"BACTERIOLOGICAL PROFILE OF ICU PATIENTS IN A TERTIARY CARE HOSPITAL IN NORTHERN INDIA''

A

Full Term Training submitted to the Lovely Professional University

For partial fulfillment of the requirement for the award of Degree of

Master of Clinical Microbiology

SUBMITTED BY

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CERTIFICATE

This is to certifying that **Kumari Pushpalata Tiwari** bearing registration no.11310577 has completed her dissertation titled ''**BACTERIOLOGICAL PROFILE OF ICU ISOLATED PATIENTS IN TERTIARY CARE HOSPITALIN NORTHETN INDIA**.'' under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study .No part of the dissertation has ever been submitted for any other degree at any university

This dissertation is fit for submission and the partial fulfillment of the conditions for the award of M.sc Clinical Microbiology

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DECLARATION

I**, Kumari Pushpalata Tiwari**, student of **M.sc Clinical Microbiology** under Department of **Paramedical Sciences of Lovely Professional University, Punjab**, hereby declare that all the information furnished in this project report is based on my own intensive research and is genuine. No part of the dissertation has ever been submitted for any other degree at any university.

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ABSTRACT

INTRODUCTION

Throughout the world multidrug resistant nosocomial infections are one of the leading causes of death and morbidity amongst hospitalized patients accounting a major burden on patients and public health system of any country. The purpose of this study is to provide up to date data on the frequency, source and microbial etiology of nosocomial bacteria isolated in different ICU patients with the goal of improving its management. ICU is one of the potential sources of nosocomial infections even in countries where extensive infections control measures are routinely implemented. Over the past few decades, the frequency of antimicrobial resistant and its associations with serious infectious diseases have increased with alarming rate. This study provides the epidemiological data of bacterial isolates in ICU patients over 3 months of study period from January 6th 2015 to March 31st 2015 from a tertiary care hospital, Jalandhar, Punjab.

MATERIALS AND METHODS

During the period from January 6th 2015 to March 31st 2015,a total of 207 samples (blood , urine, pus, fluid ,sputum)from the patients admitted in the different ICUs were collected aseptically and processed for culture and identification of bacteria .

AIMS AND OBJECTIVE

The objective of this study was to isolate various bacteria from different ICU samples and to identify the isolated bacteria.

RESULT

Out of 207, 40 (19.32%) samples were positive. The most frequent Gram negative organism were isolated, *Escherichia coli* 15(7.24%) followed by *Pseudomonas* 10 (4.83) *Klebsiella spp* 8 (3.86%), *Acinetobacter spp* 2(0.9650) and Gram positive was *Staphylococcus aerus* 4 (1.93%) and *Candida spp* 2 (0.96%) Demographic data and other details were obtained from patients records.

CONCLUSION

This report reveals the microbiological profile of the patients in ICU. Regular microbiological surveillance help in implementing better therapeutic strategies to reduce the high morbidity and mortality associated with the patients in the critical care unit.

INTRODUCTION

Intensive care unit (ICU) patients are at higher risk of developing nosocomial infections. When compared with other hospitalized patients [1]. Nosocomial bactermia is a major subgroup of hospital acquired infections. Incidence of nosocomial bactremia in ICU patients has been reported from 1% to as high as 6.5 % compared to 0.65% in other hospitalized patients [2]. A recent epidemiological study showed that ICU patients represented 34% of all hospital Gram negative bactremia. It is reported that patients having Gram negative bactermia have high mortality rate and prolonged ICU stay. Gram negative bactremia have been associated with higher case fatality rates when compared to Gram positive bactremias (44% vs. 30%). Approximately 25000 episodes of blood stream infections occurs in the USA annually .Blood stream infections have an overall mortality rate of 18% making them one of the leading cause of death in the USA. ICU despite their apparent impact on patient outcome, have become high risk areas for nosocomial infections [3].

The ICU is dealing with the most critical cases in a hospital and a great need of higher antibiotic is there while treating patients in ICU [4]. The cases like trauma, kidney transplant, heart surgery and any other surgical cases are brought to ICU after the surgical procedure in OT. Talking about the operation which is believed to be the major place from where a multidrug resistant organisms transfer from one patient to another patients .Among them multidrug *Acinetobacter ,MRSA, VRE, Pseudomonas spp* are most popular nosocomial organism which infect the patient during surgical intervention.

The patient in the ICU has 5 to 7 fold higher risk of nosocomial infections compared with the average patient and 20 to 25% of all nosocomial infections develop in ICU [5]. Critically ill patients admitted in the ICU are always at higher risk of developing infections with various antibiotic resistant organism .Infections caused by multi drug resistant bacteria constitutes a serious problem throughout the world Antibiotic resistant.

Bacterias are the common causative organisms of health care associated infection, particularly in the ICU. Over the past several decades, the frequency of antimicrobial resistance and its association with serious infectious diseases have increased at alarming rate.

Throughout the world multi drug resistant nosocomial infections are one of the leading causes of death and morbidity amongst hospitalized patients accounting a major burden on patients and public health system of any country.[6]

Intensive care unit is one of the potential source of nosocomial infections, even in countries where extensive infection control measures are routinely implemented .The international study of infections in ICU, which was conducted in 2007 demonstrated that the patients who had longer ICU stays had higher rate of infection, especially infections due to resistant *Staphylococcus areus ,Acinauetobacte spp, Pseudomonas species* and *Candida species*. The rate of nosocomial infections in ICU is rising mainly because of increasing use of invasive procedures which are performed in the ICU[7] .The therapeutic interventions which are associated with infectious complications include indwelling catheters ,sophisticated life support intravenous fluid therapy , prosthetic devices ,immunosuppressive therapy and the use of broad spectrum antibiotic leading to a spectrum of multi drug resistant pathogens which contributed to the evolution of the problem of the nosocomial infections[8] .Moreover the ICU of infectious patients is more than twice that of non infected patients .

Nosocomial infections are responsible for morbidity and mortality in hospitalized patients.[9] They also increase the cost of treatment and prolong hospitalization. The Centre for Disease Control Prevention (CDC) defines the intensive care unit associated infections as these occur after 48 hours of ICU admissions or within 48 hours after the transfer of the patients from the ICU.[10]

Globally, patients in the ICU have encountered an increasing emergence and spread of antibiotic resistant pathogen .The world wide incidence rate is 23.7 infections per 1000 patient's days. The rate of nosocomial infections range from 5% to 30% among ICU patients.[11] Among the pathogenic bacteria some were able to gain resistant to the antibiotic and have become antimicrobial resistant organism and now those bacteria have become resistant against most of the antimicrobial agents these days. These bacteria are especially affecting the ICU of a hospital and so as the community [12].

Although ICU generally comprises <5% of all hospitals bed, they accounts for 205 to 25 % of all nosocomial infections.[13] The increased risk of infections is associated with the severity of the

patient's illness. Length of stay exposure to invasive devices and procedure increased patients contact with health care personnel and length of stay in ICU [14].

Hospital acquired infections are most commonly associated with mechanical ventilation, invasive medical devices or surgical procedures[15] Gram negative bacteria are responsible for more than 30% of hospital acquired infections and predominantly in hospital acquired *Pneumonia*. They are highly efficient at up regulating or acquiring mechanism of antibiotic drug resistant in the presence of selection pressure. In ICU, Gram negative bacilli are responsible for considerable percentage of all nosocomial bacteremia. The overall or crude rate of death does not distinguish between the contribution of the patients underlying disease and the contribution of blood stream infections.[16]The prognosis of post bactremia is very variable. In recent years many studies have analyzed the mortality in relation to pathogen , source of infection, patients age and underlying disease .A large number of bacteria are potential pathogen in hospitalized patient *Klebsiella, Proteus, Morgonella, Enterobacter, Citrobacter, Serratia, Acinetobacter* ,and *Pseudomonas spp,* are commonly associated with the hospital environment and may also be isolated from the patients with underlying disease example who are more likely to be permanently colonized with bacteria.[17]

Lower respiratory tract infections (LRTS) are the most common bacterial infections among patients in the (ICU) occurring in 10 to 25% of all ICU patients and resulting in high overall mortality which may range from 22to 71% most common bacterial isolates in LRTS in ICU are *Pseudomonas , Acinetobacter, Klebsiella spp , E coli* [18,19].

In almost all cases , there is a need to initiate empirical antimicrobial treatment before obtaining the microbial results, but the situation is further complicated by the emergence of multi betalactamase producer and multi drug resistant pathogen [20,21].In a recent report ,infectious disease society of America specifically addressed three categories of Gram negative bacilli , namely extended spectrum betalactamase (ESBL) producing *Escherichia coli* , and *Klebsiella spp*. Multi drug resistant (MDR) *Pseudomonas spp* ,and carbapenem resistant *Acinetobacter spp,* as high alert bacterial pathogen .All these major reports indicate the need for obtaining data on prevalent strains in the ICU with the susceptibility pattern ,to help in revising antibiotic policy and guiding clinicians for the better management of the patient .[22,23]

The purpose of this study is to provide up to date data on the frequency, source microbial etiology and susceptibilities of bactremia with the goal of improving its managements and we hope to emphasize the importance of frequent surveillance for both nosocomial infectious and increasing antimicrobial resistance.

REVIEW OF LITERATURE

Various microorganisms have survived for thousands of years by their ability to adapt to microbial agents. They do so by via spontaneous mutation or by DNA transfer .Intensive Care Unit despite their apparent impact on patient outcome have become high risk area for nosocomial infections. The ICU patients has 5.7 fold higher risk of nosocomial infections compared with the average patients and 20-25% of all nosocomial infections develop in ICUs.[24,25]

Nosocomial bactremia is a major subgroup of hospital acquired infections .Gram negative bactermia is particularly associated with significant morbidity and mortality [26]. Incidence of nosocomial bactremia in ICU patients has been reported from 1% to as high as 6.5% compared to 0.65% in other hospitalized patients [27]. Furthermore a recent epidemiologic study showed that ICU patients represented 34% of all hospital Gram negative bacteriamia[27]. In addition Gram negative bactremia in the setting of critical illness is determined by admission to ICU has been associated with higher mortality rates and prolonged ICU stay. [28]

Microbial infections are more in ICU patients due to prolonged stay because of critical illness and increasing multidrug resistance pattern .Infection caused by multidrug-resistant bacteria constitutes a serious problem for ICU patients throughout the world. A Study was performed in 1993; the aim of the study was to find the microbial infections and antibiotic resistant patterns among Jordanlan ICU. All clinical isolates were first identified by conventional methods in a routine microbiology laboratory. A large representative number of Gram –negative isolates were identified at the species level with API 20E and API 20NE (Bio- merieux system France). microbial infections were observed in 155 patients(30%) among 519 patients admitted to ICU .Gram negative bacteria were involved in 110 (49%) , Gram positive bacteria in 68 (31%) ,mixed bacteria species in 25 (11%) and *Candida spp* in 19 (9%) of all 233 infection episodes. Five species were isolated most frequently: *Staphylococcus aureus* (40) ,*Acinetobacter spp* (28) *Pseudomonas spp* (22) , *Enterobacter spp* (20), and *Klebsiella spp* (17). All most Gram negative bacteria were sensitive to Imipenem and ciprofloxacin.[29]

Studies of Gram negative bactremia provide epidemiological data and may improve empiric antimicrobial therapy based on local pattern of susceptibility. Such information may also leads to

infection control measures designed to decrease infection rates. Therefore regular surveillance of important pathogen and its resistance pattern is mandatory [30].

