Prospective Study on Prevalence and Characterization of Microorganisms causing Bloodstream Infections (BSIs) In Hospitalized Patients

Internship Training Report

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Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:

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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of **Ms. Rimi Kour (lecturer)** (Internal supervisor), Lovely Professional University and **Dr. Raman preet kaur**(External supervisor),MD Microbiologist Tagore Hospital,Jalandhar. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Amandeep Kaur

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Place: Phagwara

CERTIFICATE

This is to certify that **Mr./Ms. Amandeep Kaur** bearing **Registration Number** 11405912 has completed his/her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

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ABSTRACT

BSIs are a major threat to the health of hospitalized patients and leading causes of morbidity and mortality. Around 200,000 cases of bacteremia occur annually with mortality rate ranging from 20-50% worldwide. Main objective of this study is to know the prevalence and characterization of different microorganisms causing BSIs. A total of hundred blood samples were processed for isolation and identification of causitive organisms of BSIs. Samples were subculture on Blood agar and MacConkey's medium and their antibiotic sensitivity on MH agar. Bacterial pathogens were detected in 34 positive cultures. Male and Females were found to be almost equally effected (1:1) with maximum effected age group was 61-70. gram positive organisms are major cause of bloodstream infections then gram negaitive organisms with *Staphylococcus aureus* being the predominant isolate in BSI followed by *Escherichia.coli, Enterococcus spp., Pseudmonas spp.*and *Klebsiella spp.* Moreover, in the present study the antibiotic sensitivity pattern for gram positive organism S.aureus has shown the highest sensitivity towards Linezolid(100%),Glycopeptides(100%).while in case of *E.coli* Tigecycline was 100% sensitive followed by Amoxy/clav(90%), Cefoperazone/sulbactam(90%) while Ampicilin show 100% resistance

followedbyCefepime(82%),Quinolones(LE,OF(82%),Carbapenems(MRP,IPM).Increasing antimicrobial resistance among blood pathogens is a matter of great concern to start empirical antibiotic treatment and to monitor continuously the sensitivity pattern of isolates so as to make a rational use of antibiotics. Information on trends and antibiotic resistance is needed for optimizing the use of antimicrobial therapies as well as development of new antibiotics and vaccines.

KEYWORDS: BloodStream Infections, *S.aureus, Escherichia coli,* Biochemical Analysis, Antimicrobial Susceptibility.

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Amandeep Kaur

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INTRODUCTION

1. Blood Stream Infections (BSIs) can be defined as the presence of viable bacteria in the blood analyzed by a positive blood culture result. [(1)] Few important terms associated with BSIs are:
2. Primary BSIs, which do not have a documented primary source of infection, whereas, secondary BSIs occur secondary to a localized focus of infection, such as pneumonia, biliary tract infection, skin and soft-tissue infection, and wound infection.[(2)]
3. Bacteremia is the presence of viable microorganisms in the bloodstream of patients, which could in the form of transient, intermittent or persistent bacteremia.[(3)]
4. Septicemia is a clinical syndrome characterized by fever, chills, malaise, hyperventilation and toxicity which occurs when the circulating bacteria multiply at a rate that exceeds their removal by phagocytosis.[(4)]

   BSIs are a major threat to the health of hospitalized patients due to the increasing use of indwelling medical devices these days. Though this device usage has helped a lot in curing patients but there is a dark side of this achievement too. The microorganisms colonize the external surface of indwelling devices and can gain entry to sterile sites such as bloodstream through insertion. This ultimately leads to increased morbidity, mortality as well as increased length of stay in the hospital and also puts financial pressure on the patient. Also prolonged stay is usually associated with prolonged antibiotic usage which might be the cause of increasing antibiotic resistance. [(5)]

The other risk factors associated with these BSIs are prolonged hospitalization, Immunosuppression, premature infants, Chronic diseases like Diabetes Mellitus, patients undergone Bone marrow transplantation, etc.[(6)]

