedia, Mumbai)**:**

Trypticase peptone	17g
Phytone peptone	3g
NaCl	5g
K_2HPO_4	2.5g
Glucose	2.5g
Distilled water	1 liter
pH	7.4g

Media used for klebocin typing:

Nutrient agar (Himedia, Mumbai)**:**

Peptone	10g
Meats extract	10g
Sodium chloride	5g
Agar	20g
Water	1 liter
pH	7.4

Klebsiella stains:

Four Klebocin producer strains, MTCC 109, MTCC 432, MTCC 618 and MTCC 39 were kindly supplied by Institute of Microbial Technology, Sector 39-A, Chandigarh - 160 036, India in the form of freeze dried cultures in ampoules. Two more klebocin producers (L2 and L1) were included in the producer strain set. These last two laboratory strains were selected for typing set using standard procedures as described by Hall et al, [59] Bauerfeind et al [62] and Dykes et al. [63] Collectively, the set of producer strains used in this study comprised of six klebocin producers as follows: MTCC 109 (producer 1), MTCC 432 (producer 2), MTCC 618 (producer 3), MTCC 39 (producer 4), L2 (producer 5) and L1 (producer 6).

The indicator strain isolated in our laboratory, sensitive to all six klebocins was used as a positive control in klebocin typing.

Recovery of freeze dried cultures from ampoule:

The intactness and the identification label of the ampoule were checked initially. The ampoule was marked with a file transversely over the cotton-wool plug. The tip of a glass rod was heated red-hot in a Bunsen flame and applied firmly to the file mark on the ampoule, which could crack at that point. Air was allowed to enter via the plug; then carefully the drawn-out end of the ampoule and the plug were put into a container of disinfectant, for subsequent autoclaving. With aseptic precautions, a small amount of sterile Nutrient Broth was added to the ampoule with a sterile Pasteur pipette, expressing and taking up the broth several times to bring the contents of the

ampoule into suspension. The suspended material in the broth was seeded into Trypticase Soy Broth to ensure recovery of the organism after appropriate incubation.

Induction of klebocin production: [9]

To each broth culture (about 0.7ml) of producer strains, 3.3 ml Trypticase Soy Broth (TSB) and 1 ml of mitomycin C (0.5mcg/ml) was added and incubated at 37° C for 5 hours with intermittent shaking.

The cells were killed by addition of 0.25 ml chloroform. The suspension was centrifuged at 3000 rpm for 10 min in cold centrifuge. Supernatant containing respective klebocins was aliquoted in screw cap vials and stored at -20° C. The purified preparations of klebocins were subjected to SDS-PAGE which showed them to be proteins having two peptides, one of 85kDa and the other of 11kDa.

Klebocin titration: [61]

The klebocins were serially diluted eightfold in Nutrient Broth, ranging from 1:8 to 1:512. With micropipette 0.025ml of each dilution was delivered onto an NA (2% agar) plates (15 by 100 mm) streaked with cotton tipped applicator that had been moistened in a culture of the Indicator strain. This culture was prepared by sub culturing from a work stock culture into 2ml of Nutrient Broth and incubating at 37° C for 6 hours. The plates were incubated overnight at 37° C.

The highest Dilution of klebocin yielding completely clear inhibition of the indicator strain was defined as the Inhibitory Dilution (ID). Control spots

of chloroform treated TSB containing 1µg of mitomycin C per ml showed no inhibition of the indicator strain.

Typing procedure: [9, 61]

The test strains were inoculated by a straight needle from NA slants into 2 ml of Nutrient broth and incubated at 37° C for six hours. These test cultures were swabbed over a nutrient agar plates and 12µl of each klebocin was spot inoculated on marked sectors with a micropipette. The plates were incubated overnight at 37° C.

A positive control plate swabbed with a six hours culture of the indicator strain was included with every typing.

The readings were made by grading the inhibition seen, using a scale of no reaction to 4+.

1+ - a partial inhibition with confluent growth.

2+ - a partial inhibition showing patches of semi confluent growth or more than 10 colonies.

3+ - a clear zone containing no more than 10 distinct colonies.

4+ - a completely clear zone of inhibition.

All reactions that were 1+ or greater were designated as being positive, and those that were less than 1+ were designated as negative. The negative and positive reactions to six klebocins were recorded, using a mnemonics system proposed by Farmer (1976).

The six klebocins were divided into three pairs. Each combination of reactions for a given pair was assigned a mnemonic notation (Table 3). Thus the klebocin type for a given isolate is reported as a mnemonic composed of three numbers

Table 3. Mnemonic system for reporting klebocin sensitivity patterns:[61]

[a] Inhibition of isolate by both klebocins.

[b] Inhibition of isolate by first klebocin and no inhibition by second klebocin.

Reproducibility of klebocin typing:

The reproducibility was determined by using the formula: R = Nr / N, where Nr is the number of isolates assigned the same type on repeat testing and N is the number of isolates tested. [43]

Table 4. Examples of klebocin sensitivity pattern using mnemonic system:

Producer Strains	Example 1 (Type 211)		Example 2 (Type 314)	
	Reaction	Mnemonic notation	Reaction	Mnemonic notation
MTCC 109	+	2	–	3
MTCC 432	–		+	

MTCC 618 MTCC 39	+ +	1	+ +	1
L2 L1	+ +	1	– –	4

RESULTS

During the one-year study period, among 2658 patients admitted to the IMCU, only 2454 cases were followed and included in this study. The remaining 204 cases were excluded. (118 died within 48hrs of admission, 86 were discharged or went home against advice).

Among 2454 patients, 64% of patients (n=1570) were males and 36% (n=884) were females. The mean age was 59.96 with the range of 15 to 89 yrs old. 35% of the patients were more than 60yrs of age. Out of 2454 cases, 253(10.3%) patients developed HAP. The highest incidence of HAP (55.73%) was observed in the age group more than 60 years. (Ref table 5, 6)

The primary reason for IMCU admission was due to neurological events (31.1%), cardiac and pulmonary emergencies (26.0%), acute infections (12.%5), poisoning (5.3%), envenomation (0.5%), etc and the incidence of HAP was greater in patients with diseases requiring prolonged mechanical ventilation and in patients with those diseases that predispose to pulmonary infection such as sepsis and prolonged stay in IMCU.

Out of 1352 patients on mechanical ventilation, 62.0% of patients (n=157) developed HAP and only 38.0% of patients (n=96) developed HAP out of 1102 non ventilated patients. (Ref table 7)

Totally 145 sputum samples, 70 BAL and 38 ETA were collected and processed. Isolates in pure growth or mixture of two organisms at quantitative threshold were considered as significant isolates. All 253 specimens in this study showed significant growth of organisms. About 274 organisms were isolated from 253 samples.