One study was conducted which provides epidemiological and antimicrobial susceptibility data for nosocomial Gram negative bacteria in general Intensive Care Unit over periods of 5 years. Positive blood cultures in ICU patients were identified by the clinical microbial laboratory (BACTEC 9240) and reviewed daily .All cases of aerobic Gram negative bacteria from January 1st 1999 to December 31st 2003 were reviewed .Demographic data, time from admission to bacteremia, causative organisms ,anti microbial susceptibilities ,choice of empiric antibiotic therapy, ICU length of stay and mortality rate were collected . 45 nosocomial Gram negative bacterias occurred in 44 patients. Infections rate of 6.9 /1000 admissions and 11.3 /1000 patient's days remained stable. Seven bacterial species were identified *Pseudomonas aurginosa*, *Enterobacter spp* , were most common source of bacteria including *Pneumonia* 48% and central venous catherization 22.2% .Antimicrobial susceptibility were highest imipenem, gentamicin, tobramycin piperacillin tazobactum .Mortality rate were 53.3%in the ICU and 60% overall hospitalization .Average length of ICU stay was 50.5 days compared 6.13 days for all comers[31].

Hospital acquired infections are a serious health care problem worldwide associated with significant morbidity and mortality. It accounts for10 to 15 % of acquired infections and increase the cost of hospitalization. In United States, 10 -20% of nosocomial are estimated to involve the bloodstream. A study was conducted to evaluate the Frequency and Profile of microbial infection in burn Intensive care from 1st January 2000 to 31st December 2007 and to determine the prevalence of different bacteria involved in such infections and their microbial susceptibility .During the time of study 995 patients were admitted to BICU. Blood cultures were collected from each septicemia case and reviewed for age, sex, total body surface area burned, isolated microorganism and antibiotic sensitivity. There were 430 episodes of BSI among 830 cases, the annual true positive rate varied between 40.0 and 59.4% the majority (87.9%) being caused by one species only. However 22% had two or more episodes with different pathogens during hospitalization. The leading isolates was *Staphylococcus aureus* (40.4%), *Pseudomonas spp* ranked second (23.9%) , *Klebsiella spp* were third responsible for (7.4 %) and *Candida albicans* were forth most common pathogens .Identification of BSI bacterial pathogens combined with

determination of antimicrobial susceptibility of the bacteria can help clinicians to select appropriate agents for rigorous empirical treatment of BSI .Although eradication of infection in burn patients is impossible, a well conducted effective surveillance, infection control and preventive measures program etiology and prognosis have been performed worldwide.

Hospital acquired blood stream infections are a preventable source of morbidity and mortality .Critically ill patients in ICU are at particular risk for nosocomial blood stream infections because of their debilitated condition and frequent need for invasive procedure .The use of intravascular devices is the most important risk factors for the development of primary blood stream infections. It has been estimated that catheters related blood stream infection occurs in 3%- 7% of cases in which central venous catheters are used affecting >200,000 patients in United States annually.[32]

A study conducted in March 1998 to 31st December .The aim of the study was to observe the nosocomial blood stream infection in ICU of non teaching community hospital. Trained data collectors including infection control and research nurses and the data include patient's history, date of ICU admission and severity of illness. Nosocomial infections occurred > 24 hours after admission to the ICU. Sample were collected and processed. The result shows that, during the 21 months 3,163 patients were admitted to medical and surgical ICU, the incidence of primary bloodstream infection was 38(1%) of 3,163 patients , patients with central venous catheter was 34 (4%) of 920 patients or 4.0 infections per 1000 catheters- day. Among them Crude Mortality Rate was 53%. It was concluded that the incidence of risk factors and serious outcomes of blood stream infections in a non teaching community hospital is similar to those seen in tertiary care hospital[33].

It is important to know the pathogens causing nosocomial bactremia in the ICU and their antimicrobial sensitivity to formulate the appropriate treatment and guidelines. The microbial profile varies between institutions and also the various wards and ICUs of hospitals .The debilitated condition of the patients due to underlying disease, invasive diagnostic and therapeutic procedure and contaminated life supporting equipments used in ICUs predispose the patients to life threatening. A study was conducted to obtain information on pathogen causing nosocomial blood stream infections in ICU of tertiary care hospital in north India from July to December 2001. All patients with one or more positive blood cultures during the study period

were included .The age, sex, ICU types, underlying diseases, culture reports and possible portal of entry were recorded for each patients. Bacterial isolation and identification were done using standard culture methods and antimicrobial sensitivity testing was done by disk diffusion method. It was observed that during the study 1253 patients were admitted in ICU. A total 152 episodes of nosocomial bacteremia occurred in 140 patients during this period of 152 episodes 148 (97%) were monomicrobial and 4 (3%) were polymicrobial. A total 156 bacterial isolates were obtained. *Pseudomonas aeurginosa* (21%) being the commonest isolates. Over all Grams negative bacteria accounted for the majority (71%) isolates. A source of bactremia was identified in 49 (32%) episodes with lower respiratory tract being commonest 49% source [34].

Patients admitted in ICU are at higher risk of acquiring nosocomal infections compared with the patient admitted in general ward. This required for is partially because of the severity of the underlying illness and iatrogenic factor related to high frequency of invasive procedure required for monitoring and treatment [39]. Bacteremia continuous to be a major cause of morbidity and mortality in hospitals. In ICU, Gram negative bacilli are responsible for a considerable bacteria's.

A cohort study was conducted prospectively for 15 months .The aim of the study was carried to identify the risk factor for morbidity and mortality .This study was study was conducted in ICU of emergency, surgery and general surgery of Istanbul faculty of medical Hospitals between 1 October 2001 and 31 December 2002. A total 1, 450 patients were admitted during the study period. A case control study was conducted to identify the risk factor for mortality of nosocomial infection patients. Nosocomial was defined as the isolation of one or more organism from blood cultures taken at least 45 hours after admission .An assessment of whether or not the isolated organism represented true bactremia rather than contention was made by clinically or laboratory evidence of infection. Blood cultures (Bact/Alert, organon Teknika Durahan, N.C, USA) were generally taken from the patients in ICU only when systemic infection was suspected . The culture bottle were incubated and tested for a minimum of 7 day. The isolation of bacteria from two or more bottles was accepted as significant. All isolated micro organism were identified by standard laboratory methods. Antimicrobial susceptibility test were performed. The following data were collected for each patient were recorded age, sex , including diagnostic categories leading to ICU admission diagnosis , need for and duration of mechanical ventilation , presence of central vascular catheter , ICU length of stay , organ transplantation , all hematological and

biochemical values were included in the analysis . It was observed that two hundred and fourteen bactremia spp episodes occurred in 176 patients (64 female, 122 male; 51.3+_ 21.3) year's old .90 of whom died and 86 survived. The bactremia s rate was 12.1% the most common etiological agent of bactremia were *Klebsiella pneumonia*. 46 (21.5%), *Methicillin resistant Staphylococcus aureus* ; 46(21.55%) *Pseudomonas aurogenosa* 32 (14.9%) and E coli 20 (9.3). multivalent analysis showed that the requirement of mechanical ventilation for more than 7 days ($p<0.001,0$ and increased creatinine level ($p=0.034$) were independent risk factor for mortality of nosocomial in ICU . It was concluded that nosocomial Gram negative bacteria continue to be one of the major source of morbidity and mortality. [35]

Nosocomial infection or blood stream infection is a serious and potential preventable complication of hospitalization and has been estimated to be the eight leading cause of death in USA. Critically ill patients are particularly vulnerable to hospital acquire infections which are two to seven times more common in USA and can account for approximately half of all hospital acquired infections . ICU acquired has been estimated to complicate between 1.2% and 6.7 % of all ICU admission. 4.4% to 6.8% of admission longer than 48 to 72 hours in duration and have an incidence of between 5to 19 per 1000 patients days.A retrospective analysis of the incidence of blood stream infection acquired during ICU admission at two universities affiliated hospitals in Melbourne, Australia. Data were obtained from prospectively collected electronic data bases of ICU admission and hospital microbiology records of positive blood culture standard protocol for the collection, analysis and reporting of blood culture were employed .Complete data were taken for 11 years since January 1998 to February 2009. And six years from January 2003 to December 2008 in other Monasa Medical Centre. The first positive culture after the third ICU day was used to define the occurrence of ICU acquired blood stream infection. They studied 6339 admission of greater than 72 hours in two universities affiliated to ICU .They reported *Enterococci* and Gram negative infections both approximately double the risk of death and Candida was associated with an over four fold risk of dying in hospital.[36]

Ventilator associated pneumonia (VAP) is a leading health care associated infection among critical ill patients accounting for 25% of all type of Intensive Care Unit (ICU) acquired infection. In addition to its huge impact on morbidity and health care cost , VAP is consider the most deadly hospital acquired infection with a VAP associated mortality rates ranges from 24 to

50% . Increased drug resistant rate among Gram negative pathogen that frequently causes VAP may compromise treatment and results in prolongation of hospital stay inflation of inpatient health care cost and further increase in hospital mortality. A study conducted in the adult intensive care unit, this study was conducted up to five years starting from October 2004 and 2009 were examined. A total of 248 isolates including of different pathogens were included. *Acinetobacter spp* was highly 60 to 80% resistant to all other tested antimicrobials. *Pseudomonas aeruginosa* followed by *Klebsiella spp, Staphylococcus aerus* were isolated from the study [37].

Lower respiratory tract infections (LRTI's) are the most frequent infections among patients in intensive care units. The consequences of increased drug resistance are far reaching since bacterial infection of the lower respiratory tract (LRT) is a major cause of death from infectious disease. The study was conducted with the aim of determining the bacterial etiology of LRTI in the neuro intensive care unit (NICU) as well as to update the clinicians with the various antimicrobial alternatives available in the treatment of LRTI. The study was conducted for the period of 3 years from January 2010 to December 2012 in the Microbiology Department of a Teaching Tertiary Care Hospital. The LRT specimens from 230 patients admitted in a NICU during the study period were processed. Following culture, the isolated organisms were identified and antimicrobial sensitivity was performed by standard methods. Out of the 230 LRT specimens evaluated, 198 (86.08%) were culture positive. A total of 254 pathogens were recovered with a predominance of Gram-negative isolates (n = 243; 96.05%) *Pseudomonas aeruginosa* was the most dominant pathogen followed by *Klebsiella pneumonia*.36]For effective management of LRTI's, an ultimate and detailed bacteriological diagnosis and susceptible testing is required to overcome global problem of antibiotic resistance[]

Nosocomial infections are one of the leading causes of morbidity and mortality in hospitalized patients especially the critically ill patients in the intensive care unit (ICU) where a large number of drugs are administered to the patient' which in turn leads to the generation of antibiotic resistant pathogens. The present study was conducted to identify the prevalence of predominant bacterial microorganisms and their drug sensitivity and resistance in ICU of a teaching hospital in Eastern India[38].

A retrospective record based study was conducted in the ICU of Hi-Tech Medical College and Hospital, Odisha, from November, 2011 to October, 2012. The rate of nosocomial infection was 28.2%. Urinary tract infection was the most common infection (54.9%). The predominant isolate was *E. coli* (52.7%) followed by *P. mirabilis* (15.4%) and *Pseudomonas aeruginosa* (13.2%). E. coli was highly sensitive to Polymyxin B, Gatifloxacin and Ceftriaxone and showed high degree of resistance to Cephalexin, Cefadroxil, Tobramycin and Prulifloxacin[39].

Antibiotic resistance is a major world-wide problem in the intensive care unit (ICU) It has been realized that the spread of drug resistant organisms in the ICU is related to the widespread use of antibiotics [37,38]. A cross-sectional study was carried out based on reports of bacteria isolates from the ICU of Fatmawati Hospital, from January 2009 to March 2010. The most common locations for infection were respiratory tract (78.7%), urinary tract (7.6%), surgical site (7.5%), blood (3.8%), and peritoneal fluid (2.4%). *Pseudomonas aeruginosa (P. aeruginosa*) was the most frequently isolated bacteria (26.5%), followed by *Klebsiella pneumoniae (K. pneumoniae)* (15.3%), *Staphylococcus epidermidis* (14.9%), *Enterobacter aerogenes (E. aerogenes*) (13.3%), *Klebsiella ozaenae (K.ozaenae)* (8.4%), *Escherichia coli (E. coli*) (5.2%), *Serratia liquifaciens (S. liquifaciens*), respectively [40].

