

PATHOGENIC MICROORGANISMS ISOLATED FROM BLOOD SAMPLE

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Microbiology

By

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SCHOOL OF BIO & CHEMICAL ENGINEERING**

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(DEEMED TO BE UNIVERSITY)**

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APRIL - 2021



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work **T. SURYA** (38300018) who carried out the project entitled **"PATHOGENIC MICROORGANISMS ISOLATED FROM BLOOD SAMPLE"** under my supervision from November 2020 to March 2021.

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DECLARATION

I **SURYA T (38300018)** hereby declare that the Project Report entitled **“PATHOGENIC MICRO ORGANISMS ISOLATED FROM BLOOD SAMPLE ”** done by me under the guidance of **Mr.G..Narendrakumar** at Sathyabama institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Microbiology.

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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr. V. Ramesh kumar, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Mr. G. Narendrakumar for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

The importance of bacterial isolates from blood sample environment as a resource and a potential source of novel resistance genes to the clinical pathogen. This study is aimed at isolated and characterized public health important bacteria, from a blood sample in hospital and nosocomial infection and evaluate the pathogenic microorganisms further study of the area. 25 blood samples collected from Dharmapuri government hospital of a paediatric and medical lab from patients (Male, Female, and infant) of age range between (1day-60 days) during the period from (02.11.2020 to 07.03.2021). Out of 22 samples were screened, only 20 sample isolates Collected in 2 samples identified as *Staphylococcus aureus*, 3 identified *Klebsiella pneumoniae*, 5 *Staphylococcus* spp. Depending on cultural, Microscopically, and biochemical characteristics, and antibiotic sensitivity test. Identified in *Staphylococcus aureus*, and *Klebsiella pneumoniae* microorganisms. Bloodstream infections can lead to life-threatening sepsis and required anti repaid antimicrobial treatment. The organism implicated in these infections varies with the geographical altered.

KEYWORDS: Bacteriological, pathogenic, nosocomial infection, Blood

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LIST OF ABBREVIATIONS AND SYMBOLS:

BSI	Blood stream infections
HAI	Hospital acquired infection
AMT	Antimicrobial resistance
CBI	Central line associated blood stream infections
PCT	Procalcitonin
SEPSIS	Blood poisoning.
<i>S.aureus</i>	<i>Staphylococcus aureus</i>
TB	Thrombocytopenia
EST	Erythrocyte sedimentation rate
%	Percentage
ALT	alanine transaminase
H ₂ SO ₄	Sulphuric acid
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i> .
AST	Aspartate transaminase

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CHAPTER 1

INTRODUCTION

Bloodstream infection (BSI) is a major cause of morbidity and mortality worldwide (Atlas, 2010). The mortality rate associated with BSI is estimated at 15–25% (Aljanaby and Alhasani, 1992). Administration of appropriate antimicrobial therapy can reduce mortality risk (Leibovici et al. 1998; Paul et al. 2010); however, it requires timely reporting of blood culture results to an attending physician (AP) as part of antimicrobial stewardship (AS) activities. Rapid identification methods, including molecular testing with direct communication, may help ensure a timely response and reduce the mortality rate (Bearman et al. 2005). Several challenges have been identified as hindering rapid reporting of positive blood cultures. In general, infectious disease physicians (IDP), microbiological technologists (MT) and other AS staff report blood culture results to an AP. However, the specific personnel protocol is often unclear in such cases. Furthermore, dealing with cases of positive blood culture on weekends is problematic due to fewer staff being present on site (Bell and Redelmeier, 2001).

Hospital-acquired infections (HAI), also called nosocomial infections, affect the clinical outcomes in hospitalized patients and represent a serious concern worldwide. The misuse and overuse of antibiotics in medicine and agriculture has led to the development of multidrug-resistant (MDR) bacteria, which are now recognized as a major cause of nosocomial infections. In addition to a higher risk of poor clinical outcomes and death, MDR bacterial infections are associated with increased economic burden of patients. Understanding the clinical characteristics, prevalence, and of nosocomial infections caused by MDR bacteria is crucial for effective treatment.

Bloodstream infection is the most common cause of sepsis, so there are more than 45% of BSI are caused by single bacterial species, which can introduce in the blood, such opportunistic pathogens (e.g., *Staphylococcus aureus*,

Escherichia coli, Acinetobacter spp, klebsiella pneumonia), and fungi species (Berg, 2008) Most of the blood pathogenic organism identified as immuno compromised disease, such as chronic disease, HAI, SEPSIS.

Hospital-acquired infections (HAI), also called nosocomial infections, affect the clinical outcomes in hospitalized patients and represent a serious concern world wide

1.1 .BLOOD STREAM INFECTION:

Septicemia is a serious bloodstream infection. It's also known as blood poisoning. Septicemia occurs when a bacterial infection elsewhere in the body, such as the lungs or skin, enters the bloodstream. This is dangerous because the bacteria and their toxins can be carried through the bloodstream to your entire body. Septicemia can quickly become life-threatening. It must be treated in a hospital. If left untreated, septicemia can progress to sepsis. Septicemia and sepsis aren't the same. Sepsis is a serious complication of septicemia. Sepsis causes inflammation throughout the body. This inflammation can cause blood clots and block oxygen from reaching vital organs, resulting in organ failure.

The National Institutes of Health estimates that over 1 million Americans get severe sepsis each year. Between 28 and 50 percent of these patients may die from the condition. When the inflammation occurs with extremely low blood pressure, it's called septic shock. Septic shock is fatal in many cases. KP-BSIs was defined as the isolation of KP in a blood culture specimen The onset of bacteremia was defined as the date when the first positive blood culture was obtained.

The primary site of infection was determined using clinical criteria and isolation of the infecting organism from sources other than blood. MDR was defined as resistance to at least 1 member of the following 3 classes of antibiotics: aminoglycosides (amikacin, gentamicin, or netilmicin), fluoroquinolones (ofloxacin or ciprofloxacin), and cephalosporins (cefazolin, cefotaxime, ceftazidime, ceftazidime, or cefepime).

Clinical variables collected from patients with bacteremia included age, gender, underlying medical conditions (including malignancy, leukemia, chronic renal

disease, and diabetes mellitus), smoking, alcohol consumption, laboratory findings (including C-reactive protein [CRP], Procalcitonin [PCT], erythrocyte sedimentation rate [ESR], leukopenia, thrombocytopenia [TB], hemoglobin, blood glucose, serum creatinine [Scr], bilirubin, albumin, cereal third transaminase [ALT], and glutamic-oxaloacetic transaminase [AST]), and insertion of invasive devices (i.e., drainage catheter, central venous catheter, mechanical ventilation, and urinary catheter). TB was defined as platelet count less than $150 \times 10^9/L$. Neutropenia was defined as a peripheral absolute neutrophil count of less than 500 cells/mL.

Blood stream infection (BSI) is the main cause of deaths attributable to infectious diseases]. Especially, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) are important healthcare-associated infection causative s of BSIs, both in view of (HAI) surveillance antimicrobial resistance (AMR) organism and in modern global health. The current standard for diagnosis of bloodstream infections, including device associated infections (e.g., central line-associated bloodstream infections, , requires sequential bacterial detection, identification (ID), and antibiotic susceptibility testing (AST) via traditional blood cultures (Da Silva et al., 2005).

This culture-based three-step diagnostic approach is not optimal with profound clinical implications. First, the time to result for blood cultures typically ranges from 24 to more than 48 hours. Typical AST requires an additional 16–24 hours of culture of the isolated pathogens. Keeping in mind the high mortality and morbidity associated with septicemia, right choice of empiric therapy is of importance. Therefore, the present study was undertaken to analyze the various organisms causing septicemia and their antibiotic resistance patterns, as it would be a useful guide for clinicians initiating the empiric antibiotic therapy

1.2 SOURCE NOSOCOMIAL INFECTION IN BLOOD:

Nosocomial infections, also called health-care-associated or hospital acquired infections, are a subset of infectious diseases acquired in a health-care facility. To be considered nosocomial, the infection cannot be present at admission; rather, it must develop at least 48 hours after admission. These infections can lead to serious problems like sepsis and even death.