The etiology behind these BSIs is multifactorial including bacterial, viral as well as fungal and parasitic causes. The most common being bacterial and among these *Staphylococcus aureus* is

the most often causative agent followed by gram-negative pathogens like *Pseudomonas spp., Klebsiella spp., Escherichia. Coli, Proteus spp.* and other gram negative organisms. *Candida albican*s is the most common fungal cause followed by *C.tropicalis, C.glabrata* and *Trichosporon beigelii*. [7]

Prevalence of BSIs varies across different regions of the world. These infections are more common in United States. Each year in United States, an estimated 250,000 patient's develop bacteremia or fungemia with estimated mortality16%-40 %.[8] Around 200,000 cases of bacteremia occur annually with mortality rate ranging from 20-50% worldwide. [9] In India, Incidence of *Candida albicans* BSI in 27 ICU 6.51 cases per1000 ICU admissions was seen. [10] An estimated 2, 00,000 to 3,000,000 cases of Nosocomial BSIs occur every year, with mortality rates varying from 17.5%-50%. [3]

With such a background of BSIs it seems essential to properly diagnose these BSIs so as to initiate timely management in the patients. Microbiological diagnosis holds important place in this aspect as it helps in decreasing the menace of antibiotic resistance. Information on trends and antibiotic resistance in bacteremia is needed to inform prescribing and infection control policy and to guide development of new antibiotics and vaccines. [11]

The diagnosis method for BSIs includes:

1. Manual or conventional system
2. Automated BACTAC system
3. Polymerase chain reaction
4. Fluorescence in situ hybridization[12]

Blood culture is the most accurate and routinely performed diagnostic test in clinical microbiology laboratory. Blood cultures are considered to be the gold standard for the detection of microbial pathogens related to bacteremia and sepsis despite new molecular techniques. Blood culturing in an appropriate medium eventually permits its detection and identification, as well as the determination of appropriate antibiotic therapy. [13]

AIM:

The aim of this study is to know about the prevalence of various microorganisms causing BSIs, various methods used for their identification and to determine their antibiotic susceptibility pattern.

OBJECTIVE:

- To know the Prevalence and Characterization of different Microorganisms causing BSIs
- .To determines the Antibiotic susceptibility pattern of different Microorganisms.

LITERATURE REVIEW

All research sources used for this review of literature was conducted within the studies of 1990-2016

1. . Weinstein et al., in their study, on clinical significance of positive blood culture in the 1990 observed that the most common isolate from blood culture were Coagulase negative *Staphylococci* and ranked as third common cause of bacteremia due to their higher prevalence. [14]
2. In a study done by Reimer et.al.(July 1997) they observed that The 10 most frequent microorganisms causing bacteremia and fungemia in adults from 1992 to 1993 were *S.aureus*, E.*coli, Coagulase-negative staphylococci , K. pneumonia, Enterococcus spp., P. aeruginosa, S. pneumonia, Viridans group streptococci ,C. albicans and E. cloacae.*[6]
3. A Study done by Weinstein et al, (1997) observed that among gram negative aerobic and facultative bacteria *E. coli* was the most common isolate and second most cause of septicemia after S.*aureus. Enterobacteriaceae* and *Pseudomonas aeruginosa* represent true bacteremia >95% times when isolated from blood and *Clostridium perfringens* isolation usually represent contamination of blood. [14]
4. According to a study by Mark H Reacher et.al. (Jan 2000) on bacteraemia and antibiotic resistance of its pathogen, it was observed that *S.aureus* was among the top five causes of bacteraemia in every age group, whereas *E coli*, Coagulase negative s*taphylococci*, *S.pneumoniae*, and *Enterococcus* species were ranked lower at certain ages.[11]
5. Erik L. Munson et al. (2002) in their study found that the six most common causes of bacteremia were *Staphylococcus aureus* (20% of episodes), *Escherichia coli* (14%), Coagulase-negative s*taphylococci* (13%), *Enterococci* (12%), *Pseudomonas aeruginosa* (6%), and *Klebsiella pneumoniae* (5%). The purpose of this study was to evaluate the association between positive blood culture reporting by the clinical microbiology laboratory and the antimicrobial management of patients with BSI. [15]
6. M.M. Loureiro et al. (2002) mentioned in their study that Gram-positive strain isolated from blood culture of hospitalized newborns show high resistance to Ampicillin and Penicillin, intermediate resistance to Amikacin, Erythromycin, Oxacillin, and a low