Microbiological infections plays vital role in determining the outcomes as well as cost and duration of the hospital stay for patients admitted in the ICU setup41,42]. Regular surveillance of important pathogens and its resistant pattern is mandatory. The objective of this study was to find out organisms causing infections in patients admitted in the ICU and to know the resistant pattern of isolates. During the period from January 2012 to June 2012. A total of 583 samples, 228 (39.10%) samples were culture positive. The number of Gram negative and Gram positive samples were 182(79.82%) and 46(20.18%) respectively. The most frequent Gram negative organism isolated were *Pseudomonas spp* (29.12%) followed by *Acenetobacter* spp(24.72%)*Klebsiella spp*(28.08) E coli (19.23%) .(.8.16%) isolates of *Pseudomonas spp* and *Acenetobacter spp* were carbapenem resistant. Extended spectrum Beta lactamase (ESBL) production was detected (39.28%.of *Enterobacteriaceae*[41].

Throughout the world multi drug resistant nosocomial infections are one of the leading causes of death and morbidity among hospitalized patients.A study was conducted in different ICU of

tertiary care hospital in Ahmadabad during January 2012 to April 2012.The commonest isolated organisms were *E coli*, *Acinetobacter* , *Pseudomonas spp* and *Klebsiella spp*[.42]

AIMS AND OBJECTIVE

The aim of the study is to know the bacterial profile of ICU patients in Tertiary Care Hospital

MATERIALS AND METHODS

A prospective study was conducted in the Intensive Care Unit of Tertiary care hospital in Northern India (Jalandhar, Punjab) over a period of three months. It included 14 bedded adult ICU that admits general medical trauma and surgical patients. The study was conducted in the department of microbiology from January 6th to March 31st 2015.

STUDY DESIGN AND DATA COLLECTION

Total 207 samples were collected in 3 months .From each patient the following data were collected ; name , sex, age, primary diagnosis , date of submission in the hospital and ICU. The pathogens were identified based on the standard bacteriological procedures including Gram staining, wet mounts, routine microscopy, and colony morphology on blood agar and MacConkey agar and biochemical reactions.

SAMPLE COLLECTION

Specimen were collected and transported to clinical microbiology laboratory for diagnosis. Samples like (blood, body fluid, ascetic fluid, plural fluid, urine and miscellaneous samples) were collected taking full precaution and following standard aseptic precautions and method as recommended by Hospitals' Infection Control department.

1. BLOOD SAMPLE

Sterile syringe was used to draw blood from two sites of the body in around 30 minutes of difference between drawings of two samples. All the procedures were under aseptic condition .After wearing gloves and cleaning the site with the alcohol swab properly. After drawing blood they were filled in supplied culture bottles which were Bact /ALERT 3DHealth Care Culture Media and were sent to laboratory as soon as possible. And the Blood samples cultured manually as well on MacConkey and blood agar.

2. BODY FLUID

All the body fluids for microbiology investigation were collected either during the surgical procedure or for the investigation purposes. All the samples were collected using ascetic measures. Sterile containers were used for sample collection.

3. URINE SAMPLE

Urine samples were mostly collected by the urine drainage port of Foley's catheter because most of the patients were in the Foley's catheter. For other patients, sterile recommended technique was used to collect samples in a sterile container and send to the laboratory

4. PUS

A sterile swab was generally used to take pus sample or the pus was drawn with the sterile syringe and sent to the laboratory immediately.

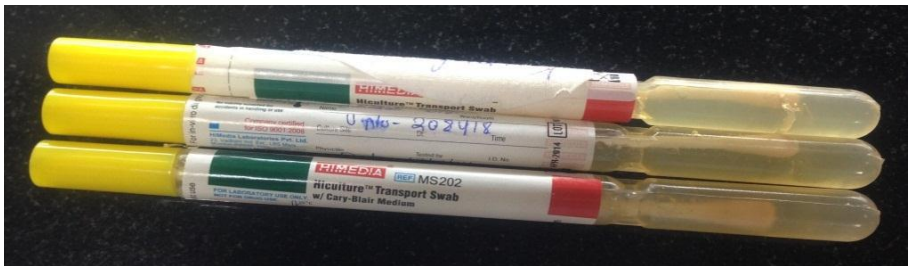


Figure: 1 Himedia Cary Blair Swabs

5. SPUTUM

A wide mouth sterile container was used to collect the sample aseptically after explaining it to the patient and sent to laboratory for investigation immediately.

PROCESSING OF THE SAMPLE

First of all samples were received with the prescription form of the patient and the entry of the samples were done in separate register as their type and unique ID was given to the sample.

The unique unit ID was bearing all the information sent with the sample.

First Day;

The samples were cultured in appropriate culture media as their type.

A Gram stain was also done. (Except the urine and stool unless specified).

I. Urine:

Two agar plates were used for urine culture.

1. Blood agar plate

2. MacConkey agar plate

Then a wet mount of urine was also prepared and observed under 40 x objectives for any abnormalities and noted down. Observed for presence of:

1. Pus cells

2. RBCs

3. Microorganisms

II. Blood:

The blood culture vials of **BacT/ALERT 3D** are immediately feed in the system and will remain there unless a positive result won't come up to 5 days.

If any of them is indicated as positive by the system then only a culture is done on the Blood agar and MacConkey agar plate.

III. Samples from Upper respiratory tract:

For these samples we used Blood agar plate and MacConkey agar plate for culture.

IV. Gram stain: Along with the culture we also observed the gram stain of the pus, tissue, and other body fluids.

INOCOULATION ON MEDIA

Culture media is required to grow the organisms from infected material to identify the causative agent. The basic constituents of culture media are water, electrolytic, peptone, meat extract, blood or serum and agar.

Samples were processed on three different media.

1. MacConkey agar
2. Blood agar

PREPARATION AND COMPOSITION OF THE MEDIA

1. **MacConkey Agar**;

Ingredients	**Grams/Liters**
Peptic digest of animal tissue	20.00
Lactose	10.00
Sodium taurocholate	5.00
Neutral red	0.04
Agar	20.00

Final pH at (25°C) 7.4± 0.2.

MaCconkey agar is also an indicator medium. Due to fermentation of lactose, there is acidic pH which forms the pink colonies in the presence of neutral red indicator. It is used for cultivation and differentiation of bacteria .The osmosis of sodium chloride prevents the spreading of *Proteus* colonies.

DIRECTIONS:

1. Suspend 55.04gm in 1000ml distilled water .Heat to boiling to dissolve the medium completely.
2. Sterilize by autoclaving at 15 lbs pressure (121°c) for 15 minutes.

PROCEDURE OF INOCULATION

1. Taking full precaution, sterilize an inoculating loop by placing it on a flame till it turns red hot.
2. Specimen was taken colony was made
3. Then again the loop was sterilized and primary streaking was done from the mother colony.
4. Then again the loop was sterilized and secondary streaking was done.
5. Then tertiary streaking was done with the help of sterilized loop.

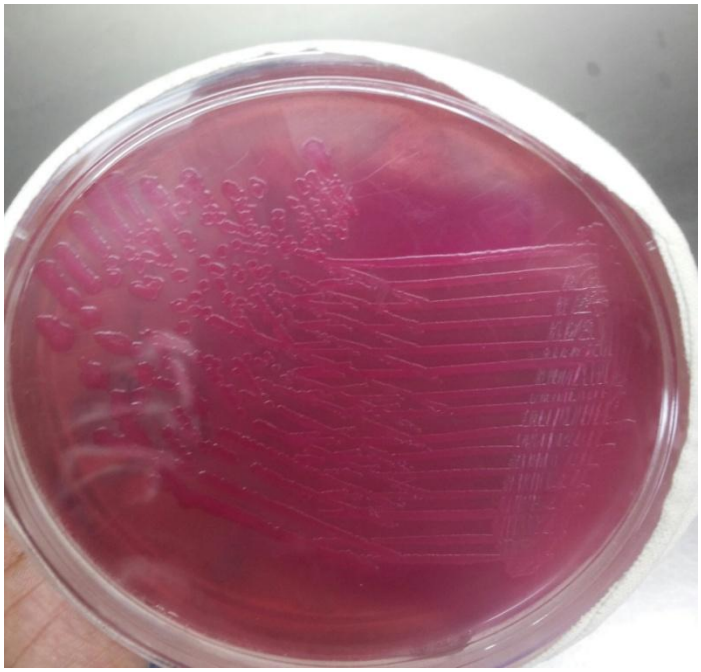


Figure: 2 Macconkey plate

BLOOD AGAR;

It is the enriched medium .When basal medium is added with some nutrient such as blood serum or egg, it is called enriched media. Blood is added to nutrient agar. It may be used for growing numbers of bacteria but one specific example is streptococcus which requires blood for growth. Blood agar is bright red, opaque medium. The variety of complex nutrients found in blood supports the growth of most bacteria, fastidious. Bacteria growing on blood agar can be classified in part on what they do to the red blood cells incorporated into the medium. As the

hemoglobin is exposed to the chemicals in the agar, its characteristics red color is altered. This type of alpha –hemolytic, turns the medium under the bacterial growth brown green. Other bacteria are capable of digesting the hemoglobin released as they destroy red blood cells. The result of this complex hemolysis termed beta hemolysis is clearing of the medium under the bacterial colonies .The medium is altered from opaque to transparent. Other bacteria leave on red blood cells essentially untouched. The medium is not discolored or cleared by growth. Such bacteria are called gamma hemolytic.

COMPOSITION

Ingredients	**Grams/Liters**
Beef heart infusion	5gm/L
Trypton	10gm/L
Sodium chloride	5gm/L
Agar	15gm/L

Final pH (at 25°c) 7.3±0.2

Procedure of Inoculation

- After taking full precaution , the loop was sterilized
- Specimen was taken, mother colony was made.
- Again the loop was sterilized, primary streaking was done.
- Then tertiary streaking was done with help of the sterilized loop.
- Incubate the media for 24 hours at 37°C

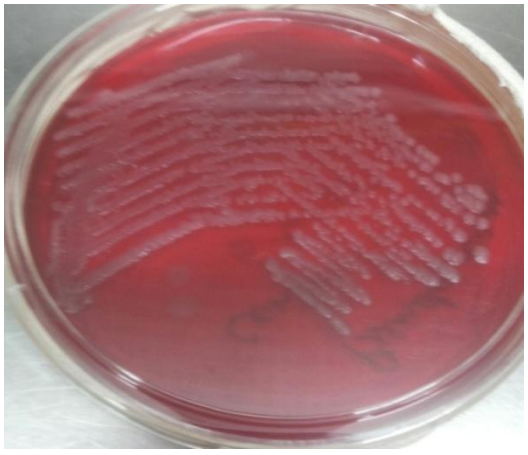


Figure: 3 Growth of *Pseudomonas aeruginosa* on Blood agar plate

NURTIENT AGAR:

When 2-3% agar is added to nutrient broth, it becomes nutrient agar. It is a simple media routinely employed medium in laboratory for diagnostic purposes. If concentration of agar is reduced 0.2-0.4% semi solid medium is obtained which enables motile bacteria to spread.

COMPOSITION:

Ingredients	**Grams/liter**
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.00

Final pH (at 25°C) 7.4 ± 0.2

Uses: for cultivation of less fastidious microorganisms can be enriched with blood or other biological fluid.