The microbes cause nosocomial infections originated in hospitals, clinics, and medical care center.

The method of transfer of hospital-acquired infection can be either by direct or by indirect contact. Touching an infected person, animals or reservoir of infection count to be in direct contact. Contaminated hands are one of the principal routes of disease transmission. Communicable infectious agents cause indirect disease transmission does not require direct connection of an infected person with a healthy one.

Pathogens present in inanimate object and utilization of these objects can lead to transmission of pathogens. Aerosols originated by coughing and sneezing of patients may carry pathogens that can transmit the diseases . *Klebsiella* is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics (40) and showing characteristic radiographic abnormalities (81) due to a severe pyogenic infection which has a high fatality rate if untreated (Doughari et al., 2011).

The vast majority of *Klebsiella* infections, however, are associated with hospitalization. As opportunistic pathogens, *Klebsiella* spp. primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying diseases such as diabetes mellitus or chronic pulmonary obstruction. Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus. To a much lesser degree, *K. oxytoca* has been isolated from human clinical specimens. It is estimated that *Klebsiella* spp

CHAPTER 2

LITERATURE SURVEY

Bloodstream infection is the most common cause of sepsis, so there are More than 45% of BSI are caused by single bacterial species, which can introduce in the blood, such opportunistic pathogens (e.g., *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* spp, *klebseilla pneumonia*), and fungi species (Elsner *et al.*, 2000).

Staphylococcus aureus have different virulence factors which give the bacteria the ability to invade the host, such as surface proteins that promote colonization and invasiveness (leukocidin, kinases, hyaluronidase) and surface factors that inhibit phagocytic engulfment (capsule, ProteinA).

Klebseilla pneumonia have different virulence factors which gave the bacteriathe ability to invade the host, such as capsular polysaccharide, lipopolysaccharide, serum resistance, siderophore production, fimbriae and other factors such as the production of urea and enterotoxin (Aher *et al.*, 2012).

The pathogenicity of *Staphylococcus aureus*, is related to production of wide variety of exoproteins, including alpha and beta haemolysins which contributes to its ability to cause diseases in humans (Dinges *et al.*, 2000).

Beta haemolysin or alpha toxin considered to be a main pathogenicity factor because of its haemolytic, dermonecrotic and neurotoxic effects. Additionally, beta-haemolysin contains sphingomyelinase that more active against sheep and bovine erythrocytes (De-Silva *et al.*, 2005).

Staphylococcal enterotoxin B (SEB) is one of the 20 exotoxin excreted by the *Staphylococcus aureus* bacterium, Staphylococcal enterotoxin B (SEB) is the toxin most commonly associated with classic food poisoning, It has also been demonstrated to cause a nonmenstrual toxic shock syndrome (TSS) (Hennekinne *et al.*, 2012)

2.1 BLOOD PATHOGENS:

Using Koch's postulates for the identification of pathogenic microbes, Ogston identified the etiological agent of suppurative abscesses (Ogston, 1883). The name *Staphylococcus aureus* was chosen to distinguish this species with its characteristic yellow colony pigment from another staphylococcal commensal that forms white colonies (*Staphylococcus albus*, now designated *Staphylococcus epidermidis*) (Fluit et al., 2000).

S.aureus displays several striking microbiological properties, e.g., the microbe binds immunoglobulins and agglutinates with or coagulates blood and plasma (Kempf et al., 2011). These traits have been useful for the early and rapid diagnosis of *S. aureus* infections

All *Staphylococci* grow in clusters, a feature that can be visualized by microscopy and accounts for the Greek name σταφυλοκοκκος or grape-like berry. Clustering is caused by the incomplete separation of daughter cells following division in three alternating perpendicular planes (Hussein et al., 2013). *S.aureus* cells appear perfectly spherical with a diameter of ~1 μm (Giesbrecht et al., 1998). *S. aureus* also produces catalase; when applied to colony material, the catalase test is a rapid, useful test to distinguish *Staphylococci* from other Gram-positive bacteria such as *Streptococci*.

S.aureus is a facultative anaerobe that grows by aerobic respiration or by fermentation, which yields principally lactic acid. The bacterium metabolizes glucose via the pentose phosphate pathway (Høiby et al., 2010).

There is no evidence for the existence of the Entner-Doudoroff pathway; however, enzymes of the entire tricarboxylic acid cycle and a typical F₀F₁-ATPase are encoded by the genome of *S. aureus* (Gwendolyno, 1988).

Upon glucose depletion, *S. aureus* cells growing in aerobic conditions oxidize D-galactose, acetate, succinate and malate. An excellent summary of these metabolic pathways was recently published (Goldman et al., 2015)

CAUTION: *S. aureus* is a highly virulent and adaptable pathogen with the ability to infect, invade, persist, and replicate in any human tissue including skin, bone, visceral organs, or vasculature (Lowy, 1998).

The organism has been placed in Risk Group Level 2. All manipulations with *S. aureus* strains must be performed following biosafety level 2 measures including experimental work in certified biosafety cabinets.

Guidelines for BSL2 practice can be obtained from the latest edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL, 5th Edition) via the following CDC Web link: <http://www.cdc.gov/biosafety/publications/bmbl5>.

2.2. *Klebsiella pneumoniae*

The genus *Klebsiella* was named after the German microbiologist Edwin Klebs (1834–1913). It is also known as Friedlander's bacillum in honor of Carl Friedländer, a German pathologist, who proposed that this bacterium was the etiological factor for the pneumonia seen especially in immunocompromised individuals such as people with chronic diseases or alcoholics.

Community-acquired pneumonia caused by *Klebsiella pneumoniae* may be called Friedländer's bacillus. In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia, and sepsis.

For patients with an invasive device in their bodies, contamination of the device becomes a risk; neonatal ward devices, respiratory support equipment, and urinary catheters put patients at increased risk. Also, the use of antibiotics can be a factor that increases the risk of nosocomial infection with *Klebsiella* bacteria. Sepsis and septic shock can follow entry of the bacteria into the blood. (Hennekinne *et al.*, 2012). *Klebsiella pneumoniae* (KP) has been reported as the second overall cause of gram-negative bloodstream infections (BSIs) after *Escherichia coli*. KP is a common pathogen that causes infections of the bloodstream, urinary tract, lungs, intra-abdominal, and other sites (Meatherall *et al.* 2002)

2.3 *Staphylococcus epidermidis*:

S. epidermidis is a biofilm-producing commensal organism found ubiquitously on human skin and mucous membranes, as well as on animals and in the environment.

Biofilm formation enables this organism to evade the host immune system. Colonization of percutaneous devices or implanted medical devices allows bacteria access to the bloodstream. Isolation of this organism from blood cultures may represent either contamination during the blood collection procedure or true bacteremia. *S. epidermidis* bloodstream infections may be indolent compared with other bacteria. Isolation of *S. epidermidis* from a blood culture may present a management quandary for clinician.(Sharon kleinschmidt, 30.oct.2015).

2.4 Blood culture history:

Early blood culture methods were labour-intensive. One of the first known procedures, published in 1869, recommended that leeches be used to collect blood from the patient.

A microbiology process from 1911 noted that decontamination of the draw site and equipment could take over an hour, and that due to a lack of methods for preserving blood, the cultures would sometimes have to be prepared at the patient's bedside. In addition to subculturing the broth, some protocols specified that the blood be mixed with melted agar and the mixture poured into a petri dish.

In 1915, a blood culture collection system consisting of glass vacuum tubes containing glucose broth and an anticoagulant was described. Robert James Valentine Pulvertaft published a seminal work on blood cultures in 1930, specifying—among other insights—an optimal blood-to-broth ratio of 1:5, which is still accepted today.