The commonest organism isolated was Klebsiella pneumoniae (48.2%) followed by Pseudomonas aeruginosa (15.3%), E.coli (8.4%), Acinetobactor spp (7.7%), Proteus spp (6.9%), MRSA (6.2%), MSSA (5.1%), Serratia spp (0.7%), Enterobactor spp (0.7%), Strep.pneumoniae(0.4%) and Candida albicans (0.4%). (Ref table 8)

Twenty one samples had showed mixed growth of two organisms likewise Klebsiella pneumoniae and Pseudomonas aeruginosa in 15 cultures (7 Sputum, 6 BAL & 2 ETA), Klebsiella pneumoniae and E.coli in 3 cultures (2 Sputum &1 BAL), Klebsiella pneumoniae and MRSA in 2 cultures (1 Sputum & 1 BAL), Klebsiella pneumoniae and MSSA in 1 sputum culture.

Klebsiella pneumoniae, E.coli and P. aeruginosa had a maximum sensitivity pattern to Imipenem followed by Cefepime, Gatifloxacin and Amikacin. All Gram negative bacterial isolates had 100% sensitivity to Imipenem. Among 132 K.pneumoniae isolates 82% (n=108) were found to be ESBL producers. Among 23 E.coli isolates 52% (n=12) were ESBL producers.

Staphylococcus aureus had a maximum sensitivity to Vancomycin followed by third generation cephalosporins. No Vancomycin resistant

Staph.aureus was detected. 54.84% (n=17) of Staph.aureus were Methicillin Resistant strains. (Ref table 9)

Klebocin typing was performed on a total of 160 K.pneumoniae strains isolated from patients (n=132) and IMCU sites & IMCU staff members (n=28). The overall typability of the strains was 87.5% and only 12.5% of strains were untypable. Nineteen klebocin types were observed. The most common mnemonic type found among patient's isolates was 211 followed by 111, 112 and 441. (Ref table 10) Among the isolates from IMCU sites & staff the common mnemonic type was 211. (Ref table 11)

The Inhibitory Dilution (ID) for all klebocin preparations was 1:256 except MTCC 432 klebocin preparations, for which the ID was 1:128.

With the exception of klebocin preparations from MTCC 432, the reproducibility of klebocin typing was 83% even after four weeks of storage at -20°C.

Klebocin typing and antibiogram of patient's isolates was compared with those from IMCU sites & staff, isolated during the period of infection. In 101 (76.51%) cases, the mnemonic types as well as antibiogram of Klebsiella pneumoniae isolates from patients matched with those of IMCU sites and staff. All the klebocin type 211 of both patient's and environmental isolates were 100% sensitive to Imipenem, 97% sensitive to Cefipime and Fluroquinolones, 95% sensitive to Amikacin and 76% sensitive to Gentamicin. 100% of untypable strains (444) (15 patient's isolate and 5 environmental isolates)

were sensitive to Imipenem, 93% sensitive to Amikacin, Cefipime, Ofloxacin and Gatifloxacin, 80% to Ciprofloxacin. The klebocin type 111 which was isolated from suction tube had similar antibiotic pattern like that of Patient's isolates (n=9). All klebocin type 111 were resistant to Cotrimoxazole, Cephalexin, Cefuroxime, Cefotaxime, Ceftazidime and Ceftriaxone and 100% sensitive to Imipenem. This antibiotic sensitivity pattern was similar to that of klebocin type 112 (1 from throat of the IMCU staff and 7 from HAP patients). (Ref table 12)

Table 5. Age -wise distribution of patients.

Age (yrs)	Total patients	Patients with HAP (n) (%)
0-15	10	0(0)
16-30	349	7(2.77)
31-40	414	13(5.14)
41-60	882	92(36.36)
>60	859	141(55.73)
Total	2454	253

Table 6. Age and sex- wise distribution of patients.

Age(yrs)	Male		Female	
	Total	With HAP	Total	With HAP
<15	6	-	4	-
16-30	223	5	126	2
31-40	288	9	126	4
41-60	551	59	271	33
>60	502	102	357	39
Total	1570	175	884	78

Table 7. Influence of mechanical ventilation on HAP.

Ventilator	Total	Patients with HAP (%)
Yes	1352	157(62.0)
No	1102	96(38.0)
Total	2454	253

Table 8. Pathogens isolated from HAP patients.

Pathogens isolated	No of isolates (n=274) (%)
K.pneumoniae	132 (48.2)
P.aeruginosa	42 (15.2)
E.coli	23 (8.4)
Proteus spp	19 (6.9)
Acinetobactor spp.	21 (7.7)
Serratia	2 (0.7)
Enterobactor spp.	2 (0.7)
MSSA	14 (5.1)
MRSA	17 (6.2)
Strep. pneumoniae	1 (0.4)
Candida albicans	1 (0.4)

Table 9. Antimicrobial susceptibility pattern of Bacterial isolates.

ANTIMICROBIAL AGENT Disc content(µg)	K.pneumoniae (132)	P.aeroginosa (42)	E.coli (23)	Acinetobactor(21)	Proteus sp (19)	Serratia (2)	Enterobactor sp (2)	Staph.aureus (31)	Str. Pneumoniae (1)
Gentamicin (10)	75	21	13	13	15	2	1	14	1
Amikacin (30)	121	27	22	17	19	2	2	19	1
Ampicillin (10)	-	-	-	-	-	-	-	16	1
Oxacillin (1)	-	-	-	-	-	-	-	14	1
AmoxyClav(20/10)	29	0	7	-	-	-	-	24	1
Cotrimoxazole(25)	44	4	11	4	11	1	2	7	0
Cephalexin(30)	8	16	8	12	10	1	0	26	1
Cefuroxime(30)	8	16	8	12	10	2	1	26	1
Cefotaxime(30)	21	39	13	21	18	2	2	29	1
Ceftazidime(30)	21	39	14	21	19	2	2	27	1
Cefepime(30)	127	42	23	21	19	2	2	-	-
Ciprofloxacin(5)	102	30	12	12	17	2	1	23	1
Ofloxacin(5)	123	31	12	17	17	2	2	25	1
Gatifloxacin(5)	127	40	20	18	19	2	2	-	-
Imipenem(10)	132	42	23	21	19	2	2	-	-
Erythromycin(15)	-	-	-	-	-	-	-	26	1
Vancomycin(30)	-	-	-	-	-	-	-	31	1

Table 10. Mnemonic types of Klebsiella pneumoniae isolates.