Directions: Suspend 28.0 grams in 1000ml distilled water. Heat till boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C for 15 minutes).If desired enriched with 5-10% of blood or other biological fluids. Mix well and pour into sterile Petri plates.

MULLER HINTONE AGAR

Muller Hinton agar is microbial growth medium used for antimicrobial susceptibility testing. This agar is recommended for disc diffusion method of antibiotic susceptibility testing. This is recommended by FDA, World Health Organization and NCCLS for testing most commonly encountered aerobic and facultative anaerobic bacteria in food and clinical material.

COMPOSITION:

Ingredients	Grams/liter
Beef infusion solids	4.0
Starch	1.5
Casein hydrolysis	17.5
Agar	15.0

Final pH 7.4 ± 0.2 at 37°C

Uses: for determination susceptibility of microorganisms to antimicrobial agents

Directions: suspended 38.0 grams in 1000 ml distilled water. Heat till boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure at 121°C for 15 minutes. Mix well before pouring

CULTURE TECHNIQUE:

A loop full of sample is transferred to the plate and a well is prepared. Using the same inoculating loop and after sterilizing it, the quadrant streaking method is used to culture it. An intermittent sterilizing is used in between all steps.

- Streak culture
- Lawn culture
- Stroke culture
- Stab culture

STREAK CULTURE: (Surface Plating);

It is the routine method employed for bacterial isolation in pure culture. A platinum or nichrome wire loop of 2-4mm internal diameter is used. Due to high cost of platinum, loops for routine laboratory work are made of nichrome wire (24 S.W.G. size). This loop is first sterilized in the Bunsen burner by making it red hot and cooled by touching and uninoculated part of the medium then a loop full of specimen is smeared onto the surface of dried plate near the peripheral area. This is named as primary inoculums. From the primary inoculums, it is spread thinly over the

plate by streaking with the loop in parallel lines. The loop is flamed and cooled in between the different set of streaks. It is done to obtain isolated colonies over the final series of streaks. The culture plate incubated at 37°C for overnight. Confluent growth occurs at the primary inoculums and well separated colonies are obtained on the final streaks. Single isolated colony is the best to study the various properties of bacteria.

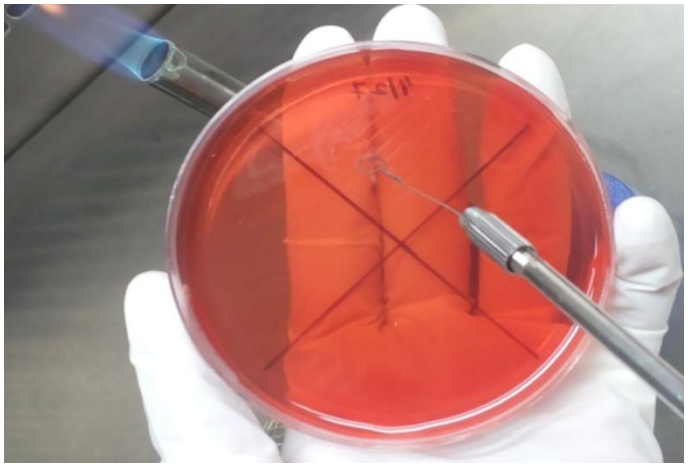


Figure: 4 Streaking Method

LAWN CULTURE;

This type of culture method is employed in antibiotic sensitivity testing (disk diffusion method) and in bacteriophage typing. It may also employ for preparation of bacterial antigens and vaccines where a large amount of bacterial growth is required. Lawn cultures are obtained by flooding the surface of the plate with a liquid culture or suspension of the bacterium. Culture plate is kept for a minute and then excess material is poured off. Alternatively the culture plate may be inoculated by a sterile swab soaked in liquid bacterial culture or suspension. Plate is then incubated at 37°C overnight to obtain bacterial colonies.

STROKE CULTURE:

Stroke culture is done in tubes containing agar slope and is employed for providing a pure growth of the bacterium for slide agglutination and other diagnostic test.

STAB CULTURE:

Stab culture is performed by straight wire charged with culture material by puncturing deep inside the agar. This technique is employed to demonstrate gelatin liquefaction, oxygen requirement of the bacterium and to maintain stock cultures for preservation of bacteria.

INOCULATION OF CULTURE MEDIA:

Most of the pathogenic organisms grow best at 37°C i.e. human body temperature. The inoculated culture media are incubated at 37°C in an incubator.

SECOND DAY:

OBSERVATION OF THE COLONIES

1. All the plates were observed for the growth. Those plates with no growth were further incubated and those growths were observed.
2. The morphology of the colony and their size their odor consistency etc were observed and noted down.
3. The plates were also observed for colony count. If the growth is significant then the plates were selected for pure culture and if not then further incubated.

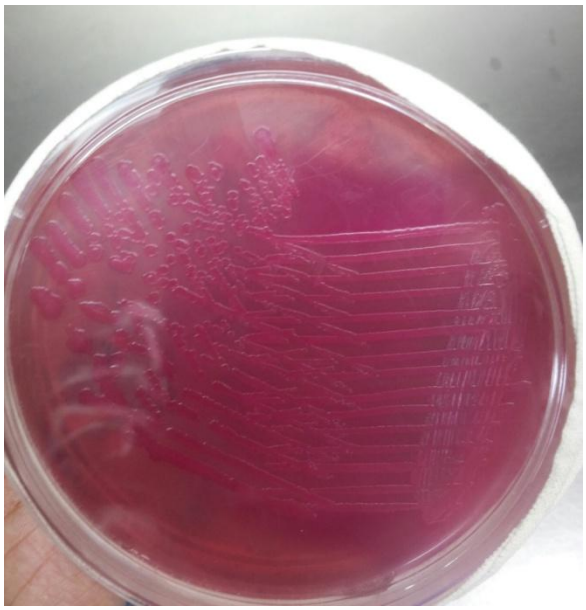


Figure: 5 Colony Morphology *of E. coli* on MacConkey Agar Plate

STAINING: GRAM STAINING

PRINCIPLE

Gram staining is a method of differential staining bacterial species into two large groups (Gram positive and Gram negative). It is based on the chemical and physical properties of their cell wall. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram positive bacteria. A Gram positive results in purple / blue color while Gram negative results in pink red color. The word Gram is always spelled with a capital referring to Hans Christen Gram the inventor of Gram Staining .Gram positive bacteria cell wall has large amount of peptidoglycan and Gram negative bacteria have less amount of peptidoglycan in their cell wall. They have lip polysaccharide containing a compound known as lipid A or endotoxin .The organism that retain purple color with crystal violet and are not decolorize by acetone iodine are called Gram positive bacteria . The organisms that lose their color of crystal violet after being treated with acetone iodine are called gram negative bacteria.

PREPARATION OF SMEAR;

Smear was prepared on a clean and dry slide. Drop of normal saline was taken on the slide then with the help of sterilized straight wire loop, colonies were taken and mix with normal saline on the slide and the smear was prepared.

PROCEDURE;

1. Smear of the specimen was fixed by heating.
2. Smear was flooded with crystal violet stain for one minute.
3. Gram's Iodine was poured on the smear for one minute. Again washing of the smear was done.
4. Decolorize the smear with acetone smear was washed
5. Counter stain saffrainine was added for one minutes
6. The smear was washed, dried and observed under oil immersion objective (100 x) microscopically.

BIOCHEMICAL PROFILE

Bacterial species differ in their capacity to break down different sugars. Some biochemical tests are based on presence of specific enzymes such as Catalase, coagulase, oxidase, urease, gelatianase, Indole test , TSI Test, MR Test, VP Test etc. Some of the widely used biochemical tests are described as follows.

1. SUGAR FERMENTATION

PRINCIPLE

To determine the ability of an organism to ferment a specific carbohydrate (sugar) incorporated in a medium producing acid or acid with gas.

PROCEDURE

Test organism is inoculated in a sugar medium and incubated at 37 ° C for 18 to 24 hours. Glucose, lactose, sucrose and mannitol are widely used sugars. Sugar media contain 1% sugar. Indicator used is Andrade's indicator (a solution of acid fusion to which is added sodium hydroxide)

INTERPRETATION

Positive –pinkish red (acidic)

Negative- yellow to colorless (alkaline)

Gas production can be seen as bubbles in Durham's tube

Examples of fermentative bacteria;

Glucose fermenters -All members of the *Enterobacteriaceae*

Glucose and lactose fermenter- *Escherichia coli, Klebsiella spp.*

Glucose and mannitol fermenters- *salmonella spp*

2. INDOLE PRODUCTION

Principle:

To determine the ability of an organism to decompose aminoacid tryptophan into Indole. Tryptophan is decomposed by an enzyme tryptophanase produced by certain bacteria.

PROCEDURE: Indole production is detected by inoculating the test bacterium into peptone water (tryptophan rich) and incubating it at 37°C for 48 -96 hours. 0.5% Kovac's regents is added to the bacterial growth and gently shaken.

INTERPRETATION:

Indole positive- a red colored ring near the surface of the medium (example *Escherichia. coli, Proteus sp.* other than *P.mirabilis, Edwardsiella*)

Indole negative-yellow colored ring near the surface of the medium (example *Klebsiella sp., Proteus mirabilis)*

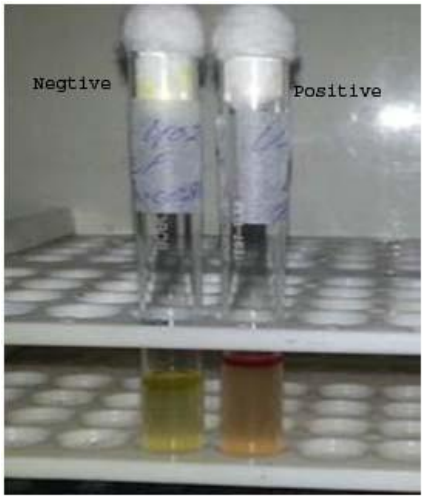


Figure: 6 Indole Test

UREASE PRODUCTION:

PRINCIPLE:

To determine the ability of an organism to produce an enzyme urease, this splits urea to ammonia. Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/ red in color.

PROCEDURE:

The test is done in Christensen's urease medium. The test organism is inoculated in the entire slope of the medium and incubated at 37°C. It is examined after four hours and after overnight incubation.

INTERPRETATION:

Positive - pink color (*Klebsiella sp, Proteus sp*.)

Negative-pale yellow color (*Escherichiae.coli)*

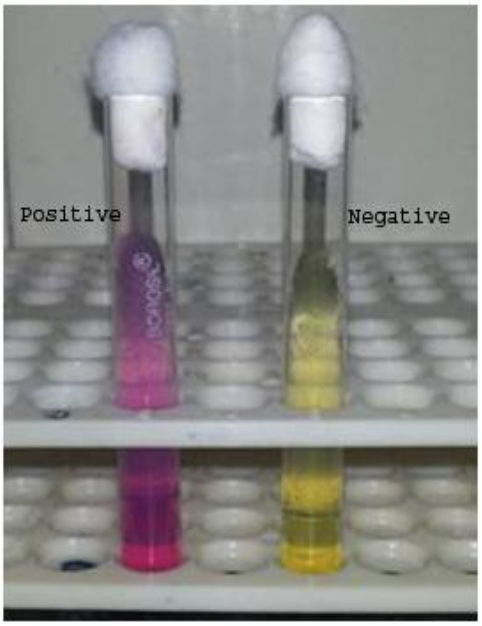


Figure: 7 Urease Test

CITRATE UTILISATION TEST:

PRINCIPLE:

It is the ability of an organism to utilize citrate as the sole source of carbon for its growth with resulting alkalinity.