The use of SPS as an anticoagulant and preservative was introduced in the 1930s and 40s and resolved some of the logistical issues with earlier methods. From the 1940s through the 1980s, a great deal of research was carried out on broth formulations and additives, with the goal of creating a growth medium that could accommodate all common bloodstream pathogens.

CHAPTER :3

AIM AND SCOPE

3.1 AIM:

Therefore this study was aimed to isolate and identified pathogenic bacteria in the blood.

3.2 OBJECTIVES:

- i. To perform further gram staining.
- ii. To isolation and identification cultural character.
- iii. To perform biochemical test characters.
- iv. To perform antibiotic susceptibility of Pathogenic isolated some conventional and traditional antibiotics.

3.3. Source of the data

22 samples of blood were collected from patients suspected of having bloodstream infection and certain clinical symptoms. these sample were collected and different age group in gender patients for the present study were those attending Government hospital Dharmapuri District, Tamilnadu.

CHAPTER 4: MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 *Equipment's used*

1. Test tubes
2. Auto clave
3. Incubater
4. Laminor air flow
5. Culture botteles(aerobic,anaerobic)
6. Microscope
7. Sterile syirange
8. Inoculation loop ,
9. Petri plates
10. Brain heart infusion agar
11. Gram stain solution
12. Biochemical solution
13. Antibiotic equipment
14. Centrifuge
15. MacConkey agar
16. Mueller- Hinton broth
- 17. Antibiotic discs.**

METHODOLOGY

4 .2 *Source of the data*

22 samples of blood were collected from patients suspected of having blood stream infection and certain clinical symptoms. these sample were collected and different age group in gender patients for the present study were those attendinggovernment hospetial Dharmapuri District, Tamilnadu.

4.3 .*Specimen collection:*

- i. Draw blood by blood collection procedure in an aseptic conditions.
- ii. Remove the tip of the aluminium in the bottle and wipe the top with alcohol (Wipe and let dry prior to collection of specimen).

- iii. Sample volume:
- iv. 1-3 ml of blood in case of paediatric patients (25mL).
- v. 3-10 ml of blood in case of adult patients (50mL).
- vi. Inject blood into aerobic and anaerobic bottles.
- vii. NOTES: It is important to obtain the correct amount of blood. An inadequate volume may lead to false negative



Fig: 1.1 Blood collection tube

4.4 Sample processing

- i. The sample collection period was from Dec 2020 to march 2021. Blood sample collected from admitted patients were brought to the pathology laboratory
- ii. Blood is drawn from patients by using a syringe (5 ml).
- iii. It is immediately Blood was transferred to clean sterilized brain heart infusion broth tube, the blood is then allowed to clot for at least 10 to 15 minutes at room temperature,
- iv. To review the steps which can help in prevention of broth, and incubated at 37 C for 24 hours.
- v. Then kept in an incubator for 24 hours for further laboratory investigations .
- vi. In case of no growth, incubation period was extended for another 24 hours Growth was sub cultured on Blood agar and MacConkey agar plates, and incubated for ten days at 37 C.
- vii. For broth with no growth even after 48 hours were still incubated upto ten days.



Fig 4.1. Brain heart infusion agar broth

4.5 BLOOD CULTURE

The blood specimens were inoculated on blood agar, McConkey agar and chocolate agar plates by direct streaking method using a loop to deliver a loopful of the blood specimens. After incubation overnight at 37°C the bacterial growth is examined, if there were no growth, the plates were re-incubated for another 24 hours before they were considered as a negative culture (Novak-Weekley and Dunne, 2016).



Fig : 4.2. Blood culture bottle.



Fig :4.3 .Lab work

4.6. Isolation and identification of bacteria

Bacteria have been isolated from pure colonies and cultured on blood, McConkey, and chocolate agar then isolated bacteria were examined microscopically by using Grams stain technique for referred to as Gram-positive or Gram-negative bacteria. The identification tests include cultural, morphology, and physiological characteristics of each bacterial isolates were done (Brown, 2005).

4.7 Identification of Morphological characteristics

Colonies of the bacterial isolates that cultured on blood agar and MacConky media were described according to their shapes, color, diameter, odor, and other characteristics (Macfaddin, 2000).

MICROSCOPIC OBSERVATION

4.8 Physical Characterization

4.8.1 GRAM'S STAINING

A loopful of overnight grown culture was taken and a uniform smear was made on a clear glass slide and it was heat fixed.

Crystal violet solution was added on to the slide and kept for a minute. The slide was then washed with water and flooded with a solution of Gram's iodine. After a minute, 95% ethanol was used on the slide for decolorization. Then, the washed slide was counter stained with saffranine and observed under a light microscope.

The Gram-positive organisms appeared violet in colour and Gram-negative organisms, pink in colour.

4.8.2 MOTILITY TEST

A loopful of culture was stabbed onto semisolid Agar tubes. The tubes were incubated at room temperature for 24 hrs. Motile culture showed diffuse growth and non-motile culture showed growth only along the line of inoculation.

4.9 BIOCHEMICAL CHARACTERIZATION

4.9.1 CATALASE TEST

This test was performed to know whether aerobic bacteria possess Catalase enzyme or not. A small quantity of bacterial culture was scrapped out using a capillary tube and transferred to a test tube containing H₂O₂. Evolution of gas

bubbles from H₂O₂ is positive for the presence of Catalase enzyme in the bacteria (Karlowsky et al.,2002).

4.9.2 OXIDASE TEST

This test was performed in order to know whether the bacteria possess Oxidase enzyme or not. A loopfull of bacterial culture was rubbed over the Oxidase disc (Hi-media) and development of purple blue colour within 30 seconds was taken as oxidase positive.

4.9.3 INDOLE PRODUCTION TEST

This test was performed to check the ability of bacteria to produce indole. The medium consisted of the following ingredients

Indole medium

Tryptone	-	10.0 g/l
Sodium chloride	-	0.5 g/l
Distilled water	-	1000 ml
pH	-	7.4 ± 0.2

Kovac's reagent

p-dimethyl amino benzaldehyde`	-	5.0 g/l
Amyl alcohol	-	750 ml
HCl	-	250 ml

Bacteria was inoculated on the indole medium and incubated for about 24-48 hrs at 37°C. To the bacterial culture, few drops of Kovac's reagent was added and shaken well. Development of pink or red coloured ring was recorded as positive for indole production.

4.9.4 PROSKAUER TEST-Methyl red and voges

This test was performed to know whether the organism produced has acid end products or neutral end products. The medium consisted of the following ingredients

Peptone	-	7.0 g/l
Dextrose	-	5.0 g/l
Dipotassium phosphate	-	5.0 g/l

Distilled water	-	1000 ml
pH	-	6.9 ± 0.2

5 ml of the above medium was poured into a test tube and autoclaved at 15 lbs for 15 minutes.

Methyl red reagent

Methyl red	-	0.1 g/l
Ethyl alcohol	-	300 ml
Distilled water	-	200 ml

Five drops of methyl red were added and development of red colour was recorded as positive for MR test.

Voges-Proskauer reagent

VP – Reagent A

Alpha-naphthol	-	5.0 g
Ethyl alcohol	-	100 ml

VP – Reagent B

Potassium hydroxide	-	40.0 g
Distilled water	-	100 ml

To another set of bacterial cultures, few drops of VP reagents A and B were added and mixed well. Development of pink colour was positive to VP test and indicated the production of acid by fermentation.

4.9.5 CITRATE UTILIZATION TEST

This test was performed to know whether the organism utilized citrate as the sole source of carbon. The medium consisted of the following ingredients

Sodium citrate	-	2.0 g/l
Magnesium sulphate	-	0.2 g/l
Sodium chloride	-	5.0 g/l
Ammonium dihydrogen phosphate	-	1.0 g/l
Dipotassium phosphate	-	1.0 g/l
Bromothymolblue	-	0.08 g/l
Agar	-	15.0 g/l

Distilled water	-	1000 ml
pH	-	6.8 ± 0.2

Slants of the above medium were prepared in test tube and bacteria were inoculated and incubated at 37°C for 24 hrs. Change of the colour of the medium from green to blue and it was taken as positive for utilization of citrate as a sole carbon source for growth (Schaeffer, 2002).