resistance to Cephalotin. While Gram-negative strains were highly resistant (70-100%) to Cephalosporin, Intermediate resistance (35-70%) to amino glycosides, Tetracycline, Chloramphenicol and Trimethoprim-Sulfamethoxazole., a little resistant to Imipenem and Ciprofloxacin. (16)

7. A Study by Beekmann et.al. (July 2003) ranked Bloodstream infections (BSIs) as the 10th leading cause of death in the United States, with a recent increase in age-adjusted death rates. Acc. to this study Gram-positive pathogens caused the majority of BSIs, with *Staphylococcus aureus* being the most common pathogen followed by *Escherichia coli* and Coagulase negative *staphylococci*.(5)
8. According to study done by Harald Seifert Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany (2009) it was found that Bloodstream infections are associated with high morbidity and mortality. In the United States, an estimated 250,000 patients develop bacteremia or fungemia every year and many infections are acquired in hospitals. (8)
9. Karam El-Din et.al. (2010) observed that Yeast infection namely candidiasis is an increasingly common problem in hospitalized patients, with epidemiologic surveys revealing that *Candida spp*. are now the fourth most common pathogens isolated from the blood of hospitalized patients. According to this study, Out of the 207 cases with bacterial growth 52 (25.1%) were due to *Staphylococcus aureus*, while 60 (78.9%) cases were due to *Candida albicans* out of the 76 cases with fungal growth.(7)
10. Prakash KP et al,(2011) in his study found that *Streptococcus spp., S. aureus, E. coli, Klebsiella spp., S. pneumoniae, P. aeruginosa, K. pneumoniae, Acinetobacter spp.,* and *Enterobacter spp.* were the 10 most commonn bacterial pathogens causing BSI. *Staphyloccocus spp.* and *Streptococcus spp.* causing BSIs show resistance to Trimethoprim/Sulphamethoxazole while most of gram negative organism were resistant toAmpicillinfollowedbyTrimethoprim/Sulphamethoxazole,Cepharadine,gentamicin,imipenem(17).
11. In a study done by Alo, M. N et al, (2012) Ciprofloxacin and Amikacin showed 100% resistance to the *E. coli* isolated from blood culture of hospitalized patients while Cefoxitin showed 100 % sensitivity followed by Sulphamathroxazole (80%). *K.*

pneumonia (80%) were highly resistant to Ciprofloxacin followed by Amikacin (60%). [18]

12. Anita C et al, (2016) in her study mentioned *E.coli* being the predominant isolate in BSI followed by Klebsiella *pneumoniae,P.aeruginosa,Enterobacter spp,Citrobacter spp. and Acinetobacter spp*. and found that gram negative organisms shows highest resistance towards Amikacin (68.75%), Ceftazidime (59.4%), and Ceftriaxone (56.3%), moderate for Gentamicin (50%), Ciprofloxacin (43.75%) and least resistance (37.5%) for Ofloxacin followed by Imipenem 18.8% .[19]

MATERIALS AND METHODS:

A total hundred blood samples were processed for isolation and identification of causitive organisms of BSIs at Tagore Hospital And Heart Care Centre,Jalandhar during the study period of Jan1 to April 30,2016.

PROCESSING:

The blood culture bottles were collected containing 5-10 ml of patient blood transported to the clinical microbiology laboratory from different wards of hospital (ICCU,emergency,ICU) by hospital staff immediately after collection using aseptic conditions. The first step was to check and match the patient name,I.D. No,sex,Age,Date labelled on the culture bottle with the Requisition form of Patient.then these bottles were incubated for 24hrs at 37^0c in incubator.