Mnemonic types	Patient's strains	IMCU sites	IMCU Staff	Total no. of strains (%)
211	70	12	9	91(56.8%)
444	15	2	3	20(12.5%)
111	9	1	–	10(6.2%)
112	7	–	1	8(5.0%)
441	6	–	–	6(3.7%)
311	4	–	–	4(2.5%)
241	3	–	–	3(1.8%)
314	3	–	–	3(1.8%)
414	2	–	–	2(1.2%)
341	2	–	–	2(1.2%)
243	2	–	–	2(1.2%)
312	2	–	–	2(1.2%)
221	1	–	–	1(0.6%)
231	1	–	–	1(0.6%)
141	1	–	–	1(0.6%)
332	1	–	–	1(0.6%)
411	1	–	–	1(0.6%)
412	1	–	–	1(0.6%)
244	1	–	–	1(0.6%)
Total no. of strains	132	15	13	160
Typability	88.64%	86.66%	76.92%	87.5%

Table 11. Source and mnemonic types of Klebsiella pneumoniae.

Mnemonic type	IMCU staff			IMCU sites			
	Throat	Hand	Nose	Suction tube	Trolley	Wall	Curtains
211	5	3	1	8	3	-	1
444	2	1	-	-	1	1	-
111	-	-	-	1	-	-	-
112	1	-	-	-	-	-	-
Total	8	4	1	9	4	1	1

Table 12. Antimicrobial sensitivity pattern and klebocin type of Klebsiella pneumoniae isolates from HAP Patients and IMCU sites & staff.

ANTIMICROBIAL AGENT Disc content(µg)	Klebocin Type							
	211		444		111		112	
	Patient's(70)	IMCU(21)	Patient's(15)	IMCU (5)	Patient's(9)	IMCU(1)	Patient's(7)	IMCU(1)
Gentamicin (10)	53	16	8	3	7	1	4	1
Amikacin (30)	67	20	14	4	8	1	5	1
AmoxyClav(20/10)	16	5	8	3	4	1	3	1
Cotrimoxazole(25)	40	12	6	2	0	0	0	0
Cephalexin(30)	3	1	2	1	0	0	0	0
Cefuroxime(30)	3	1	2	1	0	0	0	0
Cefotaxime(30)	7	2	8	3	0	0	0	0
Ceftazidime(30)	7	2	8	3	0	0	0	0
Cefepime(30)	68	20	14	4	7	1	7	1
Ciprofloxacin(5)	67	20	12	4	6	1	6	1
Ofloxacin(5)	68	20	14	4	6	1	6	1
Gatifloxacin(5)	68	20	14	4	8	1	7	1
Imipenem(10)	70	21	15	5	9	1	7	1

DISCUSSION

. The present study showed that the incidence of HAP was 10.3% (n=253) out of 2454 cases admitted in IMCU, Coimbatore Medical College Hospital over a period of one year.

This incidence was lower than the study by Mukhopadhyay et al from Lucknow (53.9%), [18] Rakshit et al from Mumbai (47%), [13] Vincent et al from Europe (46.9%), [25] Dey et al from Manipal (45.4%), [15] Sopena et al from Spain (36.4%), [30] Berba et al from Philadelphia (28.2%) [33] and Merchant et al from Mumbai (16.7%). [19] This was higher than the incidence reported by Chevret et al from France (8.9%), [29] Alp et al from Netherlands(6.8%), [32] Trivedi et al from Mumbai (9.38%)[16] and Pawar et al from New Delhi (2.6%). [14]

It is possible that our incidence rate may be an over estimate of the HAP in the hospital because of the nature of the clinical criteria used. Studies based solely on clinical criteria alone are criticized because of the non-specificity of parameters like fever, leukocytosis and infiltrates on the chest radiographs. However, the stringent steps followed to make a diagnosis of HAP in this study and the close monitoring before and after the diagnosis of HAP occurred should make our estimate very close to the true HAP incidence. It is unlikely that a true HAP case would have been missed because we did quantitative culture of all specimens (BAL, Sputum and ETA) to discriminate between the true pathogen and the contaminant using the diagnostic threshold for each specimen.

High occurrence (55.73%) of HAP among the age group more than 60 years was observed in the present study. This could be due to the bulk of the study population in this study was more 60 years of age group. This was in accordance with an earlier study by Berba et al. [33] Age more than 60 years is one of the known risk factor for the development of HAP as reported in previous studies. [1, 27] But Muhammad et al reported that highest incidence was among 41 to 60 years of age group. [22]

The present study showed that the incidence of HAP was high among male patients than females. This finding was similar to the study by Mukhopadhyay et al from Lucknow. [18] But Berba et al showed that the male sex had a protective effect against the development of HAP. [33] Dey et al reported that gender had no significant role in the development of HAP. [15]

In the present study the incidence of HAP was greater in patients with diseases requiring prolonged mechanical ventilation like OP poisoning (15.0%) and considerably low in patients with diseases which presumably, had unaffected lungs before admission to IMCU like snake bite (0.4%). These findings were similar to the previous studies. [13, 15]

HAP developed in 157 out of the 1352 patients (62.0%) receiving mechanical ventilation but in only 96 out of 1102 patients (38.0%) with no mechanical ventilation. This incidence of VAP (62.0%) was higher than the study by Muhammad et al (30.7%) [22] and lower than the study by Alp et al who showed that 75.5% of all patients with HAP were VAP. [32]

Mechanical ventilation is a definitive risk factor for developing HAP that has been shown previously by many studies [15, 18] and this study also shows the significance of that risk factor causing HAP.

Klebsiella pneumoniae (48.2%) was the commonest organism isolated in this study. Most of the previous studies reported Pseudomonas aeruginosa as the commonest isolate from HAP patients in IMCU. [35, 14, 13, 18] But Pseudomonas aeruginosa was the second common organism in the present study. Acinetobacter spp (7.7%) was the fourth common isolate in this study. Dey et al, Rajasekhar et al and Alp et al reported that Acinetobacter spp as the commonest organism in their study. [15, 23, 32] E. coli was the commonest organism in the study by Tullu et al. [17] It was third commonest organism in the present study. These findings indicate that the causative pathogens always vary in different setups. The present study suggests that the colonization rate for Klebsiella pneumoniae may be higher in IMCU.

The rate of polymicrobial infection was found to be only 8.3% in this study which was lower than the study by Mukhopadhyay et al (16.3%) [18] and Singhal et al (12.3%). [83] The lower rate of colonization of IMCU environment by more than one type of organisms may be the reason for the lower incidence of polymicrobial infection.

Antimicrobial resistance among Gram negative bacilli is increasing worldwide and is of particular concern in the Intensive Care Unit setting. A direct correlation has been shown between resistance of Gram negative bacilli and patient mortality, cost of patient care and length of stay in the hospital. [76] In a study by Kaul et al about the Gram- negative bacterial antibiotic susceptibility patterns in IMCU, Christian Medical College, Vellore showed that Klebsiella resistance to cefotaxime and ceftazidime ranged from 25-50% and 14-91%, while E.coli resistance to these antibiotics ranged from 50-70% and 50-80% respectively. [81] In this study Klebsiella resistance to cefotaxime and ceftazidime was 84% and E.coli resistance to these antibiotics were 43% and 39% respectively. The resistance of K.pneumoniae and E.coli to third generation cephalosporins was higher in this study.