4.9.6 UREASE TEST

This test was performed to know whether the organism produced the enzyme urease or not. The medium consisted of the following ingredients

Peptone	-	1.0 g/l
Glucose	-	1.0 g/l
Sodium chloride	-	5.0 g/l
Mono potassium phosphate	-	0.2 g/l
Dipotassium phosphate	-	12 g/l
Phenol red (1.2%)	-	1 ml
Agar	-	15.0 g/l
Distilled water	-	1000 ml
pH	-	6.8 ± 0.2

The base was prepared and sterilized by autoclaving at 121°C for 15 lbs for 15 min. The media was cooled to 56°C, 40% Urea was added and allowed to solidify in a slant position, the slant was inoculated with a drop of 4-6 hours growth of bacterium in broth and incubated at 37°C for 24 hrs.

4.9.7 TRIPLE SUGAR IRON TEST

This test was performed to know whether the organism produced H₂S and gas formation. The medium consisted of the following ingredients

Beef extract	-	3.0 g/l
Yeast extract	-	3.0 g/l
Peptone	-	2.0 g/l
Sodium chloride	-	5.0 g/l-
Glucose	-	1.0 g/l

Lactose	-	10.0 g/l
Sucrose	-	10.0 g/l
Ferrous ammonium sulphate	-	2.0 g/l
Sodium thiosulphate	-	3.0 g/l
Phenol red	-	2.5 g/l
Agar	-	15.0 g/l
Distilled water	-	1000 ml
pH	-	7.4 ± 0.2

The ingredients were dissolved, autoclaved at 121°C for 15 lbs for min. Then pour the media in sterile test tubes in such a way that about 1 inch butt and slope is obtained. Colonies were picked up with a straight wire loop, and inoculate by stabbing down the centre of agar butt carefully. Incubate at 37°C for 24-48 hrs for 1 to 2 days.

4.9.8 NITRITE TEST

This test was performed to know whether the organism reduced nitrate. The medium consisted of the following ingredients

Beef extract	-	0.3 g/l
Peptone	-	0.5 g/l
Potassium nitrate	-	0.1 g/l
Distilled water	-	1000 ml

Before adding potassium nitrate and glucose the other ingredients of the medium were boiled to dissolve them, and the pH was adjusted to 7.4. Then the medium was poured in test tubes and autoclaved at 15 lbs for 30 min. The bacteria were inoculated and incubated at 37°C for 24 hrs.

To 5 ml of the bacterial culture, 0.5 ml of 1% sulphanilic acid prepared in 5M acetic acid, followed by 0.5 ml of 0.6% dimethyl-naphthalamine in 5M acetic acid was added.

Development of red colour indicated the reduction of nitrate into nitrite. If no colour was developed, 5 mg of zinc dust was added to each test tube. Development of red colour indicated that the nitrate has not been broken down by the bacterial isolates.

4.9.9 CARBOHYDRATE FERMENTATION TEST

Carbohydrate fermentation test is used to identify the fermentation of sugars. The medium consisted of the following ingredients

Carbohydrate broth

A. Base

Peptone	-	1.0 g/l
Beef extract	-	1.0 g/l
NaCl	-	0.5 g/l
Distilled water	-	100 ml.

B. Carbohydrate Solution

Carbohydrate	-	10 g/l
Distilled water	-	100 ml

C. Indicator

Bromocresol purple	-	1.6 g/l
Ethanol	-	100 ml
Solution A	-	900 ml
Solution B	-	100 ml
Solution C	-	1ml

One ml of mixed solution was taken in a test and autoclaved for 10 min at 121°C. Filter sterilized (5-10%) carbohydrates were added aseptically to the sterile base to make 1% Solution. Lactose, Sucrose, Glucose, Mannitol and Maltose were used for sugar fermentation.

4.10 SENSITIVITY TEST

A sensitivity analysis is a test that measures a bacteria's "sensitivity" to an antibiotic. It also determines the drug's ability to destroy bacteria. Your doctor will use the results of the test to assess which medications are most likely to be successful in treating the infection (Garau et al.,1999).

Take a sterile Muller Hinton agar plate . The proper labelling should be done in the plate . Let sterilize our cotton swab for swabbing. After sterilization , using cotton swab take some colonies. After that open the plate wipe the swab inside gently .After completely swabbing the plate in one direction slightly rotate the plate and continue swabbing. Close the plate and then dispose the swab in a biohazard container. The next step is to place antibiotic disc on the plate for that first select the antibiotics .After that sterilize the tip of the forceps .Pick up the disc carefully from one antibiotic disc and place the disc onto the swabbed plate. Each disc should be slightly pressed down to ensure the contact with agar. The disc should not keep closer and keep atleast 25 millimeter distance from this to disk maximum 6 discolour should be placed on a 90 mm plate. Close the plate and keep the plate in an incubator at 37 C in inverted position for 12 hours. After 12 hours we can observe the result.

CHAPTER 5:

RESULTS AND DISCUSSION;

5.1 Isolation of bacteria

Blood samples from a total of 25 clinical different blood samples were collected from Dharmapuri government Hospital of paediatric and Medical city.

Table (1) samples were collected from different age groups and gender during the period from 05/12/2020 to 05/3/2021. ten blood were clinical blood positive samples while the rest 15 sample were negative blood samples. The relationship of BSI with the age of patients was investigated in this study and the patients were grouped into three categories according to their age as shown table.

.5.1Table (): Total number of samples used for the isolation of bacteria

Clinical sample	Positive (growth)	Negative (growth)
25 sample	05(20%)	20(80%)

5.2. Incidence of blood stream infection (BSI)

Blood samples from a total of 25 clinical different blood samples were collected, their ages ranging from, (adult: 19 - 75 years).

The results have showed that 05 of blood samples contained heavy bacterial growth while 20 of samples had no bacterial growth as demonstrated in table

5.2 Table): Distribution of incidence of routine symptomatics in relation to age of patients

Age group (Specimen) -25	(19-75) years
Male	Female
15(60%)	10(40%)

5.3.IDENTIFICATION OF BACTERIAL ISOLATES

Several morphological, physiological and biochemical tests were made to identify bacterial isolates. 10 isolates were obtained from one 25 samples. Results showed that constitute identified as *K. pneumoniae* (3 isolates) and, . Constitute, *Staphylococcus aureus* constitute (2 isolates) , *e.coli* (05 isolates). The other

bacterial isolates were constituted Figure (1) illustrates the percentages of each bacterial species found in the collected samples.

Bacterial isolates were identified according to their cultural, microscopical and biochemical characteristics that were in agreement with (Holt *et al.*, 1994; Atlas *et al.*, 1995; Collee *et al.*, 1996).

5.3.1 Microscopic observation:

Gram's staining:

Gram's staining was performed for the identification of bacteria. While the gram staining there are totally 3 types bacteria were observed from both gram positive and gram negative. The identified bacteria which is appear in gram positive cocci and gram negative rods.

Motility test:

Motility test were performed for the identification of presence of flagella in the bacteria. Flagella used to for movement of bacteria. It may identified by hanging drop technique. Overnight incubated culture used for the motility test. Movement of bacteria was observed under the high power objectives of the microscope. One is motility positive and two are negative

5.3.2 Colony Morphology Selective media characterization of bacteria:

There were three sample bottles only produce hemolysis in the blood culture bottle. These samples were transferred into nutrient agar and some selective media for the further studies.

Selective media such as MacConkey agar, Eosin methylene blue (EMB) agar, Mannitol salt agar (MSA) were used for the identification of confirmed bacteria. Bacterial growth results were showed in figure . Based on the result of biochemical analysis and growth in media, the bacteria was identified and the results were tabulated in the table .

First identification of bacterial isolates were done after incubated aerobically on MacConkey agar, blood agar and EMB agar plates and anaerobically on chocolate agar plates at 37 C° for 24-48 hrs.