MANUAL SYSTEM

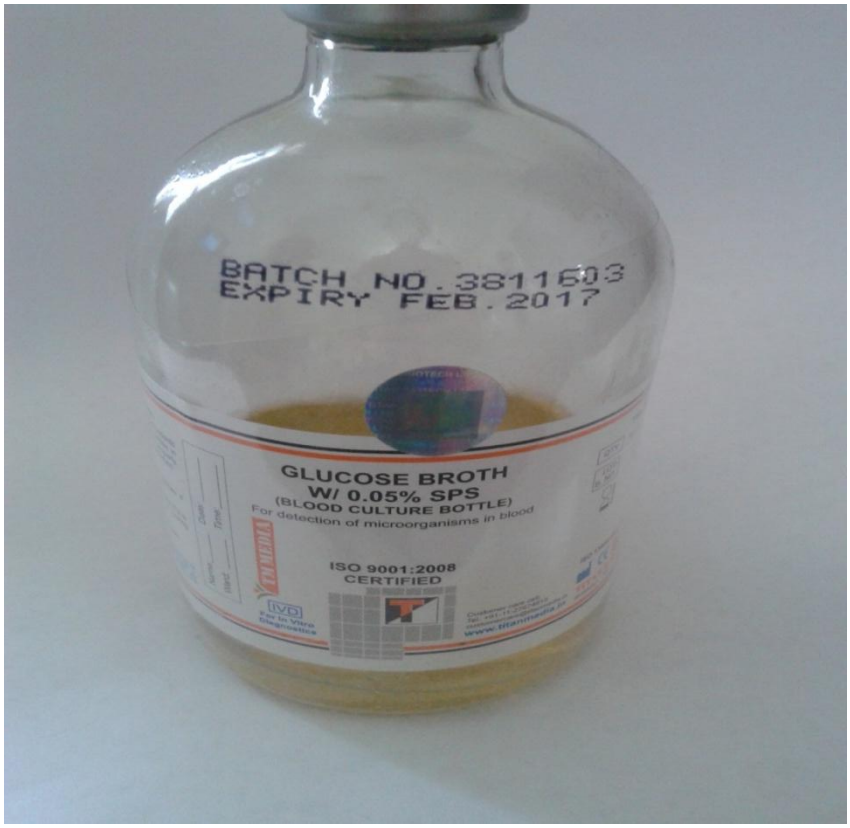

Fig.1. Showing Bloodculture bottle used in Manual system

In Manual system glass bottles were used which contain 50ml of glucose broth. 5-10ml of blood from patient collected by sterile syringe is transferred into these broth containing glass bottles which are then incubated at 37^0c for 24 hours in incubator. After 24hrs subculture was done on Blood agar and MacConkey's agar culture plates and they were incubated for 24hrs in the incubator. After 24hrs colonies morphology were observed in the plates. Repeated subcultures were done in case no growth was observed within first 24hrs and again incubation was done.

PROCEDURE:

1. Conventional broth bottles after 24hrs incubation were removed and blood was subcultured on solid media plates i.e Blood agar plates and MaCconkey agar plates by streaking using innoculating loop.
2. These subcultured plates were incubated for 24hrs in incubator. Bottles were further incubated for 48,72hrs and 7 days and subculture was done after 24,48,72hrs respectively in case no growth was observed after first 24hrs.
3. Subculturing was done by streak plate method. The streak plate is a most practical way of obtaining discreate coloniesand pure culture.
4. One loopful of inoculum from sample was placed near the periphery of the plate.With a loop the inoculum was spread over the uper portion of the plate.The loop was stab into the agar several times and streaking continued overlapping the previous streak.Loop was stab as before and streaking continued .
5. Loop was flammed and allowed to cool and overlaped the last streak and streaking completed. The loop was lifted and the centre of the plate was streaked with zigzag motions.
6. Plates were incubated for 24hrs at 37^0c in incubator.
7. Specific colony morphology was observed in the plates after 24hrs incubation in positice cases.

BLOOD AGAR: Blood agar media is an example of an enriched, nonselective, differential and general purpose medium. On blood agar plates *S.aureus* produces golden yellow or white colonies with beta hemolysis clear zone around colonies.