All isolates of Acinetobacter, Serratia and Enterobactor were sensitive to third generation cephalosporins. 92% of Pseudomonas aeruginosa and 94% of Proteus were sensitive to cefotaxime and ceftazidime. These findings were similar to the study by Kaul et al from Christian Medical College, Vellore, who reported that in Pseudomonas aeruginosa and the other non-fermenting gram-negative bacteria (NFGNB) Ceftazidime resistance decreased. Among Aminoglycosides, most of the GNB were sensitive to Amikacin than Gentamicin. Highest sensitivity rates were detected for Gatifloxacin than Ciprofloxacin and Ofloxacin among Quinolones. These findings were similar to previous studies on antimicrobial resistance among gram-negative bacteria by many authors. [21, 22, 23, 39, 64]

All Gram negative bacilli isolated in this study had a maximum sensitivity pattern to Imipenem and Cefepime. This was similar to the study by Lockhart et al about antimicrobial resistance among Gram-Negative Bacilli causing infections in Intensive Care Unit patients in the United States between 1993 and 2004 [76] and Rakshit et al from Mumbai. [13]

Gram-negative bacilli producing ESBL appear to be on the rise in Asian countries and pose a serious problem in pulmonary infections. [80] In the present study the occurrence of ESBL production among K.pneumoniae and E.coli were 82% and 52% respectively. For Klebsiella pneumoniae this finding was higher than the study by Feizabadi et al (72.8%), [74] Gonlugur et al (12.2%), [39] Hosoglu et al (68.3%), [72] Asian HAP Working group (0.9% to 40%) [80] and lower than the study by Verhamme et al (100%), [79] Vitkauskiene et al (88.9%), [84] Dey et al (100%). [15] For E.coli this finding was higher than the study by Gonlugur et al (20.8%), [39] Asian HAP Working group (2.3% to 40%) [80] and lower than the study by Hosoglu et al (74.6%), [72] Dey et al (100%). [15]

In this study ESBLs were predominantly present among K. pneumoniae compared to E. coli. Our findings are similar to that of most of the studies in Europe and USA [38] and the Indian studies by Jain et al [64] and Shanmuganathan et al. [65] But studies by Gonlugur et al, [39] Hosoglu et al [72] and Kumar et al [82] showed higher incidence of ESBLs among E.coli than K.pneumoniae. The indiscriminate use of third-generation cephalosporins has been proposed as a reason for the rise of ESBL producing strains in India. [80]

Among the Staphylococcus aureus isolates in this study, the occurrence of Methicillin resistance was observed in 54.84%. This was higher than the study by Leroy et al (33%) [35] and lower than the study by Mukhopadhyay et al (59.45%) [18] and Rakshit et al (100%). [13] No Vancomycin resistant Staphylococcus aureus was detected in the present study.

Epidemiological investigation of nosocomial infections is greatly facilitated by the use of a precise typing system for the organism involved. Our study about the hospital acquired pneumonia in IMCU suggests a high prevalence of Klebsiella pneumoniae accounting for 48.2% of total cases. In the present study, we have used Klebocin typing and antibiotic sensitivity profiles as the typing methods for the detection of source of Klebsiella infections in our IMCU. Although not comparable to molecular methods in discriminatory power, antibiogram and klebocin typing are simpler and relatively economical method of bacteriological typing.

In the present study all Klebsiella pneumoniae isolates were subjected to klebocin typing by spot inoculation method as described by Pal et al who done the klebocin typing in their study by spot inoculation method which was described by Chugh et al with some modifications. Using the spot inoculation method, we observed 87.5% typability, whereas Buffenmyer et al, [61] Chibber et al, [60] Malik et al, [43] Aggarwal et al [44] and Pal et al [9] reported 67%, 72.8%, 83.3%, 73.5% and 71.62% typability respectively. Bauernfeind et al ,[62] Hall, [59] Israil [46] and Podschun et al [58] have reported 96.3%, 77%,

85% and 96% typability respectively using streak and point method for klebocin typing.

Despite the relatively higher typability observed in streak and point method, we followed the spot inoculation method. In view of the high induction of klebocins by mitomycin C, storage stability at -20°C and simplicity of the technique, spot inoculation method is more appropriate typing method.[9]

Nineteen klebocin types were observed in the present study using six klebocin producer strains. Pal et al reported that twenty eight klebocin types had been observed in their study using six klebocin producer strains [9] whereas Malik et al observed four patterns of klebocin types in their study with six klebocin producer strains. [43]

We observed the klebocin types 211 (56.8%), 111 (6.2%), 112 (5.0%) and 441 (3.7%) were the most common types in our study. According to Buffenmyer et al and other previous studies type 444 was considered as untypable and these strains accounted for 12.5% of the total strains studied. Pal et al reported types 111 (50%), 211 (5.59%), 312 (3.08%) and 112 (2.31%) as the commonest klebocin types in their study and 28.37% strains could not be typed. [9]

Malik et al found type 312 (43.4%) as the commonest followed by type 334 (23.4%), type 112 (16.6%) while remaining 16.6% strains could not be typed.

Chibber et al reported the predominant klebocin types as 244 (14.3%), 313 (13.7%) and 113 (7.6%). [60] Aggarwal et al observed the commonest klebocin types as 111, 313 and 113. Probably there is variation in the prevalence of various klebocin types in different topographical areas and institutions. Moreover the set of producer strains used in the present study was different from other studies in which they had used a set of producer strains standardized by them in their own setups. It is therefore difficult to compare our typing patterns with other studies.

In the present study, we found that by combination of klebocin types and antibiogram of K.pneumoniae isolates, the source of infection could be traced in 76.51% cases. In a study on klebocin typing by Chibber et al [60] no significant correlation was observed between the source of isolation and the klebocin type whereas Pal et al [9] observed when the klebocin typing was used in association with antibiogram, in 86.84% cases probable source of infection could be detected.

Despite the importance of the findings presented in this study, there are some limitations that should be taken into consideration when assessing our conclusions. First, although for the diagnosis of HAP we did quantitative culture of Sputum, BAL and ETA, yet the results from this study need to be validated by comparison to gold standards such as histology of lung tissue.