PROCEDURE:

1. Solid (simmon's) or liquid (koser's) media can be used can be used a bacterial colony is picked up by a straight wire and inoculated into either of these media.
2. These media are incubated at 37°C. Simmon's citrate medium contains agar, citrate and bromothymol blue as an indicator. Original color of the medium is green

INTERPRETATION

Simmon's Citrate Medium

Positive – Growth with an intense blue color on the slant .Negative – No growth with no changes in color (green).

Koser's Citrate Medium

Positive-turbidity due to growth of bacteria

Negative – No turbidity

Citrate positive bacteria - *Klebsiella sp, Enterobacter sp, salmonella sp, citrobacter sp*

Citrate negative bacteria – *Esch coli, salmonella typhi*

Figure 8 Citrate Test

1. CATALASE TEST

PRINCIPLE**;**

Certain bacteria have an enzyme catalase which acts on hydrogenperoxide to release oxygen.

PROCEDURE;

(1) Pick up a few colonies of test bacterium with platinum loop from nutrient agar slope/ plate and mix it in a drop of H_2O_2 (10 volumes) on a clean glass slide.
(2) A positive catalase reaction produces gas bubbles immediately .Since blood contains catalase, culture on blood containing media may result in false positive reaction.
(3) Use of iron wire loop for picking up bacterial colonies may be another cause of false positive test.This test is mainly done to differentiate between genera *Staphylococcus* from *Streptococcus*.

INTERPRETATION

Positive test – immediate bubbling , easily observed (o_2 formed) examples all members of enterobactriaceae, staphylococcus

Negative test – No bubbling (no O_2 formed) *Streptococcus, Clostridium*

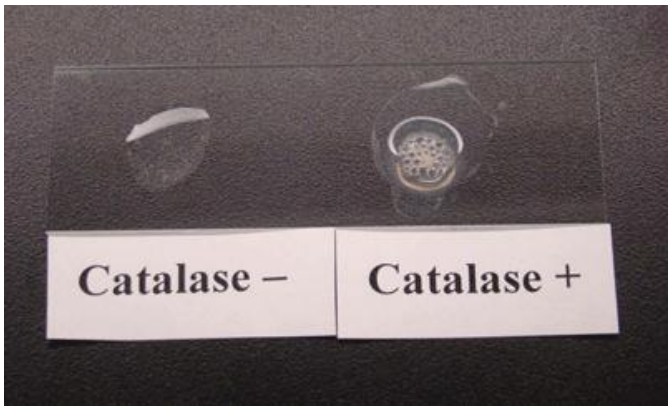


Figure 9: Catalase Test

OXIDASE TEST

PRINCIPLE:

To determine the presence of an enzyme cytochrome oxidase which catalyses the oxidation of reduced cytochrome by molecular oxygen .

PROCEDURE:

1. Freshly prepared solution of 1% tetra methyl paraphenylene diamine dihydrochloride (oxidase reagent) is used .There are different methods to perform the test.
2. A filter paper strip, soaked in the oxidase reagent is smeared with test organism. In positive oxidase reactions the smeared area turns deep purple within 10 seconds. Oxidase positive control (Pseudomonas sp) should always be included to find out the working of oxidase strip.
3. Another method is to pour oxidase reagent on the surface of the colonies, the colonies become purple within 10 – 30 minutes .This technique is useful to pick up Neisseria colonies from mixed growth on culture media. Purple colored colonies can be sub cultured to get pure culture.

INTERPRETATION

1. **Positive** – Deep purple within 10 seconds (*Pseudomonas sp, Neisseria sp*)
2. **Negative** - no color change (*Enterobacteraceae*)

Figure: 10 Oxidase Test

1. **METHYL RED (MR) TEST**

PRINCIPLE: This test detects the production of sufficient acid during fermentation of glucose by bacteria and sustained maintenance of pH below 4.5.

PROCEDURE:

i. The test organism is inoculated in glucose phosphate broth and incubated at 37°C for 2-5 days.
ii. Then add five drops of 0.04% solution of methyl red mix well and read the results immediately.

INTERPRETATION

1. Positive –Red color (*Esch coli*)
2. Negative – yellow color (*klebsiella sp, enterobacter sp*)

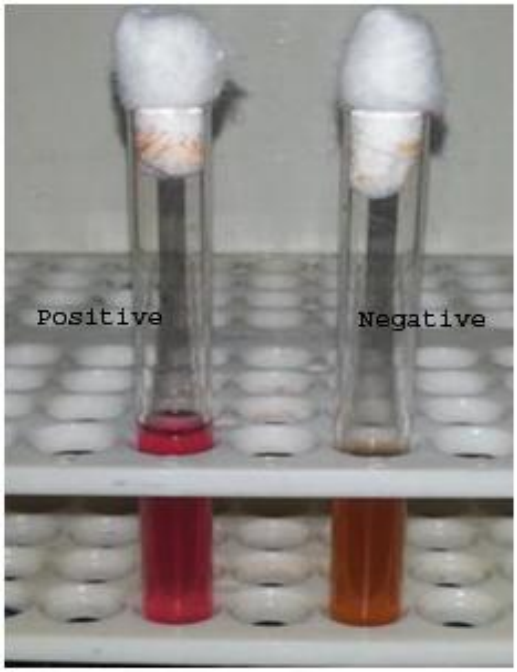


Figure: 11: MR Test

VOGES-PROSKAUER (VP) TEST OR ACETOIN PRODUCTION TEST:

PRINCIPLE: This test depends upon the production of acetyl methyl carbinol (acetoin) from pyruvic acid in the media. In the presence of alkali and atmospheric oxygen, acetoin is oxidized to diacetyl which reacts with α-naphthol to give red color.

PROCEDURE: Test organism is inoculated in glucose phosphate broth and incubated at 37°C for 48 hours .Then add 1ml of 40% KOH and 3 ml of a 50% solution of α – naphthol in absolute alcohol.

INTREPRETATION

1. **Positive**-pink color within 2-5 minutes ,deepening to crimson color in 30 minutes.(*klebsiella sp, enterobacter sp,staphylococcus*)
2. **Negativ**e-colorless for 30 minutes.*(Esch coli)*

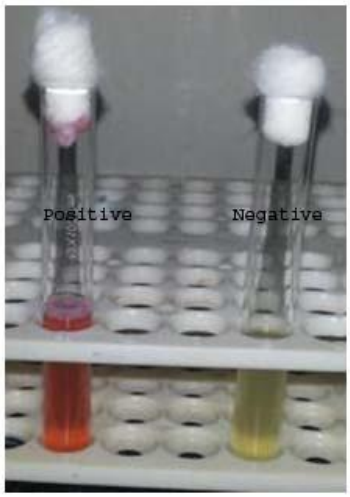


Figure: 12 VP Test

TRIPLE SUGAR IRON (TSI):

PRINCIPLE:

To determine the ability of an organism to attack specific carbohydrate incorporated in a growth medium with or without the production of gas, alone with the determination of possible hydrogen sulphide production .Triple sugar iron medium is a differential medium that can distinguish between a number of gram negative enteric bacteria based on their physiological ability (or lack thereof) to:

1. Metabolise lactose and/or sucrose
2. Conduct fermentation to produce acid
3. Produce gas during fermentation
4. Generate H_2S.

The medium contains 1.0% each of sucrose and lactose and 0.1% glucose. If only glucose is fermented, acid produced in the butt will turn it yellow, but insufficient acid products

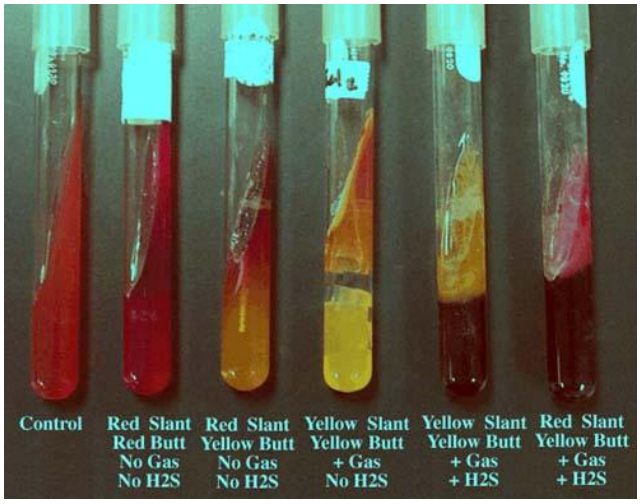


Figure: 13 TSI Test

Advanced Technology Used In Microbiology Laboratory

BACT/ALERT: The Bact/ALERT Microbial Detection System is used to determine if microorganisms are present in blood or other normally sterile body fluid samples taken from a patient suspected of having bactremia/fungemia .The Bact /ALERT System and culture bottle provide both a microbial detection system and culture media with suitable nutritional and environment condition for organism commonly encountered in blood infections and other normally sterile body fluid infections .An inoculated bottle is placed into the instrument where it is incubated and continuously monitored for the presence of microorganism that will grow in the Bact /ALERT.

PRINCIPLE:

The Bact/ALERT Microbial Detection System utilizes a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (co2) dissolved in the culture medium. If microorganism are present in the test sample, Co_2 is produced as the organisms are present in the test samples Co_2 is produced as the organism metabolize the substrate in the culture medium. When growth of the microorganisms produces Co_2, the color of the gas permeable sensor installed in the bottom of each culture bottles changes from blue green to yellow .The lighter

color results in an increase of reflectance units monitored by the system. Bottle reflectance is monitored and recorded by the instruments every 10 minutes.

Figure: 14 Bact /ALERT Microbial Detection System

Figure:15 Bact /ALERT Culture Bottles
THIRD DAY

Preparation of pure culture preparation for antibiotic sensitivity testing:

Few isolated colonies from the plates were transferred to the peptone water tubes aseptically and were incubated for 2 hours in 37 degree C.

Disc diffusion Method:

Kirby Bauer method of Disk diffusion was used.

PRINCIPLE:

Antimicrobials charged in filter paper discs with known concentration were allowed to diffuse out into seeded Mueller Hinton medium plates with test organism .The zone of inhibition is measured by scale for detecting whether the microorganism has been resistant or sensitive to the given antimicrobial drugs.

PROCEDURE:

1. Mueller Hinton Agar medium plates had prepared and inoculated using the lawn culture technique with a sterile HIMEDIA culture swabs.
2. Appropriate group of antibiotic disk were charged on the lawn culture.
3. The antibiotics were selected as the detected organisms.