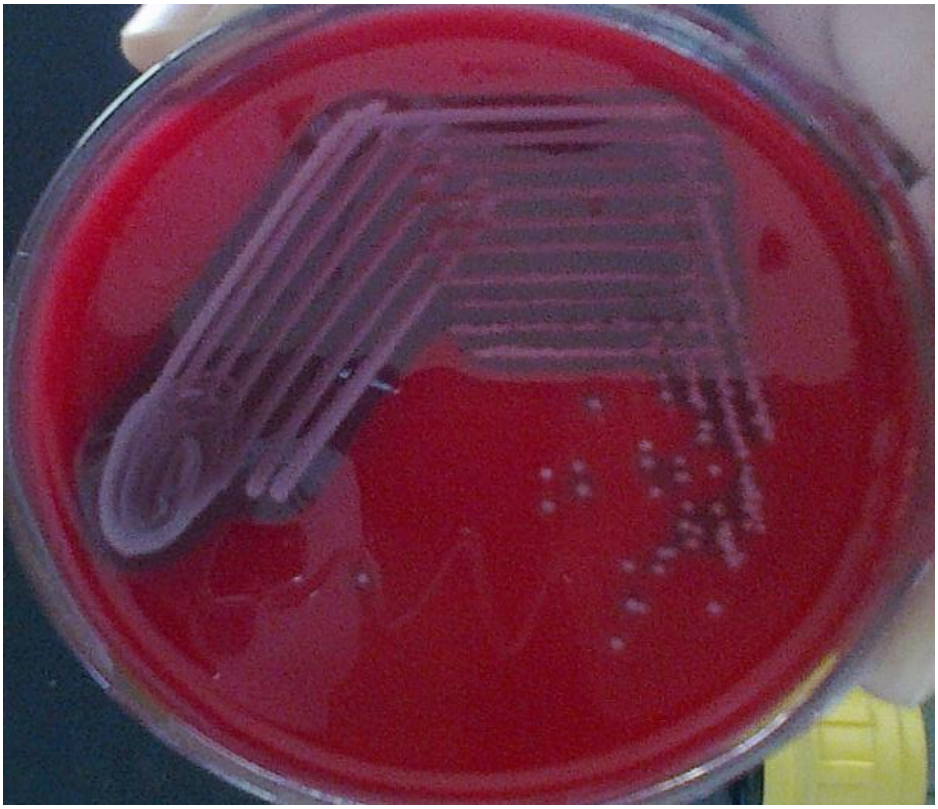

Fig.2. Showing *S.aureus* isolated on Blood Agar Plate

MACCONKEY AGAR :

It is the standard medium for the cultivation of enterobacteria. It is selective and differential medium. Lactose fermenting organisms like *E.coli* produce pink color colonies on it and non-lactose fermentors like *Pseudomonas spp*.produce colorless colonies.