Last, in the present study the reproducibility of the klebocin typing was 83%. This was higher than the previous study by Malik et al who showed in their study that the reproducibility of the klebocin typing was 73.3%. [43] This is of major concern because a useful and effective epidemiological typing system should have high reproducibility. In our study the reproducibility may be a major problem with the klebocin typing procedure. This difficulty was primarily due to the sensitivity of the test strains to MTCC 432 klebocin and was presumably associated with their low titers. This problem was a serious shortcoming since, as shown in Table 10 the commonest type in our study was 211. This pattern of typing was due to lack of sensitivity of the clinical isolates to MTCC 432 klebocin (producer 2). Replacing the producer strain of this klebocin with the strain that yields high-titer preparations may help in achieving acceptable reproducibility. Such strains may be obtained by screening Klebsiella pneumoniae isolates for klebocin activity or testing producer strains of other investigators.

In spite of these problems, this study proposed the klebocin typing in association with antibiogram as a tool for epidemiological typing of Klebsiella pneumoniae isolates in the laboratories where the facilities to perform genomic based molecular typing are not available. The advantages of klebocin typing include (i) the ready availability of required materials in most bacteriological laboratories, (ii) the ability to perform the typing procedure within the usual work day, and (iii) the ability to store the klebocins for future use.

SUMMARY

- 2454 cases admitted in IMCU, Coimbatore Medical College Hospital, Coimbatore were studied over a period of one year from May 1, 2007 to April 30, 2008.
- 1570 males and 884 females were in the study.
- The overall incidence of HAP was 10.3%.
- The incidence of VAP was 62.0%.
- The incidence of HAP was higher (55.73%) in the age group more than 60 years.
- The 274 organisms were isolated from 253 lower respiratory tract samples.
- The incidence of polymicrobial infection was 8.3%.
- Klebsiella pneumoniae (48.2%) was the commonest organism isolated. This was followed by Pseudomonas aeruginosa (15.3%), E.coli (8.4%), Acinetobactor spp (7.7%), Proteus spp (6.9%), MRSA (6.2%), and MSSA (5.1%).
- All Gram-negative bacilli were 100% sensitive to Imipenem.
- All Gram-negative bacilli were 100% sensitive to Cefepime and Gatifloxacin except Klebsiella pneumoniae which was 96% sensitive to

Cefepime and Gatifloxacin, Pseudomonas aeruginosa which was 95% sensitive to Gatifloxacin and E.coli which was 87% sensitive to Gatifloxacin.

- 92% of Pseudomonas aeruginosa and 94% of Proteus were sensitive to third generation cephalosporins.
- The screening for the ESBL producing GNB was done with Cetazidime, Cephotaxime, Ceftriaxone, Cefpodoxime and Aztreonam by Kirby Bauer's disc diffusion method.
- The confirmation of ESBL production was done by Double disk potentiation test and Double disk approximation test.
- 82% of K.pneumoniae and 52% of E.coli were extended spectrum beta-lactamase producing strains.
- Staphylococcus aureus was 100% sensitive to Vancomycin.
- 54.84% of Staphylococcus aureus were Methicillin Resistant strains.
- Number of Klebsiella pneumoniae isolated from IMCU sites and Staff was 28.
- Induction of klebocin production from six producer strains was done with mitomycin C.
- Presence of klebocins was confirmed by SDS-PAGE.

- Klebocin typing was done for a total of 160 K.pneumoniae isolated from HAP patients and IMCU sites & staff.
- Nineteen klebocin types were observed using six klebocin producer strains.
- The typability of klebocin typing was 87.5% by the spot inoculation method.
- 211 (56.8%) was the commonest klebocin type followed by 111 (6.2%), 112 (5.0%) and 441 (3.7%).
- The untypable strain (444) accounted for 12.5%.
- The reproducibility of the klebocin typing was 83%.
- The source of Klebsiella infection was detected in 86.84% of cases using klebocin typing and antibiogram.
- The commonest source of Klebsiella infection in our IMCU were suction tube (32%), throat of the IMCU staff (29 %), hands of the IMCU staff (14%) and trolley (14%).

CONCLUSION

This one year prospective study on 2454 cases admitted in IMCU, Coimbatore Medical College Hospital was conducted in order to study the incidence, the Clinical and Bacteriological profile of HAP, the antibiotic susceptibility pattern of the bacterial isolates, the role of Klebocin typing in epidemiological typing of K.pneumoniae strains and the source of infection in IMCU using antibiogram and klebocin typing.

The incidence of HAP was 10.3% with the occurrence being most in the age group more than 60 years. Mechanical ventilation was a important risk factor for the development of HAP. Klebsiella pneumoniae, Pseudomonas aeruginosa, E.coli, Acinetobacter, Proteus spp, and MRSA were the most common bacterial isolates.

The most effective antibiotics against the GNB isolated from HAP patients were Imipenem followed by Cefepime and Gatifloxacin. The antibiotic susceptibility pattern of the isolates will help the clinicians to choose the appropriate antimicrobial agents for prophylactic as well as treatment purposes.

When klebocin typing was used in association with antibiogram, in 86.84% cases of nosocomial Klebsiella infection probable source of infection could be detected. Thus a combination of these two typing methods poses a contribution in epidemiological studies.

RESULTS

During the one-year study period, among 2658 patients admitted to the IMCU, only 2454 cases were followed and included in this study. The remaining 204 cases were excluded. (118 died within 48hrs of admission, 86 were discharged or went home against advice).

Among 2454 patients, 64% of patients (n=1570) were males and 36% (n=884) were females. The mean age was 59.96 with the range of 15 to 89 yrs old. 35% of the patients were more than 60yrs of age. Out of 2454 cases, 253(10.3%) patients developed HAP. The highest incidence of HAP (55.73%) was observed in the age group more than 60 years. (Ref table 5, 6)

The primary reason for IMCU admission was due to neurological events (31.1%), cardiac and pulmonary emergencies (26.0%), acute infections (12.%5), poisoning (5.3%), envenomation (0.5%), etc and the incidence of HAP was greater in patients with diseases requiring prolonged mechanical ventilation and in patients with those diseases that predispose to pulmonary infection such as sepsis and prolonged stay in IMCU.

Out of 1352 patients on mechanical ventilation, 62.0% of patients (n=157) developed HAP and only 38.0% of patients (n=96) developed HAP out of 1102 non ventilated patients. (Ref table 7)

Totally 145 sputum samples, 70 BAL and 38 ETA were collected and processed. Isolates in pure growth or mixture of two organisms at quantitative threshold were considered as significant isolates. All 253 specimens in this study showed significant growth of organisms. About 274 organisms were isolated from 253 samples.

The commonest organism isolated was Klebsiella pneumoniae (48.2%) followed by Pseudomonas aeruginosa (15.3%), E.coli (8.4%), Acinetobactor spp (7.7%), Proteus spp (6.9%), MRSA (6.2%), MSSA (5.1%), Serratia spp (0.7%), Enterobactor spp (0.7%), Strep.pneumoniae(0.4%) and Candida albicans (0.4%). (Ref table 8)

Twenty one samples had showed mixed growth of two organisms likewise Klebsiella pneumoniae and Pseudomonas aeruginosa in 15 cultures (7 Sputum, 6 BAL & 2 ETA), Klebsiella pneumoniae and E.coli in 3 cultures (2 Sputum &1 BAL), Klebsiella pneumoniae and MRSA in 2 cultures (1 Sputum & 1 BAL), Klebsiella pneumoniae and MSSA in 1 sputum culture.