On MacConkey agar, *Klebsiella pneumoniae* are gram negative colonies which are lactose fermenting colonies and gave pink color, regular edge, round mucoid texture with large size. The first is characterized by producing pink colonies due to the conversion of neutral red indicator dye when it is below pH 6.8.

Adversely, the Non-lactose bacterial growth appears colorless or transparent (Holt et al., 1994)

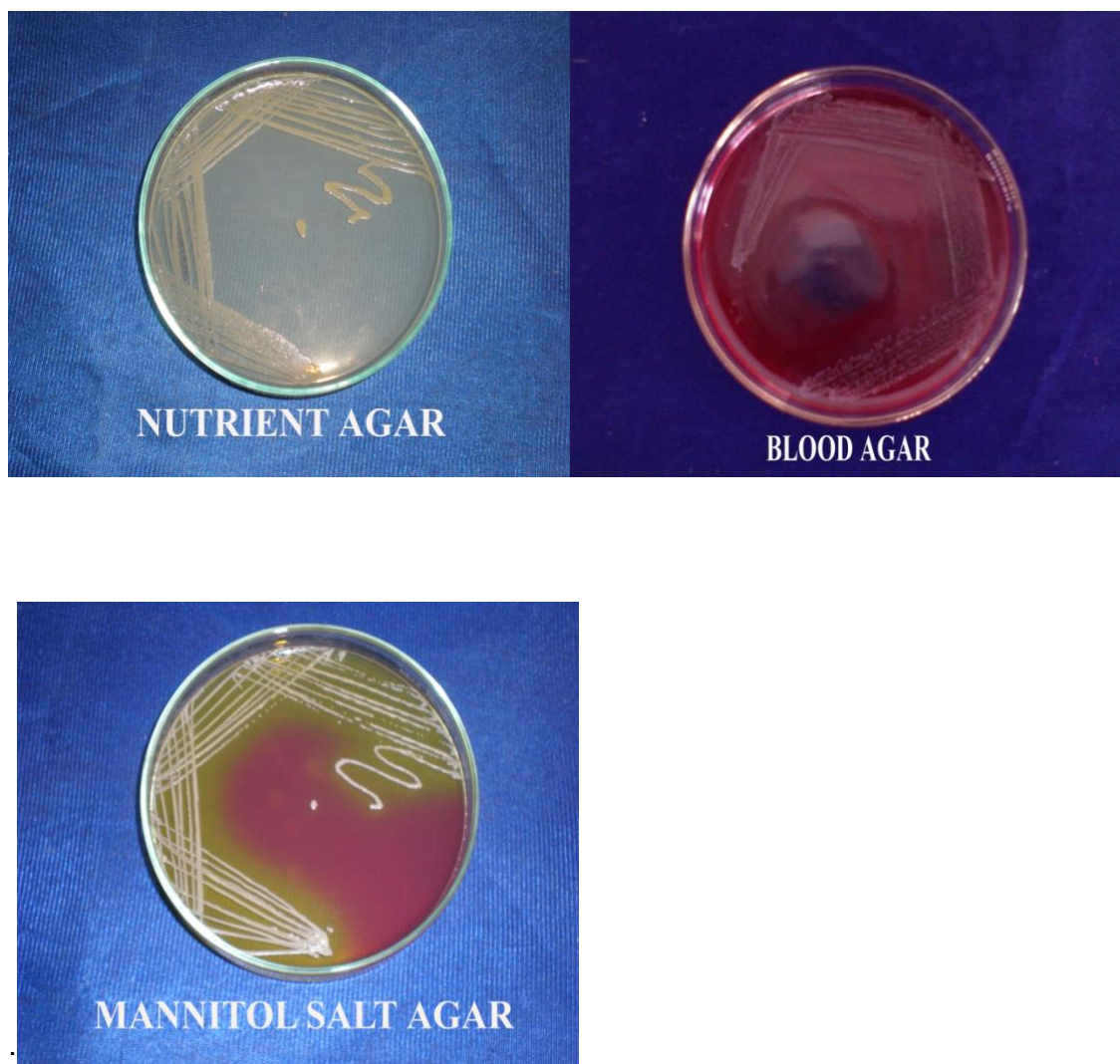


Figure5.1 : Shows that ***Staphylococcus aureus.***, in media

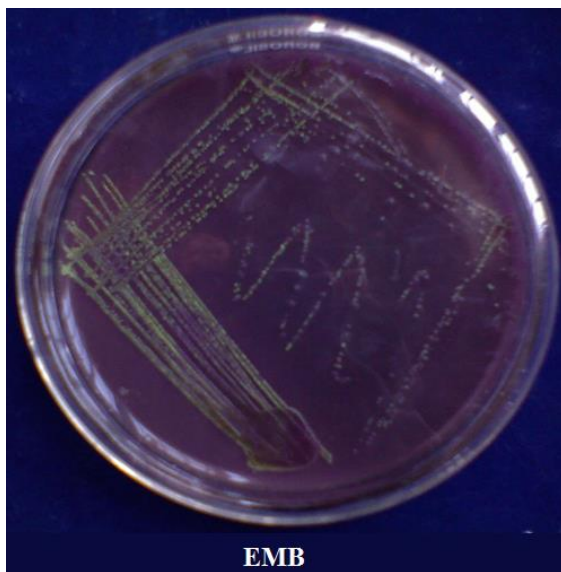
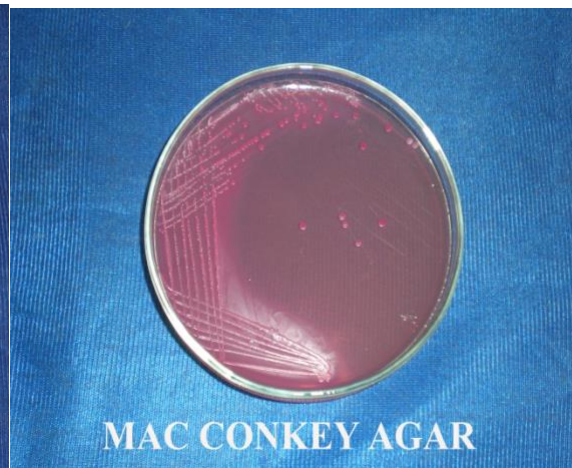
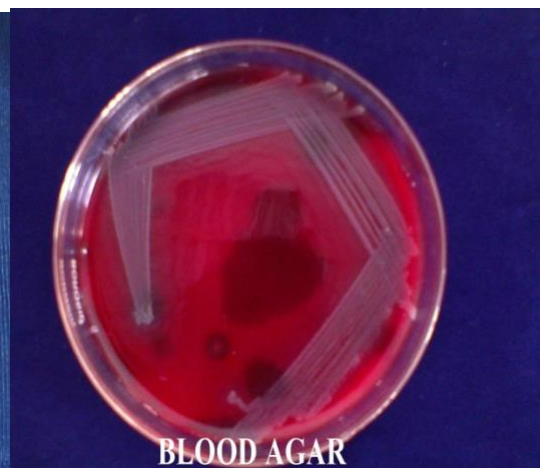


Figure 5.2 : Shows that E.coli in media



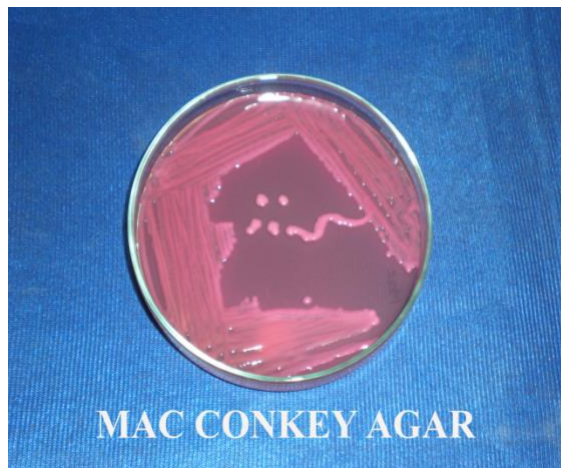


Figure5.3 : Shows that *Klebsiella pneumoniae* in media.