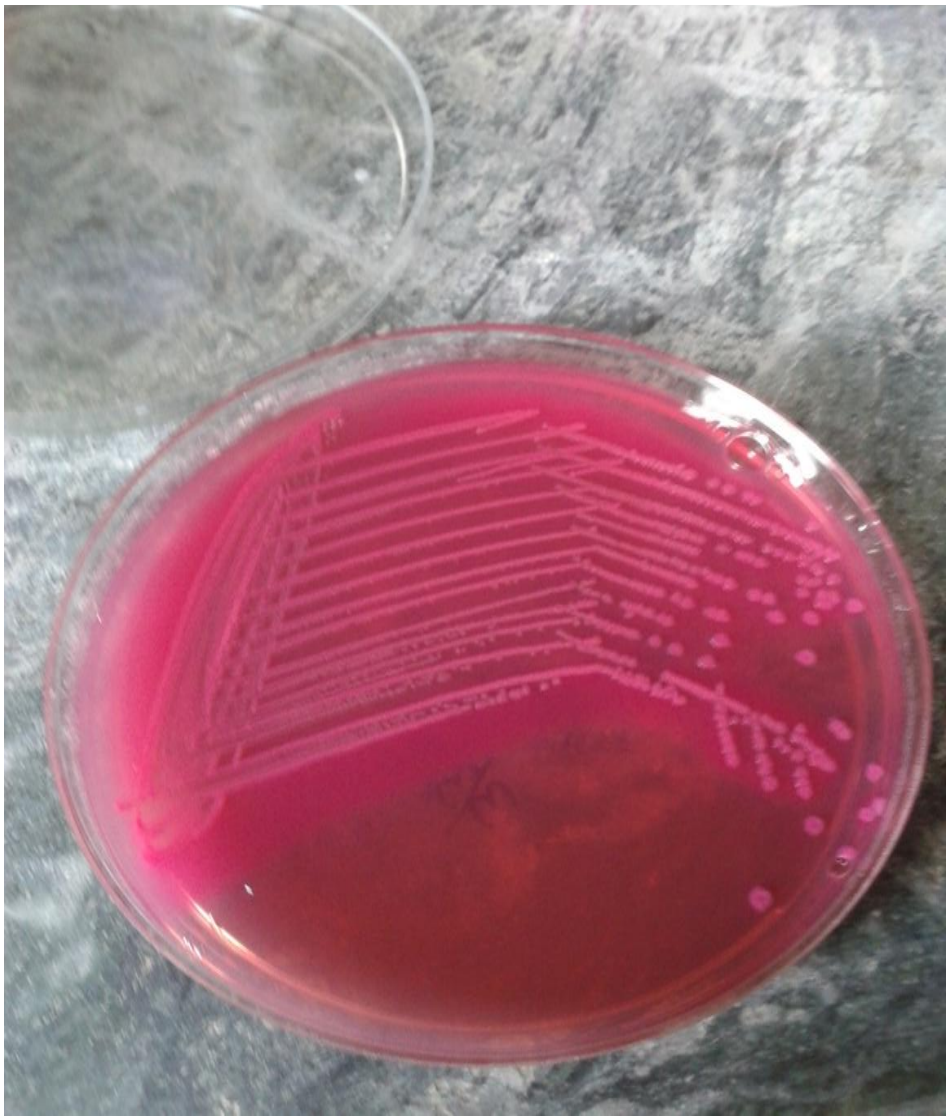

Fig.3. Showing *E.coli* isolated on MacConkey's agar plate

Fig.4. Showing Culture Bottle used in BacT/ALERT System

This system uses non-invasive fluorescent technology to detect increase in carbon-dioxide produced by the microbial growth. I had collected the blood culture bottles that are trasported to the microbiology laboratory for diagnosis. Firstly I visually inspected the bottles to check that bottles are not having any leakage,damage and deterioration. Bottles with hemolysis,turbidity, excess gas pressure, yellow sensors, or evidence of growth as positive were consider bottles after loading into instrument if show beep signal it means blood is positive or infected with microorganism. After that bottles was removed and subculture was done as mentioned in manual system and same procedure was followed. If no beep cames, incubation was done until system shows signal.

PROCEDURE:

1. Culture bottle was loaded into the instrument and incubate for five days or until designated positive.

2. After incubation bottles were taken out from instrument and subculture was done on blood agar and MaCconkey's agar plates. These plates were placed in incubator for overnight at 37^0c.
3. If growth was present in plates, smear was prepared from that growth and gram staining was done. If smear was negative it indicates a possible false positive result, then bottles were reloaded into the instrument until growth of the subculture positive. Bottles that were initially determined false positive and were redesignated positive were be subcultured and smeared.
4. Negative bottles were also checked by subculture and smear before discarding as negative.
5. BacT/ALERT bottles were not reused and disposal of these bottles was done according to laboratory protocol.

RESULTS:

Positive and negative culture bottles are determined by decision making software contained in the BacT/ALERT system. No action was required until BacT/ALERT instrument signals a bottle either positive or negative.

Fig.5. Showing BaCT/ALERT system

GRAM STAIN:

Gram stain is most widely used stain in the microbiology that differentiates bacterial species into two- groups gram positive and gram negative spp.