Klebsiella pneumoniae, E.coli and P. aeruginosa had a maximum sensitivity pattern to Imipenem followed by Cefepime, Gatifloxacin and Amikacin. All Gram negative bacterial isolates had 100% sensitivity to Imipenem. Among 132 K.pneumoniae isolates 82% (n=108) were found to be ESBL producers. Among 23 E.coli isolates 52% (n=12) were ESBL producers.

Staphylococcus aureus had a maximum sensitivity to Vancomycin followed by third generation cephalosporins. No Vancomycin resistant Staph.aureus was detected. 54.84% (n=17) of Staph.aureus were Methicillin Resistant strains. (Ref table 9)

Klebocin typing was performed on a total of 160 K.pneumoniae strains isolated from patients (n=132) and IMCU sites & IMCU staff members (n=28). The overall typability of the strains was 87.5% and only 12.5% of strains were untypable. Nineteen klebocin types were observed. The most common mnemonic type found among patient's isolates was 211 followed by 111, 112 and 441. (Ref table 10) Among the isolates from IMCU sites & staff the common mnemonic type was 211. (Ref table 11)

The Inhibitory Dilution (ID) for all klebocin preparations was 1:256 except MTCC 432 klebocin preparations, for which the ID was 1:128.

With the exception of klebocin preparations from MTCC 432, the reproducibility of klebocin typing was 83% even after four weeks of storage at -20°C.

Klebocin typing and antibiogram of patient's isolates was compared with those from IMCU sites & staff, isolated during the period of infection. In 101 (76.51%) cases, the mnemonic types as well as antibiogram of Klebsiella pneumoniae isolates from patients matched with those of IMCU sites and staff. All the klebocin type 211 of both patient's and environmental isolates were 100% sensitive to Imipenem, 97% sensitive to Cefipime and

Fluroquinolones, 95% sensitive to Amikacin and 76% sensitive to Gentamicin. 100% of untypable strains (444) (15 patient's isolate and 5 environmental isolates) were sensitive to Imipenem, 93% sensitive to Amikacin, Cefipime, Ofloxacin and Gatifloxacin, 80% to Ciprofloxacin. The klebocin type 111 which was isolated from suction tube had similar antibiotic pattern like that of Patient's isolates (n=9). All klebocin type 111 were resistant to Cotrimoxazole, Cephalexin, Cefuroxime, Cefotaxime, Ceftazidime and Ceftriaxone and 100% sensitive to Imipenem. This antibiotic sensitivity pattern was similar to that of klebocin type 112 (1 from throat of the IMCU staff and 7 from HAP patients). (Ref table 12)

Table 5. Age -wise distribution of patients.

Age (yrs)	Total patients	Patients with HAP (n) (%)
0-15	10	0(0)
16-30	349	7(2.77)
31-40	414	13(5.14)
41-60	882	92(36.36)
>60	859	141(55.73)
Total	2454	253

Table 6. Age and sex- wise distribution of patients.

Age(yrs)	Male		Female	
	Total	With HAP	Total	With HAP
<15	6	-	4	-
16-30	223	5	126	2
31-40	288	9	126	4
41-60	551	59	271	33
>60	502	102	357	39
Total	1570	175	884	78

Table 7. Influence of mechanical ventilation on HAP.

Ventilator	Total	Patients with HAP (%)
Yes	1352	157(62.0)
No	1102	96(38.0)
Total	2454	253

Table 8. Pathogens isolated from HAP patients.

Pathogens isolated	No of isolates (n=274) (%)
K.pneumoniae	132 (48.2)
P.aeruginosa	42 (15.2)

E.coli	23 (8.4)
Proteus spp	19 (6.9)
Acinetobactor spp.	21 (7.7)
Serratia	2 (0.7)
Enterobactor spp.	2 (0.7)
MSSA	14 (5.1)
MRSA	17 (6.2)
Strep. pneumoniae	1 (0.4)
Candida albicans	1 (0.4)

Table 9. Antimicrobial susceptibility pattern of Bacterial isolates.

ANTIMICROBIAL AGENT Disc content(µg)	K.pneumoniae (132)	P.aeroginosa (42)	E.coli (23)	Acinetobactor(21)	Proteus sp (19)	Serratia (2)	Enterobactor sp (2)	Staph.aureus (31)	Str. Pneumoniae (1)
Gentamicin (10)	75	21	13	13	15	2	1	14	1
Amikacin (30)	121	27	22	17	19	2	2	19	1
Ampicillin (10)	-	-	-	-	-	-	-	16	1
Oxacillin (1)	-	-	-	-	-	-	-	14	1
AmoxyClav(20/10)	29	0	7	-	-	-	-	24	1
Cotrimoxazole(25)	44	4	11	4	11	1	2	7	0

Cephalexin(30)	8	16	8	12	10	1	0	26	1
Cefuroxime(30)	8	16	8	12	10	2	1	26	1
Cefotaxime(30)	21	39	13	21	18	2	2	29	1
Ceftazidime(30)	21	39	14	21	19	2	2	27	1
Cefepime(30)	127	42	23	21	19	2	2	-	-
Ciprofloxacin(5)	102	30	12	12	17	2	1	23	1
Ofloxacin(5)	123	31	12	17	17	2	2	25	1
Gatifloxacin(5)	127	40	20	18	19	2	2	-	-
Imipenem(10)	132	42	23	21	19	2	2	-	-
Erythromycin(15)	-	-	-	-	-	-	-	26	1
Vancomycin(30)	-	-	-	-	-	-	-	31	1

Table 10. Mnemonic types of Klebsiella pneumoniae isolates.

Mnemonic types	Patient's strains	IMCU sites	IMCU Staff	Total no. of strains (%)
211	70	12	9	91(56.8%)
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312	2	–	–	2(1.2%)
221	1	–	–	1(0.6%)
231	1	–	–	1(0.6%)
141	1	–	–	1(0.6%)
332	1	–	–	1(0.6%)
411	1	–	–	1(0.6%)
412	1	–	–	1(0.6%)
244	1	–	–	1(0.6%)
Total no. of strains	132	15	13	160
Typability	88.64%	86.66%	76.92%	87.5%

Table 11. Source and mnemonic types of Klebsiella pneumoniae.