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Staphylococcus aureus are gram positive colonies which are mannitol fermenting, about 1–2 mm in diameter and appears as grape-like clusters when viewed through a microscope, and has large, round, golden-yellow colonies. *Staphylococcus aureus* produces yellow colonies with yellow zones, whereas other coagulase-negative .

Based on the result of biochemical analysis and growth in media, the bacteria was identified and the results were tabulated in the table (5.3).

S.No	Organisms	NA	MA	EMB Agar	MSA	BA
1	<i>E.coli</i>	Large, thick, greyish white, moist, smooth, opaque or translucent discs	Dry, Bright pink color colonies	White color colonies	-	β heamolysis
2	<i>Klebsiella</i>	Circular, greyish white, translucent opaque	Circular, pink-red, opaque	Circular, Greyish white, translucent opaque	-	β heamolysis
3	<i>Staphylococcus</i>	Golden yellow color colonies	Tiny, pink color colonies	-	Smooth, shiny, golden yellow color colonies, opaque	β heamolysis

**BA_Blood agar,NA-Nutrient agar ,MA-MaConkey agar , MSA-Manitol Salt agar
EMB -Eosin methylene Blue agar**

5.3.3SECONDARY TEST OF BACTERIA:

Biochemical characterization of bacteria :

Biochemical test such as indole test, methyl red, voges proskauer test, citrate utilization test, Triple sugar iron test were used for the identification of bacteria. Based on the result the bacteria was identified and the results were tabulated.

5.3.3,1 Staphylococcus aureus biochemical test:

while *Staphylococcus aureus* isolates were oxidase negative and catalase positive (Orwin *et al.*, 2003),

.The coagulase test is specific to differentiate *Staphylococcus aureus* from other species and genera which is positive (Kateete et al., 2010).

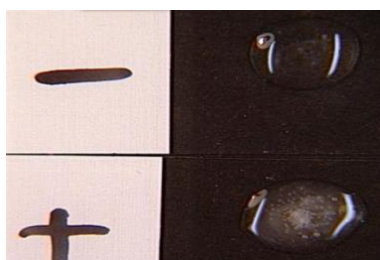


Fig 5.4 (coagulase test (A) positive result, (B) negative result

Coagulate is an enzyme that clots blood plasma. This take a look at is carried out on Gram-positive, catalase brilliant species to discover to coagulate positive *Staphylococcus aureus*. Coagulate is a virulence thing of *S. Aureus*. The formation of clot spherical a sickness brought on through using this micro organism probable protects it from phagocytosis. This take a look at differentiates *Staphylococcus aureus* from unique coagulate negative *Staphylococcus* species.

5.4 Table : Biochemical Identification of bacteria

S. No	Organisms	Morphology	Gram's staining	Motility	I	MR	VP	Citrate	TSI
1	<i>E.coli</i>	Rod	G-ve	Motile	+ve	+ve	-ve	-ve	+ve
2	<i>Klebsiella sp.</i> ,	Rod	G-ve	Non-Motile	-ve	-ve	+ve	+ve	+ve
4	<i>Staphylococcus sp.</i> ,	Cocci	G+ve	Non-Motile	-ve	+ve	-ve	+ve	-ve

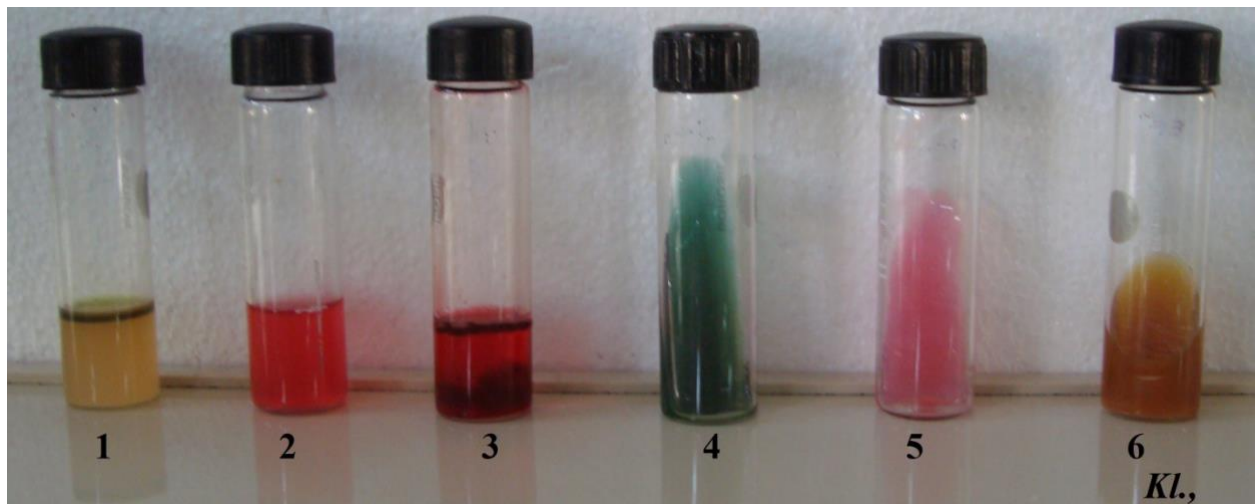


Figure 5.5 *Klebsiella pneumoniae*:

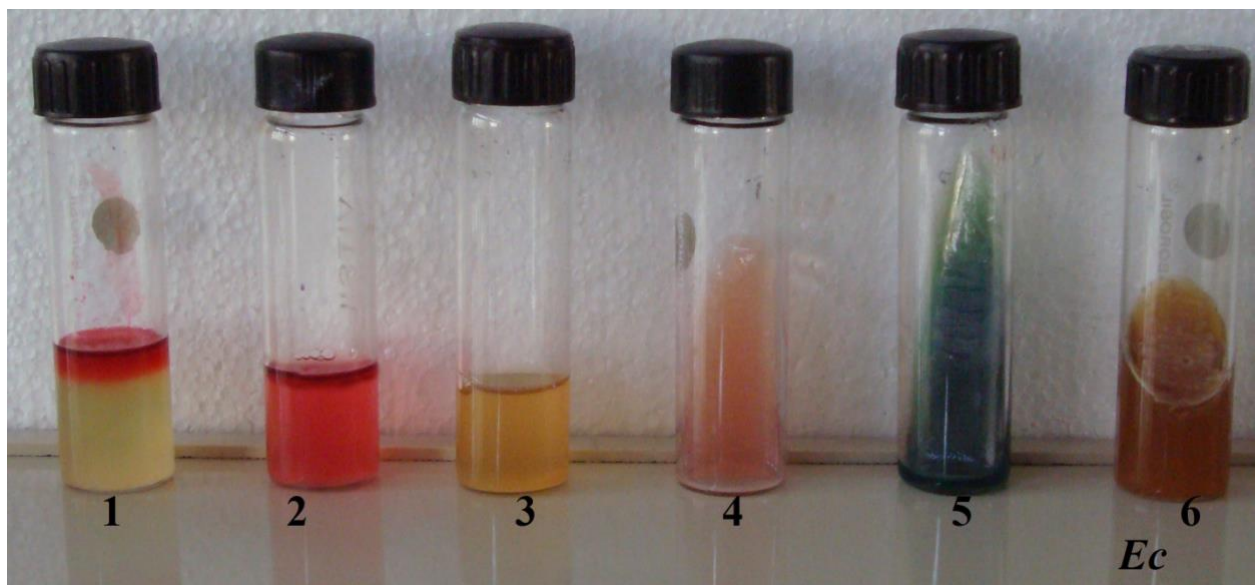


Figure 5.6 . *E.coli*

i. Methyl Red :

This take a look at is used to determine which fermentation pathway is used to utilize glucose. In the combined acid fermentation pathway, glucose is fermented and produces several natural acids (lactic, acetic, succinic, and formic acids). The steady production of enough acid to overcome the phosphate buffer will end result in a pH of below 4.9.