STAINING PROCEDURE:

- The heat fixed slide was taken and was covered with primary stain crystal violet. The slide was left for one minute.
- The crystal violet stain was washed off from the slide by covering it with gram's iodine and was left for 30 seconds.

- The gram's iodine was poured off from the slide. Then the slide was washed with acetone or 95% alcohol to decoulorise the smear.the acetone or alcohol should be poured on the smear till all the stain runs off.
- The slide was washed with distilled or tap water.
- Then the slide was covered with the secondory stain or counter stain safranine for 30-45 seconds. neutral red or 1:10 carbolfuchsin can be used .
- The slide was rinsed in water and allowed to dryin air or blotted dry by a filter paper.
- The smear was viewed under the oil immersion objective lens of light microscope.

Interpretation:

Gram positive bacteria **:** retain the color of primary stain appears purple in color.(Fig.6a)

Gram negative bacteria : takes the color of the safranin and appears pink in color(Fig.6b)

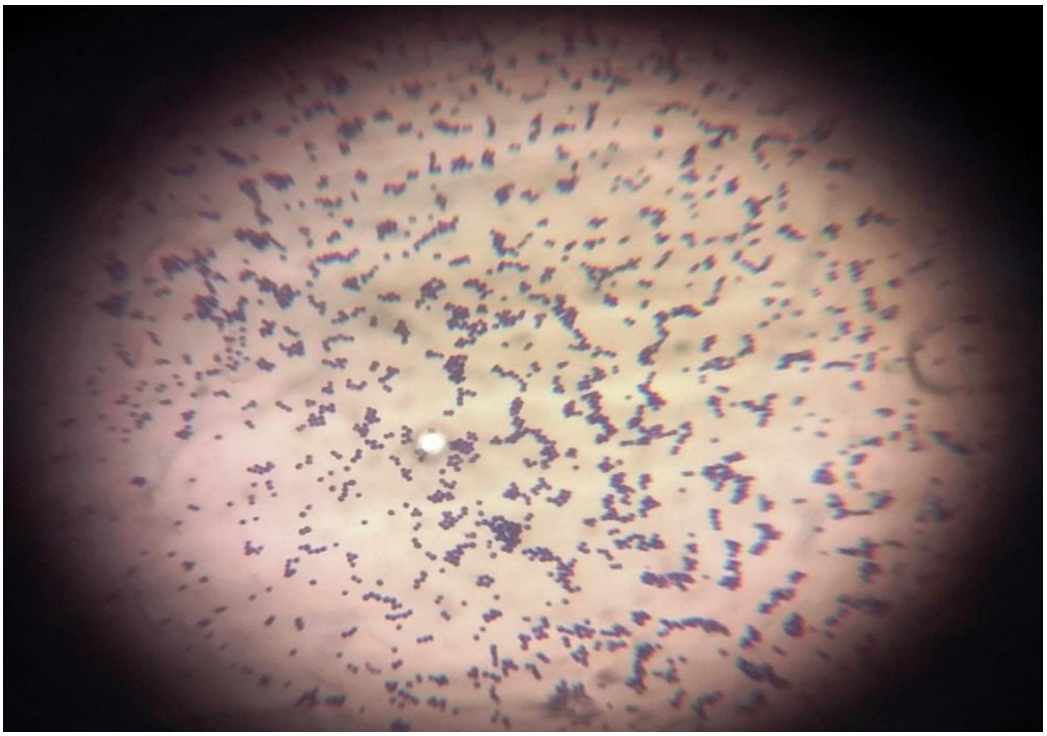

Fig.6a. Showing Gram positive Cocci under Microscope

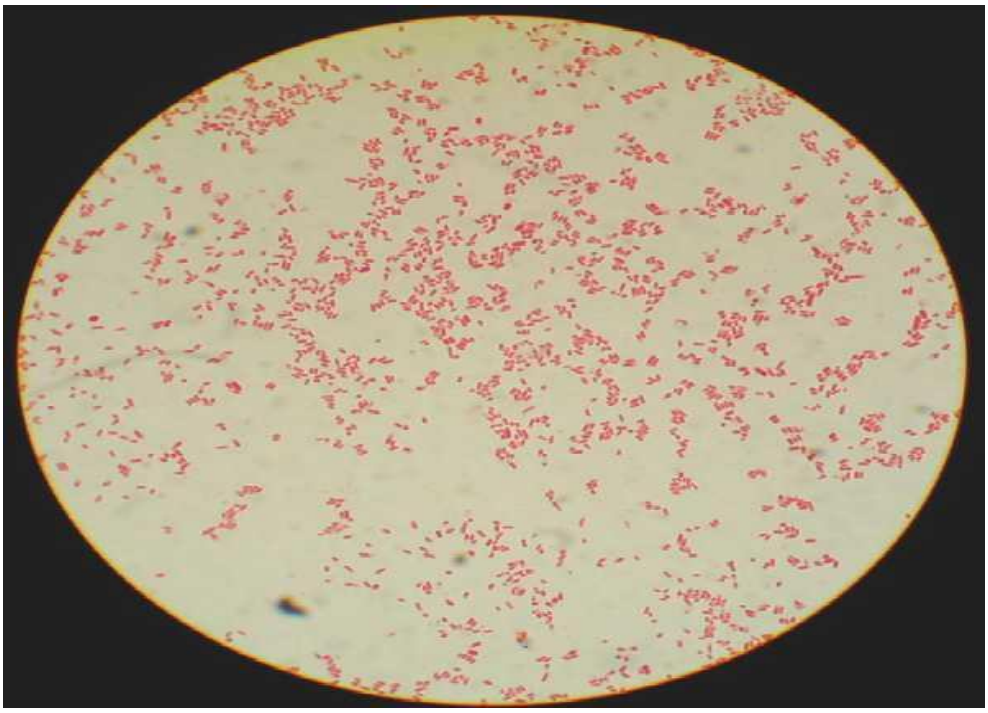

Fig.6b. Showing Gram Negative Bacilli under Microscope

MICROSCOPIC EXAMINATION:

MOTILITY TEST:HANGING DROP METHOD: It helps to distinguish motile bacteria from non-motile ones.

PROCEDURE:

1. Ist I taken the glass slide with a shallow, circular concavity in its centre.
2. Concavity was encircled with a line streak of soft petroleum jelly applied with a glass rod to the surface of the slide just outside the concavity.
3. A drop of liquid culture was placed in the centre of a coverslip.