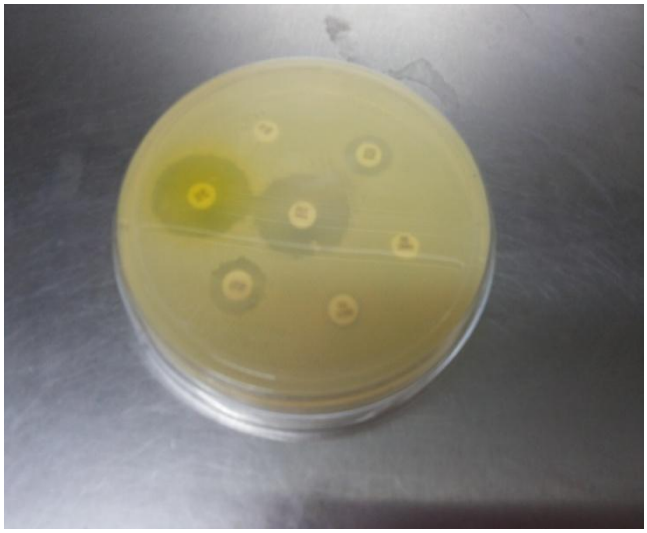


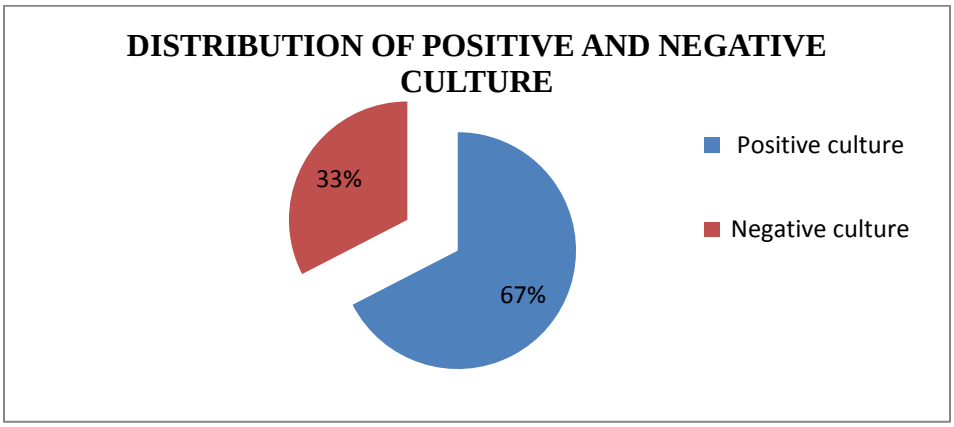
Figure: 16 Antimicrobial sensitivity testing

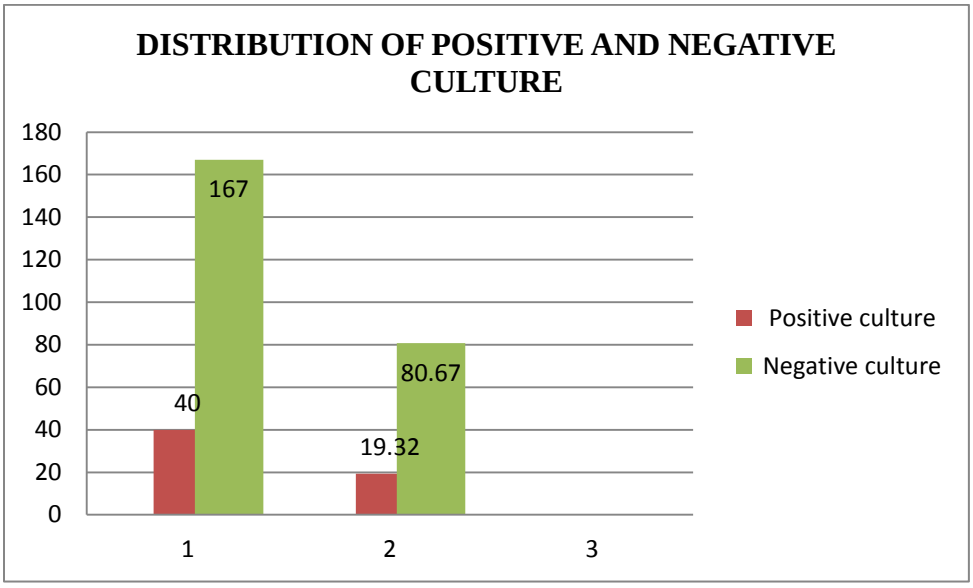
RESULTS

During the study period in the Tertiary care Hospital in Jalandhar, clinical samples were obtained from patients with various body sites infections. A total of 207 samples from ICU were processed. Among them positive and negative culture samples were listed below;

Table: Distribution of Positive and Negative culture

Samples	No of samples	Percentage
Positive culture	40	19.32
Negative culture	167	80.67



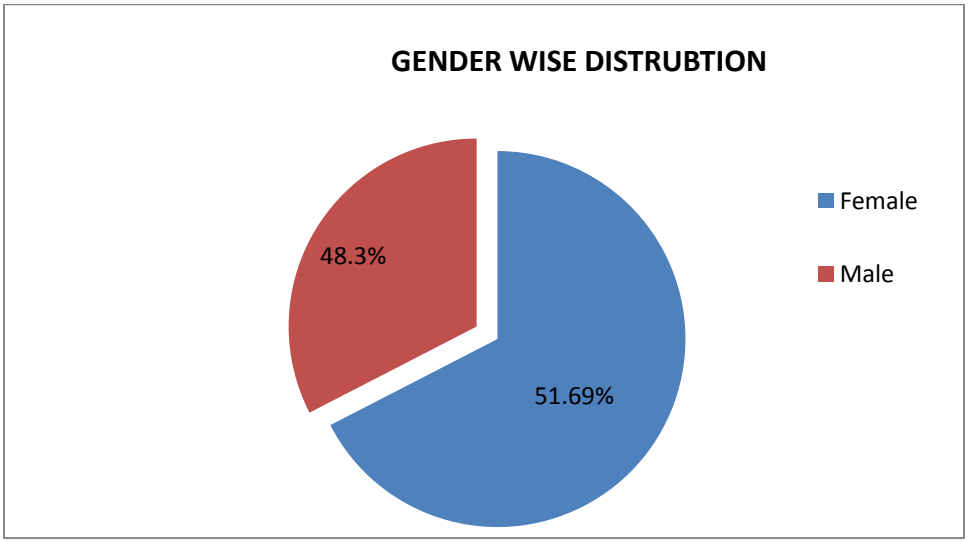


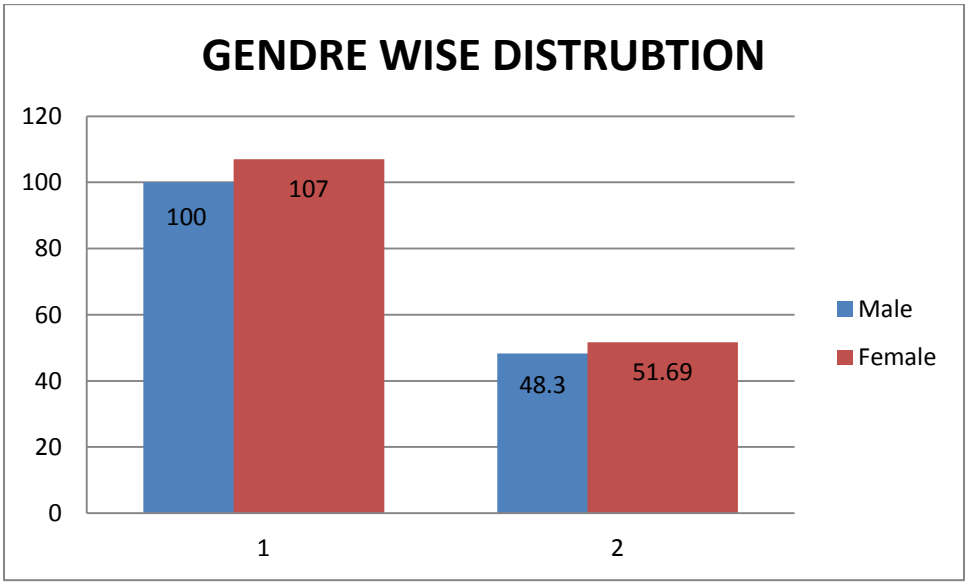
GENDRE WISE DISTRUBTION: During the study period in Tagore hospital clinical samples were obtained from both male and female patients from various body site infections. Figure shown in the Table and Pie chart below.

Gender Wise Distribution

GENDER	SAMPLES(n)	Percentage

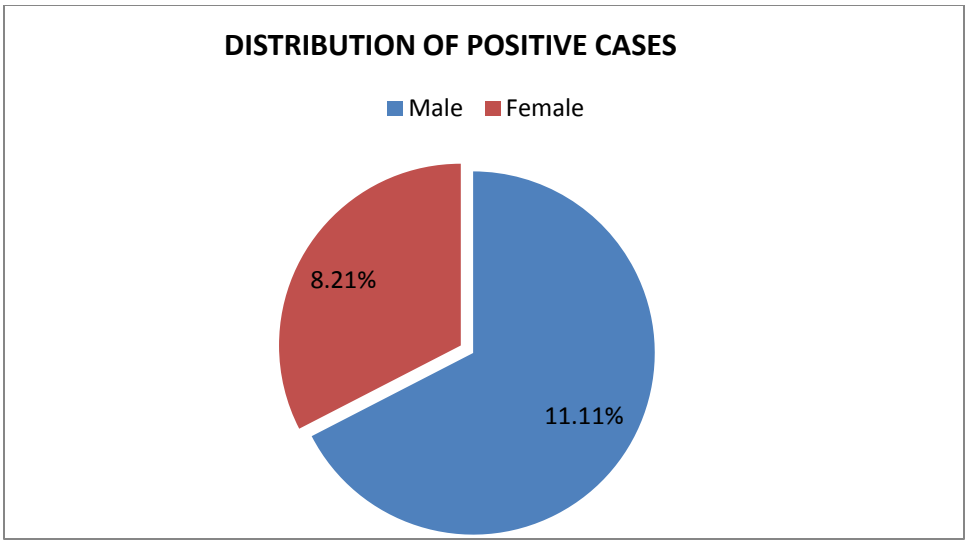
Male	100	48.3
Female	107	51.69





DISTRIBUTION OF POSITIVE CASES AMONG BOTH OF SEX

GENDER	POSITIVE	Percentage
Male	23	11.11
Female	17	8.21



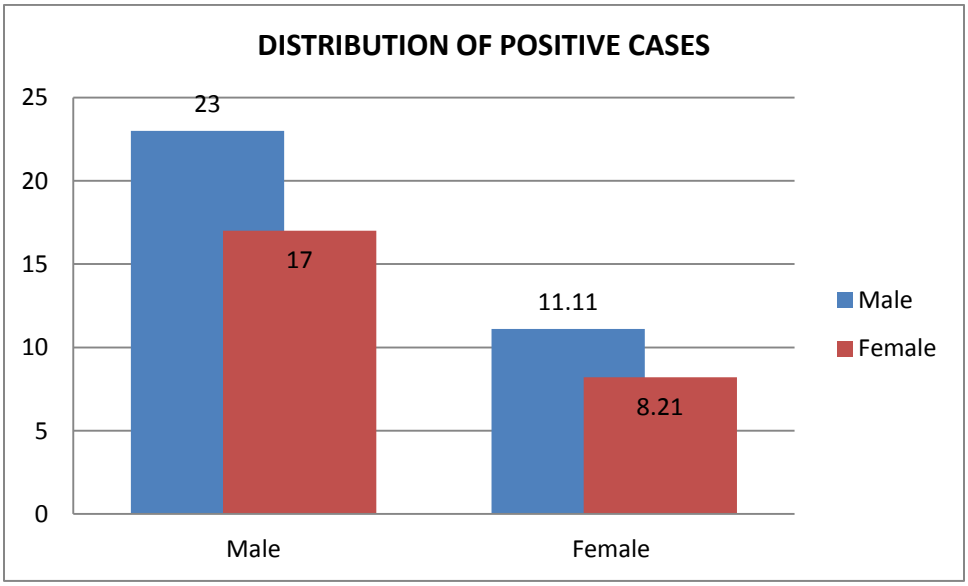
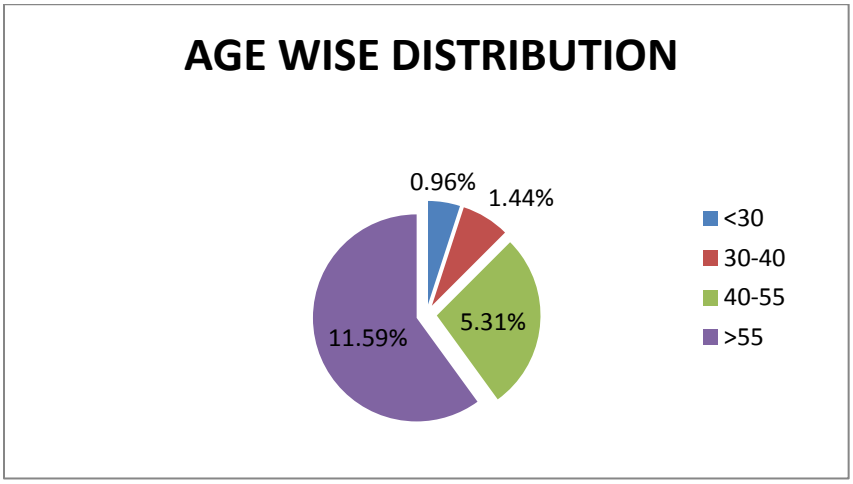


TABLE: DISTRIBUTION OF CASES IN BOTH SEX

AGE WISE DISTRIBUTION: The age of the patient having bacterial infections ranged from infancy to more than 55 years old are shown in tables and chart below.