MR and VP-. In contrast, aerogenes and *Klebsiella pneumoniae* are MR- is a glucose nonfermenter .

ii. Urease test:

This test is used to identify microorganism successful of hydrolyzing urea in the use of the enzyme urease. It is normally used to distinguish the genus *Proteus* from unique enteric bacteria. The hydrolysis of urea varieties the vulnerable base, ammonia, as one of its products. This vulnerable base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink. *Proteus Mirabilis* is a quickly hydrolyzer of urea (center tube pictured here). The tube on the lengthy way proper used to be as soon as inoculated with a urease poor organism and the tube on the identical distance left used to be inoculated.

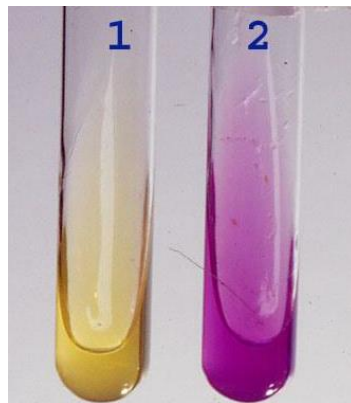


Fig: 5.7): urease test, positive result (2), and negative

Simmon citrate test:



5.3.4. The antibiotic susceptibility

The standard antibiotic discs were purchased from Hi media company. The isolated bacterial pathogens were swabbed into Muller Hinton Agar medium and antibiotic discs were placed on the surface of the medium. Subsequently incubated for about 18 - 24 hrs at 37°C for bacteria. After incubation the diameter of the circular inhibition zones were measured. This Disc Diffusion method use for identification of antimicrobial activity.

Specimen	Blood
Culture	Routine Bacteria
Gram's stain	Gram positive cells in the specimen.
Isolated organism	<i>Staphylococcus aureus</i> grow on culture
Comments	Isolate grow well in the BA. Hemolytic colonies developed in the BA. Gram positive cocci in Chain.

S.No	ANTIBIOTICS	ZONE	SENSITIVITY	DISC CONCENTRATION
	Primary Gen			
1	Amikacin	18 mm	<u>Sensitive</u>	(10 mcg)
2	Tobramycin	18 mm	<u>Sensitive</u>	(30 mcg)
3	Amoxyclav (AC)	06 mm	Resistant	(50/10 mcg)
4	Ampicillin	17 mm	<u>Sensitive</u>	(25 mcg)
5	Cefazolin	06 mm	Resistant	(30 mcg)
6	Cefixime	06 mm	Resistant	(10 mcg)
7	Imipenam	09 mm	Resistant	(10 mcg)
8	Ertapenam	19 mm	<u>Sensitive</u>	(10 mcg)
9	Piperacillin	19 mm	<u>Sensitive</u>	(75 mcg)
10	Amoxicillin	18 mm	<u>Sensitive</u>	(25 mcg)

	Secondary Gen			
1	Aztreonum	09 mm	Resistant	(50 mcg)
2	Cefotaxime	19 mm	<u>Sensitive</u>	(10 mcg)
3	Cefpodoxime	06 mm	Resistant	(10 mcg)
4	Ciprofloxacin	19 mm	<u>Sensitive</u>	(10 mcg)
5	Ceftazidime	07 mm	Resistant	(10 mcg)
6	Azythromycin	06 mm	Resistant	(30 mcg)

Age/Sex : 50 / M

Microbiology Report

Blood for Culture and Antibigram

Specimen	Blood
Culture	Routine Bacteria
Gram's stain	Gram negative rods in the specimen
Isolated organism	<i>E.coli</i> grow on culture
Comments	Gram Negative rods, Hemolytic colonies developed in BA. The isolate is not ESBL producing organism.

S.No	ANTIBIOTICS	ZONE	SENSITIVITY	DISC CONCENTRATION
	Primary Gen			
1	Amikacin	19 mm	<u>Sensitive</u>	(10 mcg)
2	Tobramycin	18 mm	<u>Sensitive</u>	(30 mcg)
3	Amoxyclav (AC)	19 mm	<u>Sensitive</u>	(50/10 mcg)
4	Ampicillin	18 mm	<u>Sensitive</u>	(25 mcg)
5	Cefazolin	08 mm	Resistant	(30 mcg)
6	Cefixime	11 mm	Resistant	(10 mcg)
7	Imipenam	06 mm	Resistant	(10 mcg)
8	Ertapenam	19 mm	<u>Sensitive</u>	(10 mcg)
9	Piperacillin	18 mm	<u>Sensitive</u>	(75 mcg)
10	Amoxicillin	19 mm	<u>Sensitive</u>	(25 mcg)

	Secondary Gen			
1	Aztreonam	06 mm	Resistant	(50 mcg)
2	Cefotaxime	19 mm	<u>Sensitive</u>	(10 mcg)
3	Cefpodoxime	06 mm	Resistant	(10 mcg)

Blood for Culture and Antibigram

Specimen	Blood
Culture	Routine Bacteria
Gram's stain	Gram negative rods in the specimen
Isolated organism	<i>Klebsiella sp.</i> , grow on culture
Comments	Gram negative rods and capsulated. The isolate is not ESBL producing organism. Multi drug resistant bacteria.

S.No	ANTIBIOTICS	ZONE	SENSITIVITY	DISC CONCENTRATION
	Primary Gen			
1	Amikacin	18 mm	<u>Sensitive</u>	(10 mcg)
2	Tobramycin	18 mm	<u>Sensitive</u>	(30 mcg)
3	Amoxycylav (AC)	19 mm	<u>Sensitive</u>	(50/10 mcg)
4	Ampicillin	17 mm	<u>Sensitive</u>	(25 mcg)
5	Cefazolin	06 mm	Resistant	(30 mcg)
6	Cefixime	08 mm	Resistant	(10 mcg)
7	Imipenam	06 mm	Resistant	(10 mcg)
8	Ertapenam	18 mm	<u>Sensitive</u>	(10 mcg)
9	Piperacillin	18 mm	<u>Sensitive</u>	(75 mcg)
10	Amoxicillin	15 mm	Resistant	(25 mcg)

	Secondary Gen			
1	Aztreonum	06 mm	Resistant	(50 mcg)
2	Cefotaxime	18 mm	<u>Sensitive</u>	(10 mcg)
3	Cefpodoxime	06 mm	Resistant	(10 mcg)
4	Ciprofloxacin	06 mm	Resistant	(10 mcg)
5	Ceftazidime	18 mm	<u>Sensitive</u>	(10 mcg)