Mnemonic type	IMCU staff			IMCU sites			
	Throat	Hand	Nose	Suction tube	Trolley	Wall	Curtains
211	5	3	1	8	3	-	1
444	2	1	-	-	1	1	-
111	-	-	-	1	-	-	-
112	1	-	-	-	-	-	-
Total	8	4	1	9	4	1	1

Table 12. Antimicrobial sensitivity pattern and klebocin type of Klebsiella pneumoniae isolates from HAP Patients and IMCU sites & staff.

ANTIMICROBIAL AGENT Disc content(µg)	Klebocin Type							
	211		444		111		112	
	Patient's(70)	IMCU(21)	Patient's(15)	IMCU (5)	Patient's(9)	IMCU(1)	Patient's(7)	IMCU(1)
Gentamicin (10)	53	16	8	3	7	1	4	1
Amikacin (30)	67	20	14	4	8	1	5	1
AmoxyClav(20/10)	16	5	8	3	4	1	3	1
Cotrimoxazole(25)	40	12	6	2	0	0	0	0
Cephalexin(30)	3	1	2	1	0	0	0	0
Cefuroxime(30)	3	1	2	1	0	0	0	0
Cefotaxime(30)	7	2	8	3	0	0	0	0
Ceftazidime(30)	7	2	8	3	0	0	0	0
Cefepime(30)	68	20	14	4	7	1	7	1
Ciprofloxacin(5)	67	20	12	4	6	1	6	1
Ofloxacin(5)	68	20	14	4	6	1	6	1
Gatifloxacin(5)	68	20	14	4	8	1	7	1

Imipenem(10)	70	21	15	5	9	1	7	1

DISCUSSION

. The present study showed that the incidence of HAP was 10.3% (n=253) out of 2454 cases admitted in IMCU, Coimbatore Medical College Hospital over a period of one year.

This incidence was lower than the study by Mukhopadhyay et al from Lucknow (53.9%), [18] Rakshit et al from Mumbai (47%), [13] Vincent et al from Europe (46.9%), [25] Dey et al from Manipal (45.4%), [15] Sopena et al from Spain (36.4%), [30] Berba et al from Philadelphia (28.2%) [33] and Merchant et al from Mumbai (16.7%). [19] This was higher than the incidence reported by Chevret et al from France (8.9%), [29] Alp et al from Netherlands(6.8%), [32] Trivedi et al from Mumbai (9.38%)[16] and Pawar et al from New Delhi (2.6%). [14]

It is possible that our incidence rate may be an over estimate of the HAP in the hospital because of the nature of the clinical criteria used. Studies based solely on clinical criteria alone are criticized because of the non-specificity of parameters like fever, leukocytosis and infiltrates on the chest radiographs. However, the stringent steps followed to make a diagnosis of HAP in this study and the close monitoring before and after the diagnosis of HAP occurred should make our estimate very close to the true HAP incidence. It is unlikely that a true HAP case would have been missed because we did quantitative culture of all specimens (BAL, Sputum and

ETA) to discriminate between the true pathogen and the contaminant using the diagnostic threshold for each specimen.

High occurrence (55.73%) of HAP among the age group more than 60 years was observed in the present study. This could be due to the bulk of the study population in this study was more 60 years of age group. This was in accordance with an earlier study by Berba et al. [33] Age more than 60 years is one of the known risk factor for the development of HAP as reported in previous studies. [1, 27] But Muhammad et al reported that highest incidence was among 41 to 60 years of age group. [22]

The present study showed that the incidence of HAP was high among male patients than females. This finding was similar to the study by Mukhopadhyay et al from Lucknow. [18] But Berba et al showed that the male sex had a protective effect against the development of HAP. [33] Dey et al reported that gender had no significant role in the development of HAP. [15]

In the present study the incidence of HAP was greater in patients with diseases requiring prolonged mechanical ventilation like OP poisoning (15.0%) and considerably low in patients with diseases which presumably, had unaffected lungs before admission to IMCU like snake bite (0.4%). These findings were similar to the previous studies. [13, 15]

HAP developed in 157 out of the 1352 patients (62.0%) receiving mechanical ventilation but in only 96 out of 1102 patients (38.0%) with no mechanical ventilation. This incidence of VAP (62.0%) was higher than the

study by Muhammad et al (30.7%) [22] and lower than the study by Alp et al who showed that 75.5% of all patients with HAP were VAP. [32]

Mechanical ventilation is a definitive risk factor for developing HAP that has been shown previously by many studies [15, 18] and this study also shows the significance of that risk factor causing HAP.

Klebsiella pneumoniae (48.2%) was the commonest organism isolated in this study. Most of the previous studies reported Pseudomonas aeruginosa as the commonest isolate from HAP patients in IMCU. [35, 14, 13, 18] But Pseudomonas aeruginosa was the second common organism in the present study. Acinetobacter spp (7.7%) was the fourth common isolate in this study. Dey et al, Rajasekhar et al and Alp et al reported that Acinetobacter spp as the commonest organism in their study. [15, 23, 32] E. coli was the commonest organism in the study by Tullu et al. [17] It was third commonest organism in the present study. These findings indicate that the causative pathogens always vary in different setups. The present study suggests that the colonization rate for Klebsiella pneumoniae may be higher in IMCU.

The rate of polymicrobial infection was found to be only 8.3% in this study which was lower than the study by Mukhopadhyay et al (16.3%) [18] and Singhal et al (12.3%). [83] The lower rate of colonization of IMCU environment

by more than one type of organisms may be the reason for the lower incidence of polymicrobial infection.

Antimicrobial resistance among Gram negative bacilli is increasing worldwide and is of particular concern in the Intensive Care Unit setting. A direct correlation has been shown between resistance of Gram negative bacilli and patient mortality, cost of patient care and length of stay in the hospital. [76] In a study by Kaul et al about the Gram- negative bacterial antibiotic susceptibility patterns in IMCU, Christian Medical College, Vellore showed that Klebsiella resistance to cefotaxime and ceftazidime ranged from 25-50% and 14-91%, while E.coli resistance to these antibiotics ranged from 50-70% and 50-80% respectively. [81] In this study Klebsiella resistance to cefotaxime and ceftazidime was 84% and E.coli resistance to these antibiotics were 43% and 39% respectively. The resistance of K.pneumoniae and E.coli to third generation cephalosporins was higher in this study.