AGE (years)	Positive growth	Percentage
<30	2	0.96
30-40	3	1.44

40-55	11	5.31
>55	24	11.59



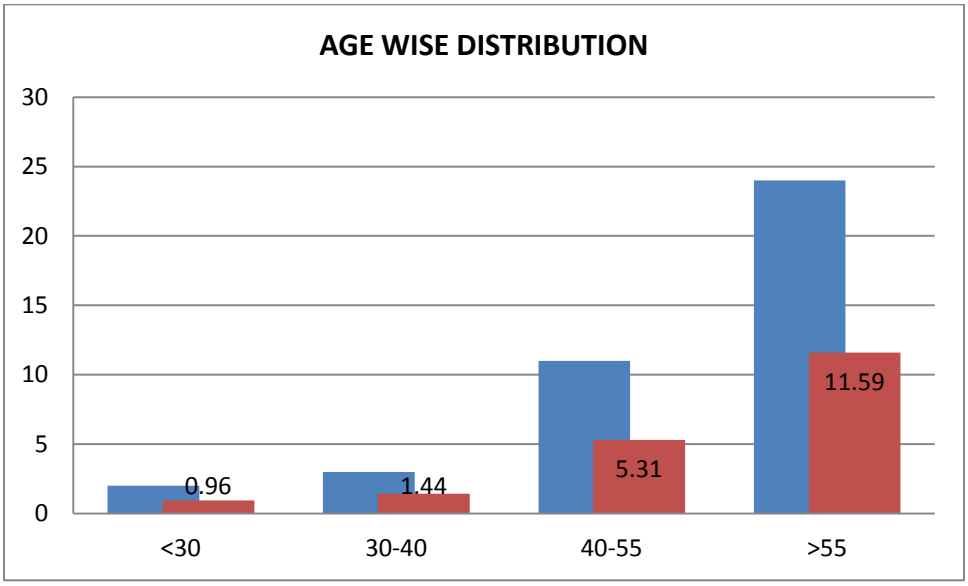
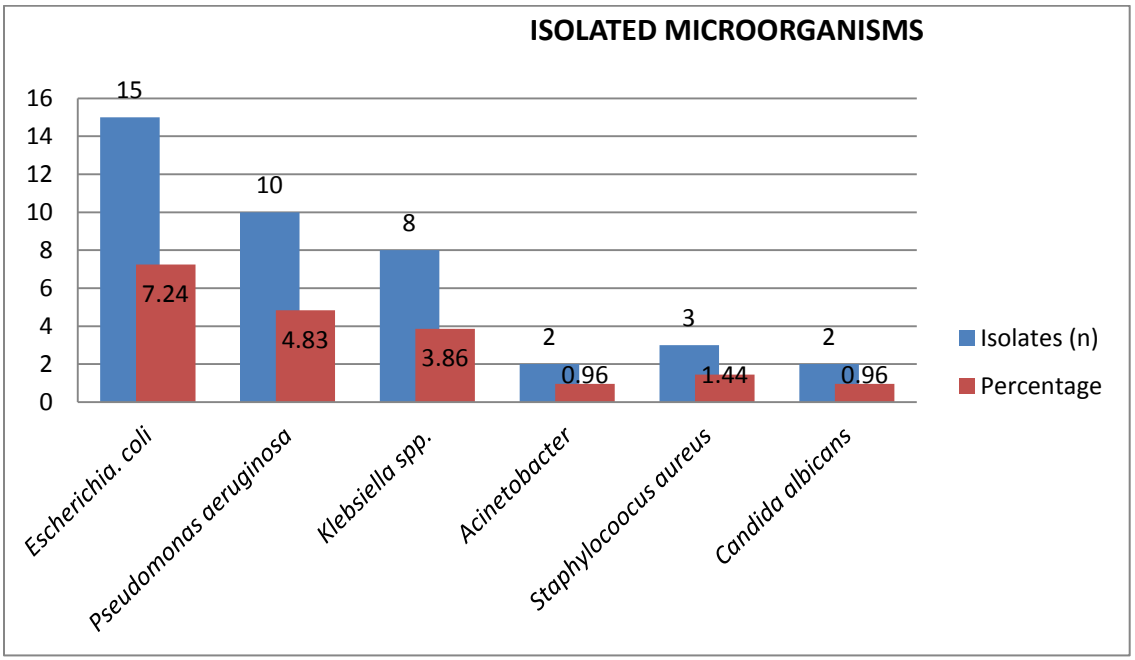


TABLE: AGE WISE DISTRIBUTION

35 samples were identified with Gram negative bacteria. Most of the Gram negative pathogens were found in miscellaneous samples. The most prevalent isolated pathogens isolated in this study were *E coli* 7.24% and pseudomonas 4.83%followed by *Klebsiella spp* 3.86%, *Acinetobacter spp* 0.96%.Isolated bacteria during the study period are shown in the Histogram below.

Isolated Microorganisms In ICU Patients

ISOLATED ORGANISMS	Isolates (n)	Percentage
Escherichia. coli	15	7.24
Pseudomonas aeruginosa	10	4.83
Klebsiella spp.	8	3.86
Acinetobacter	2	0.96
Staphylococcus aureus	3	1.44
Candida albicans	2	0.96



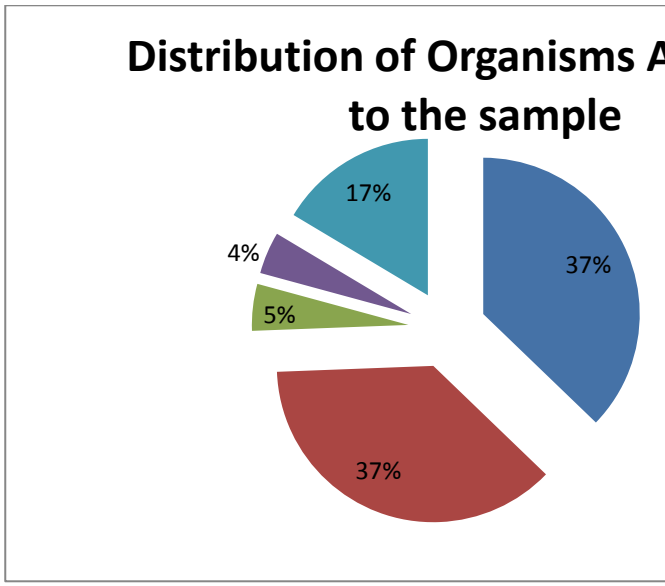
The *E coli* and *Pseudomonas* were the most common pathogen in ICU. *E coli*. According to

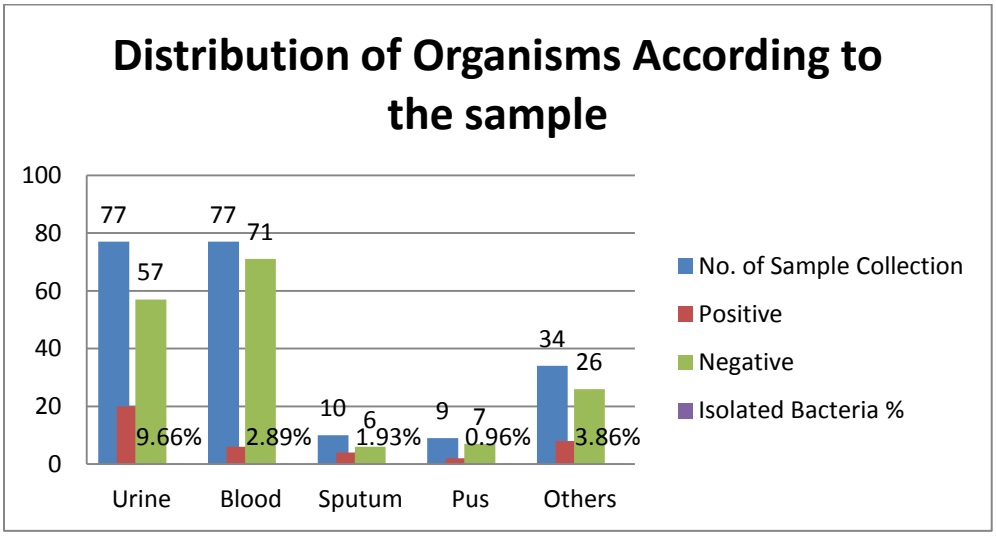
bacterial species in relation to clinical infections, the result exhibit that 35 cases with miscellaneous samples were caused four main bacterial pathogen. *E coli, Pseudomonas aeruginosa, Klebsiella and Acinetobacter*. In this case with UTI (urinary tract infection), *Ecoli* was the most frequent isolated pathogen, followed by *Klebsiella* , *Pseudomonas* and *Acinetobacter*. Similarly *Pseudomonas and Klebsiella* were the most bacterial pathogens causing respiratory tract infections and *E coli* causing blood infections.

Distribution of Organisms According to the sample

Sample Type	No. of Sample Collection	Positive	Negative	Isolated Bacteria %
Urine	77	20	57	9.66%
Blood	77	6	71	2.89%
Sputum	10	4	6	1.93%
Pus	9	2	7	0.96%
Others	34	8	26	3.86%
Total	207	40	167	19.32%

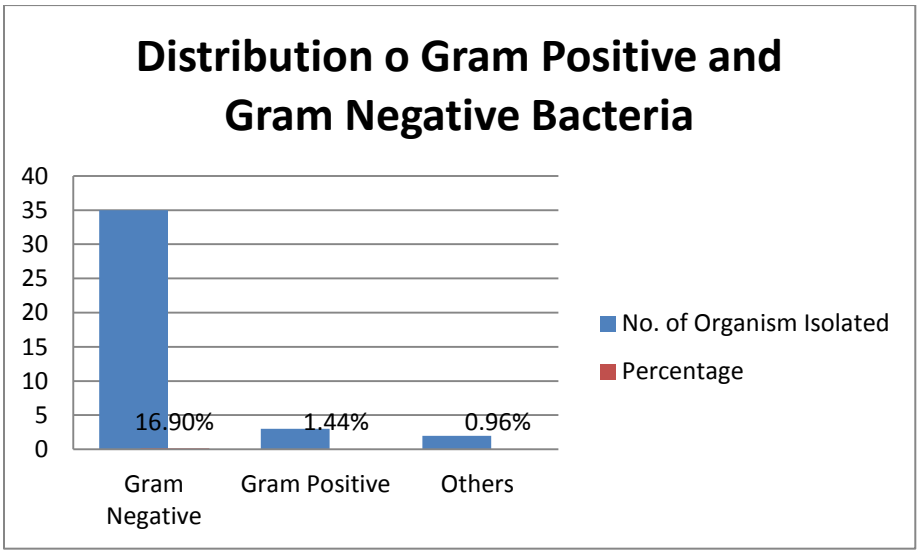
Isolated Bacteria	Total Bacteria	Percentage





Gram Negative	35	16.9%
Gram Positive	3	1.44%
Others	2	0.96%
Total	40	19.32%

Distribution o Gram Positive and Gram Negative Bacteria



DISCUSSION

The main objective of this study was to investigate microbial profile from ICU isolates in a tertiary care hospital. The result demonstrated that a total of 40 positive growth were obtained that included, *E coli* (7.24%) *Pseudomonas spp*(4.83%)) *Klebsiella spp* (3.86) *Acinetobacter* (0.96%), *Staphylococcus aureus* 1.93% *and Candida albicans* 0.96%.