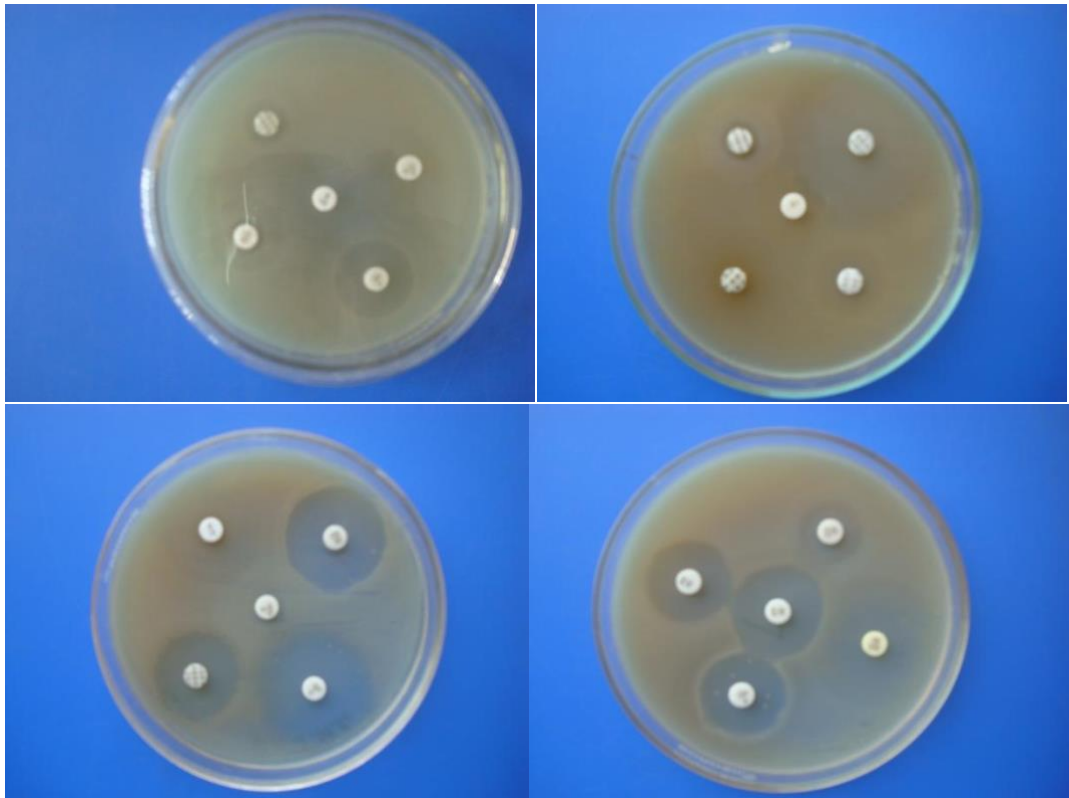


Figure 5.8 .*Staphylococcus aureus* in antibiotic test

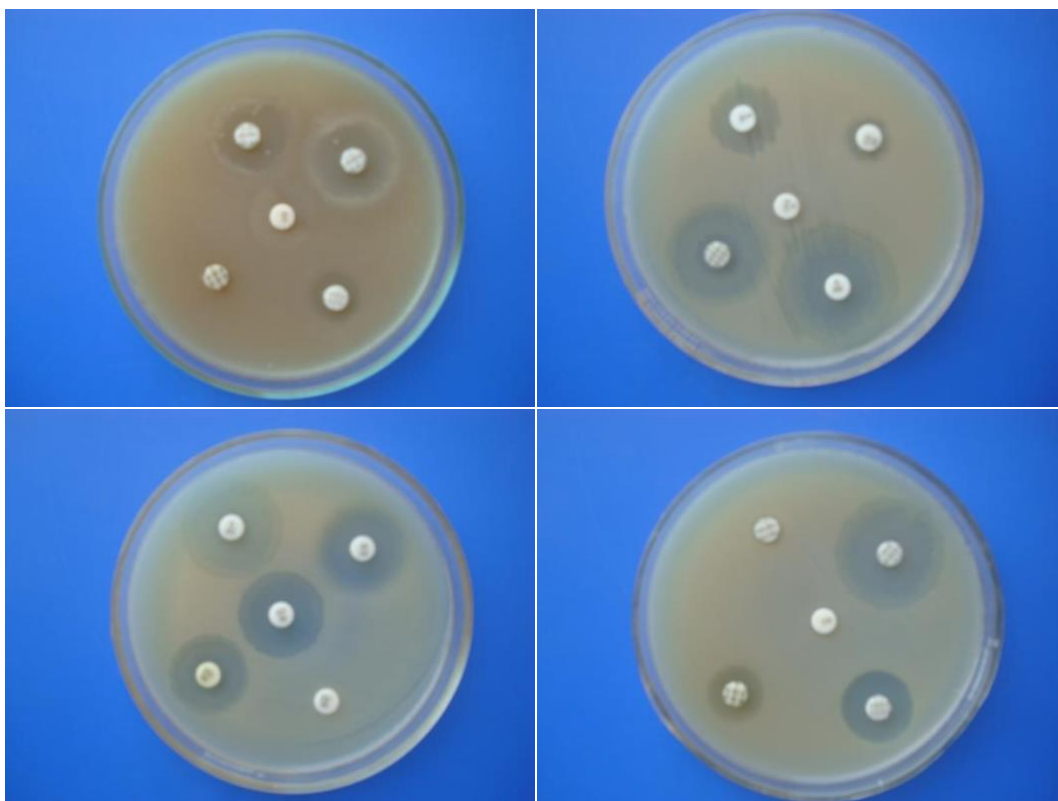


Figure 5.9. *E. coli* in antibiotic test

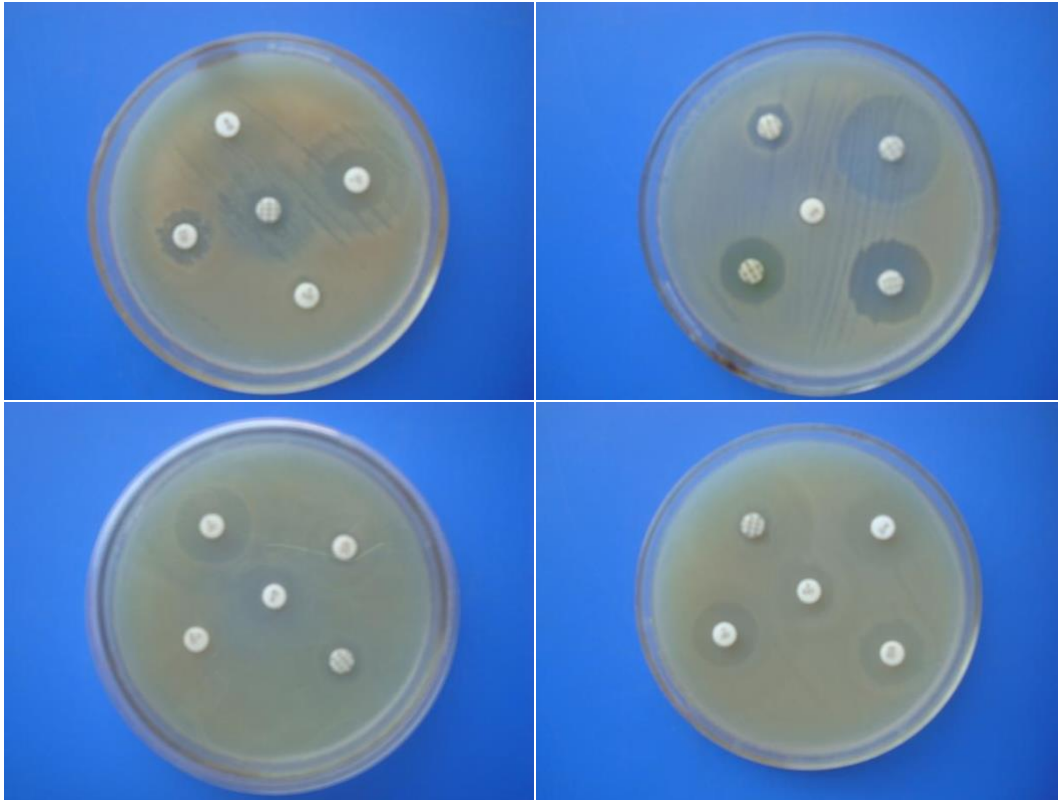


Figure 5.10. *krebsella pnuemoniae* in antibiotic test

CHAPTER 6

SUMMARY AND CONCLUSION:

SUMMARY

Results presented in that out of 25 clinical samples, 03 *Staphylococcus aureus*, 02 (*Klebsiella pneumoniae*, 05 sample e.coli isolates were recovered. Two species was the most frequently occurring among other species, when its account for 29 % of *Klebsiella pneumoniae* and of *Staphylococcus aureus* isolated clinically.

CONCLUSION

Bloodstream infections can lead to life threatening sepsis and required anti repaid antimicrobial treatment. The organism implicated in these infections vary with the geographical altered. infections caused by organisms are more likely to increase the risk of dead in the patients.

Further presented study was the profile of organisms causing bacteraemia and understand antibiotics resistance patients in hospital. The isolated were identification by standard by biochemical tests and anti-antimicrobial resistance patterns were determined by CLSI guidelines

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