All isolates of Acinetobacter, Serratia and Enterobactor were sensitive to third generation cephalosporins. 92% of Pseudomonas aeruginosa and 94% of Proteus were sensitive to cefotaxime and ceftazidime. These findings were similar to the study by Kaul et al from Christian Medical College, Vellore, who reported that in Pseudomonas aeruginosa and the other non-fermenting gram-negative bacteria (NFGNB) Ceftazidime resistance decreased. Among Aminoglycosides, most of the GNB were

sensitive to Amikacin than Gentamicin. Highest sensitivity rates were detected for Gatifloxacin than Ciprofloxacin and Ofloxacin among Quinolones. These findings were similar to previous studies on antimicrobial resistance among gram-negative bacteria by many authors. [21, 22, 23, 39, 64]

All Gram negative bacilli isolated in this study had a maximum sensitivity pattern to Imipenem and Cefepime. This was similar to the study by Lockhart et al about antimicrobial resistance among Gram-Negative Bacilli causing infections in Intensive Care Unit patients in the United States between 1993 and 2004 [76] and Rakshit et al from Mumbai. [13]

Gram-negative bacilli producing ESBL appear to be on the rise in Asian countries and pose a serious problem in pulmonary infections. [80] In the present study the occurrence of ESBL production among K.pneumoniae and E.coli were 82% and 52% respectively. For Klebsiella pneumoniae this finding was higher than the study by Feizabadi et al (72.8%), [74] Gonlugur et al (12.2%), [39] Hosoglu et al (68.3%), [72] Asian HAP Working group (0.9% to 40%) [80] and lower than the study by Verhamme et al (100%), [79] Vitkauskiene et al (88.9%), [84] Dey et al (100%). [15] For E.coli this finding was higher than the study by Gonlugur et al (20.8%), [39] Asian HAP Working group (2.3% to 40%) [80] and lower than the study by Hosoglu et al (74.6%), [72] Dey et al (100%). [15]

In this study ESBLs were predominantly present among K. pneumoniae compared to E. coli. Our findings are similar to that of most of

the studies in Europe and USA [38] and the Indian studies by Jain et al [64] and Shanmuganathan et al. [65] But studies by Gonlugur et al, [39] Hosoglu et al [72] and Kumar et al [82] showed higher incidence of ESBLs among E.coli than K.pneumoniae. The indiscriminate use of third-generation cephalosporins has been proposed as a reason for the rise of ESBL producing strains in India. [80]

Among the Staphylococcus aureus isolates in this study, the occurrence of Methicillin resistance was observed in 54.84%. This was higher than the study by Leroy et al (33%) [35] and lower than the study by Mukhopadhyay et al (59.45%) [18] and Rakshit et al (100%). [13] No Vancomycin resistant Staphylococcus aureus was detected in the present study.

Epidemiological investigation of nosocomial infections is greatly facilitated by the use of a precise typing system for the organism involved. Our study about the hospital acquired pneumonia in IMCU suggests a high prevalence of Klebsiella pneumoniae accounting for 48.2% of total cases. In the present study, we have used Klebocin typing and antibiotic sensitivity profiles as the typing methods for the detection of source of Klebsiella infections in our IMCU. Although not comparable to molecular methods in discriminatory power, antibiogram and klebocin typing are simpler and relatively economical method of bacteriological typing.

In the present study all Klebsiella pneumoniae isolates were subjected to klebocin typing by spot inoculation method as described by Pal et al who done the klebocin typing in their study by spot inoculation method

which was described by Chugh et al with some modifications. Using the spot inoculation method, we observed 87.5% typability, whereas Buffenmyer et al, [61] Chibber et al, [60] Malik et al, [43] Aggarwal et al [44] and Pal et al [9] reported 67%, 72.8%, 83.3%, 73.5% and 71.62% typability respectively. Bauernfeind et al ,[62] Hall, [59] Israil [46] and Podschun et al [58] have reported 96.3%, 77%, 85% and 96% typability respectively using streak and point method for klebocin typing.

Despite the relatively higher typability observed in streak and point method, we followed the spot inoculation method. In view of the high induction of klebocins by mitomycin C, storage stability at -20°C and simplicity of the technique, spot inoculation method is more appropriate typing method.[9]

Nineteen klebocin types were observed in the present study using six klebocin producer strains. Pal et al reported that twenty eight klebocin types had been observed in their study using six klebocin producer strains [9] whereas Malik et al observed four patterns of klebocin types in their study with six klebocin producer strains. [43]

We observed the klebocin types 211 (56.8%), 111 (6.2%), 112 (5.0%) and 441 (3.7%) were the most common types in our study. According to Buffenmyer et al and other previous studies type 444 was considered as untypable and these strains accounted for 12.5% of the total strains studied. Pal et al reported types 111 (50%), 211 (5.59%), 312 (3.08%) and 112

(2.31%) as the commonest klebocin types in their study and 28.37% strains could not be typed. [9]

Malik et al found type 312 (43.4%) as the commonest followed by type 334 (23.4%), type 112 (16.6%) while remaining 16.6% strains could not be typed.

Chibber et al reported the predominant klebocin types as 244 (14.3%), 313 (13.7%) and 113 (7.6%). [60] Aggarwal et al observed the commonest klebocin types as 111, 313 and 113. Probably there is variation in the prevalence of various klebocin types in different topographical areas and institutions. Moreover the set of producer strains used in the present study was different from other studies in which they had used a set of producer strains standardized by them in their own setups. It is therefore difficult to compare our typing patterns with other studies.

In the present study, we found that by combination of klebocin types and antibiogram of K.pneumoniae isolates, the source of infection could be traced in 76.51% cases. In a study on klebocin typing by Chibber et al [60] no significant correlation was observed between the source of isolation and the klebocin type whereas Pal et al [9] observed when the klebocin typing was used in association with antibiogram, in 86.84% cases probable source of infection could be detected.

Despite the importance of the findings presented in this study, there are some limitations that should be taken into consideration when assessing

our conclusions. First, although for the diagnosis of HAP we did quantitative culture of Sputum, BAL and ETA, yet the results from this study need to be validated by comparison to gold standards such as histology of lung tissue.

Last, in the present study the reproducibility of the klebocin typing was 83%. This was higher than the previous study by Malik et al who showed in their study that the reproducibility of the klebocin typing was 73.3%. [43] This is of major concern because a useful and effective epidemiological typing system should have high reproducibility. In our study the reproducibility may be a major problem with the klebocin typing procedure. This difficulty was primarily due to the sensitivity of the test strains to MTCC 432 klebocin and was presumably associated with their low titers. This problem was a serious shortcoming since, as shown in Table 10 the commonest type in our study was 211. This pattern of typing was due to lack of sensitivity of the clinical isolates to MTCC 432 klebocin (producer 2). Replacing the producer strain of this klebocin with the strain that yields high-titer preparations may help in achieving acceptable reproducibility. Such strains may be obtained by screening Klebsiella pneumoniae isolates for klebocin activity or testing producer strains of other investigators.

In spite of these problems, this study proposed the klebocin typing in association with antibiogram as a tool for epidemiological typing of Klebsiella pneumoniae isolates in the laboratories where the facilities to perform

genomic based molecular typing are not available. The advantages of klebocin typing include (i) the ready availability of required materials in most bacteriological laboratories, (ii) the ability to perform the typing procedure within the usual work day, and (iii) the ability to store the klebocins for future use.

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