The National Nonsocomial Infections surveillance (NNIS) of the centre for disease control of USA reports 60% of nosocomial pneumonia to be caused by aerobic Gram negative bacteria. They found Gram negative to be the predominant organism 19.32% with low isolation of

Staphylococcus aerus. The majority of Gram negative bacteria in our study originated from miscellaneous samples. UTI (urinary tract infections) followed by RTI (respiratory tract infections and blood infections.

A study conducted in Costal Karnataka south India to estimate the rate of nosocomial infections ICU for 18 month .The rate of nosocomial infections was 27.4%. The rate of the urinary tract, respiratory and the intra vascular catheter related infections were 55.52%, 35.78% and 11.52% respectively. *Klebsiella pneumonia* and were the most common isolates with the maximum susceptibility to Imipenem and vancomycin respectively.

This study on the microbial profile of the nosocomial infection showed that the rate of nosocomial infections is high. The risk of developed nosocomial infections was directly related to the length of ICU stay and the duration of use of indwelling devices such as catheter.

Another cross- sectional study was conducted based on reports of bacteria isolates from ICU of Fatmawati hospital; from January 2009 to March 2010 .During that period 722 patients were admitted to the ICU and 385 of them received antimicrobial treatment .Identification of all causative microorganisms was performed by standard microbiological method. During that period 385 specimens were culture positive and 136 showed no growth. The most common locations for infection were respiratory tract (78.7%), urinary tract (7.6%), surgical site(7.5%),blood(3.8%)and peritoneal fluid (2.4%).*Pseudomonas aeruginosa* was the most frequently isolated bacteria (26.5%) followed by *Klebsiella pneumonia* (15.3%), *Staphylococcus epidermidis* (14.9*%) Enterobacter aerogenes* (13.3%) *E coli* (5.2%) In this study almost bacteria isolated from ICU were resistant to Cephalexin (75%- 95%). These finding is comparable to our result.

In Asian countries the most frequent pathogen isolated from infections in the ICU are, *Klebsiella Pseudomonas aeruginosa, E coli* and *Staphylococcus aerus.* In 12 ICU in seven Indian cities overall 87.5% of all *Staphylococcus aerus* health care associated infections were caused by Methicillin resistant strains 71.4% of *Enterobacteraceae* were resistant to ceftriaxone and 26.1% to piperacillin – tazobactaum, 28 6 % *Pseudomonas aeruginosa* strains were resistant to ciprofloxacin 64.9% to ceftazidine and 42.0% to imipenem.

Another study performed at ICU of Tertiary care centre in Saudi Arabia showed that the most frequent pathogen are *Acenetobacter Bauman, P. aeruginosa , E coli , Klebsiella pneumonia* .

Recently similar study were conducted in hospital and several ICUs in Asian countries including Philippine , India ,Iran , China , Malaysia, Singapore , Nepal demonstrated that the most frequent microorganism derived from ICU samples were *Pseudomonas aeruginosa , Klebsiella spp, and Staphylococcus aerus*.

In Canada the Canadians National ICU study conducted during 2005 – 2006, showed that *Pseudomonas aeruginosa , Staphylococcus aerus , Enterococcus spp, Staphylococcus pneumonia , Klebsiella pneumonia* are the most common isolated recovered from clinically specimens in Canadian ICU. But in our study *E coli* and *Pseudomonas spp, Klebsiella spp* and *Acenetobacter* spp was the most common isolated organisms.

A retrospective record based study was conducted in the ICU of Hi tech MEDICAL College and hospital Odessa, Eastern India from November 2011 to October 2012. Patients who were clinically suspected of having acquired infections after 48 hours of admission to the ICU were included in the study .The clinically suspected laboratory samples were collected from the patients and subjected to testing and antibiotic sensitivity. Then it was reported that the rate of nosocomial infections was 28.2%, Urinary tract infections was the most common infections (54.9%). The predominant isolates was *E coli* (52.7%) followed by *Pseudomonas mirabilis* (15.4%) and *Pseudomonas aeruginosa* (13.2 %).

This result is very high comparing to our observations. This rate of nosocomial infections observation could be due to different clinical profile and the absence of a powerful hospital infection control program.

Similarly,, a study conducted in ICU over a period of 1 year by Dr Poonam C Sharma from 1st July – 30th June 2012 . All patients on mechanical ventilation for more than 48 hours were included. The incidence of ventilator associated pneumonia (VAP) was 39.6% which was similar to the study conducted by Dr Shalni.(35.78%) *Pseudomonas aeruginosa* was the predominant microorganism isolated with maximum resistant to Tobramycin , *E coli* was the second most common microorganism with maximum resistant to Ampicillin .Where as in our study E coli

was the most predominat microorganism followed by *Pseudomonas spp* and *Klebsiella spp* and *Acinetobacter*.

Five Years of Nosocomial Gram negative bactremia study was conducted in ICU of university Alberta Hospital (tertiary care hospital in Edimonton, Canada. In patient populations have been prospectively surveyed for nosocomial blood stream infections since 1986 as part of hospital's routine infection control surveillance Program. Positive blood cultures from ICU patients were identified by the clinical microbiological lab (BAC TECH 9240 BLOOD CULTURE SYSTEM) and reviewed daily. There were 6544 admissions to ICU from January 1st 1999 to December 31st 2003. 45 episodes of Gram negative nosocomial bactremia occurred in 44 patients. Seven bacterial species were identified; *Pseudomonas aerugionsa and Enterobacter spp* were most common. This observation can be also analysed to our results .In our observation Ecoli was the most common organism followed by *Pseudomonas spp and Klebsiella spp*. Source of bactremia included Pneumonia (48.9%) and central venous catheterization (22.2%) antimicrobial susceptibility were highest for imipenem, gentamicin, tobramicin , Ceftazidin and piperacillin tazobactum . Mortality rate were 53. 3% in ICU and 60% for overall hospitalization .Average length of ICU stay was 50.5 % days compared to 6.13ndays for all comers.

A study conducted in ICU by Patel Bhaumik v1, Patel Purav G2. During this period from January 2012 to June 2012 a total of 583 samples collected from ICU patients and processed for culture and identification. Out of 583, 228 (39.10%) were culture positive. The number of Gram negative and Gram positive organism isolated were 182 (79.82%), 46 (20.18%) respectively. The most frequent Gram negative organism isolated were *Pseudomonas spp* (29.12%), followed by *Acinetobacter spp* 45 (24.72%) , *Klebsiella spp* 42 (28.08%), *E coli* 35 (19.23%) and others 7 (3.85%).In our study also Gram negative bacteria was the most common isolates comparing to Gram positive bacteria in ICU patients.

A study conducted by Trupti Bajpai, G. Shrivastav . The aim of the study was to determine microbial profile of lower respiratory tract infections in ICU of tertiary care hospital India. The study was conducted for 3 years from January 2010 to December 2012 in the microbiological department of a tertiary care hospital .Out of 230 lower respiratory tract specimen evaluations 198, (86.08%) were culture positive. A total of 254 pathogen were recorded with predominance of Gram negative isolates (96.05%) and (3.9%) were Gram positive bacteria .The most prevalent

Gram negative pathogens isolated were *Pseudomonas aeruginosa* which was isolated (33.20%) followed by *Klebsiella pneumonia* (31.22%) whereas the most Gram positive pathogen were *Staphylococcus aerus*(3.55%) and *Streptococcus spp* (0.39%). Among 243 Gram negative bacteria (36.62%) has extended spectrum of beta lactamase (ESBL) isolates were recovered while out of 9 *Staphylococcus aereus* (55.55%) MRSA isolates were recovered. In our case also Gram negative bacteria were the most common isolates. Such as *E coli, Pseudomonas spp, Klebsiella* and *Acenetobacter* and the Gram positive isolates was *Staphylococcus aereus*.

Similarly a study conducted on bacteriological profile and drug sensitivity in ICU of a Tertiary Care Hospital in Ahmadabad by Javeri, Jitender R1 , Patel Shirishankar , the aim of the study was to identify prevalence of predominant bacterial microorganism and their drug sensitivity from January to 2012 to April 2012 . Sample were collected and processed. The commonest organism they isolated from the ICU samples were E coli 32 (25%) and *Acinetobacter* 20 (15.62%) *Staphylococci* in 21 (16.40%) *Klebsiella sp* in 18 (14.06%), *Pseudomonas* 17 (13.28) and *Candida sp* in 6 (4.68).This observation is higher than our results but the isolated organisms were similar to this report.

Another study conducted in Eastern Mediterranean Health Journal reported that E coli was the most frequent isolates 14% Whereas in our study also *E coli* and *Pseudomonas spp* was the most common organisms isolated from the ICU .

In the ICU of Fatmawati hospital Indonesia from January 2009 to March 2010. They reported the most predominant isolates were Pseudomonas spp 15 .3 % and staphylococcus epidermis (14.9%).And in our study *E. coli, Klebsiella spp* followed by *Pseudomonas* were the commonl pathogen and *Staphylococcus aerus* was few in numbers.

A previous study conducted in two main tertiary care hospital in Makkah showed that a total of 1137 Gram negative bacteria were identified in clinical specimens obtained from patients with various infections .Respiratory tract were the most common infections caused by Gram negative pathogens representing 396 cases (34.8%),followed by urinary tract infections (UTI) 285 cases (25.1%) , wound infections 280 cases (24.6%), genital infections 75 cases (6.6%) and septicemia 54 cases (5%).And the most prevalent Gram negative pathogen in this study were *E coli* (31.6%) and *Pseudomonas aeruginosa* (31.2%) , followed by *Acinetobcater baumannii*(10.8%)

,*Klebsiella spp*(6.2%) and *Proteus spp* (3.3%).. Overall susceptibility rate for E coli strain were high in this study for the most tested antimicrobial agent. *E coli* isolate showed a very low resistant rate to meropenem ,imipenem , cilastatin sodium (CS) and imipenem.The *Klebsiella* showed moderate resistant (15-40%). to most antibiotic with high resistant . Whereas in our study urinary tract infection and respiratory tract infections were most common caused by Gram negative bacteria.And the most prevalent pathogens were *E. coli , Klebsiella spp, Pseudomonas sp and Acinetobacter spp*

CONCLUSION

Nosocomial bactermia is associated marked morbidity and mortality in critically ill patients. This reports reveals the microbiological profile in ICU isolated patients .The most common isolated organism in ICU patients were *E coli* followed by *Pseudomonas aeruginosa, Klebsiella spp* and Acinetobacter .The isolation of multidrug resistant producing Gram negative bacteria in ICU patients during the study period resulted in a greater awareness to the presence of multi drug resistant among bacterial isolates in ICU .The ICU being as a critical centre where seriously ill patients are being treated should be free of these organisms unless a disastrous time will come

when we don't have any mean to treat these kind of MDR pathogens. Nosocomial bactermia is a major subgroup of hospital acquired infections. Gram negative bactremia in particular is associated with significant morbidity and mortality .ICU patients are at higher risk of developing nosocomial infections when compared with other hospitalized patients. Regular microbiological surveillance help in implementing better therapeutic strategies to reduce the high morbidity and mortality associates among the patients in critical care setting. Infections in ICU patients are important problem. Adherence of infection prevention protocols and the proper monitoring and the judicious use of antibiotic are important in in preventing such infections. Effective infection control programs such as surveillance can reduce the infection rate by up to 32%.The present results may be used as a guide to choosing an appropriate therapy particularly when treating suspected infections in ICU patients.

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