4. Glass slide was placed, with the concave surface facing down, over the coverslip and slide is pressed gently to form a seal between the slide and the coverslip.

5. Slide was turned with right side up so that the drop continues to adhere to the inner surface of the coverslip.
6. For microscopic examination, first focus was done under low power objective(10x) and then under high power objective(40x).

BIOCHEMICAL TEST:

CATALASE TEST:

1. On a clean glass slide a drop of hydrogen peroxide solution (10vol) was placed
2. From the culture plate few colonies of test bacteria were picked with the help of platinum loop and mixed with the drop of H_2O_2 .
3. In case of positive catalase reaction bubbles of free oxygen gas were produced immediately that can be easily observed.
4. Absence of bubble formation was negative catalase test .

Fig.7. Showing Catalase Test Results

COAGULASE TEST:

SLIDE COAGULASE TEST:

1. On a clean glass slide in a drop of normal saline few colonies of bacteria were emulsified and then mixed with a drop of human plasma.
2. Prompt clumping of the suspension occurs with coagulase positive stains while clumping was absent in negative stains

TUBE COAGULASE TEST:

1. 0.1 ml of overnight agr culture suspension or broth culture of organism was mixed with 0.5ml of 1in 5 dilution of human plasma.
2. Diluted plasma alone in a second tube serves as a control.
3. Tubes were incubated at 37^0c for 3-6hrs.
4. In positive cases, the plasma clots and it didn't flow when the tube was inverted.
5. If clot didn't appear it was left overnight at room temp. and was re-eximined. No clot formation occurs in coagulase negative species.

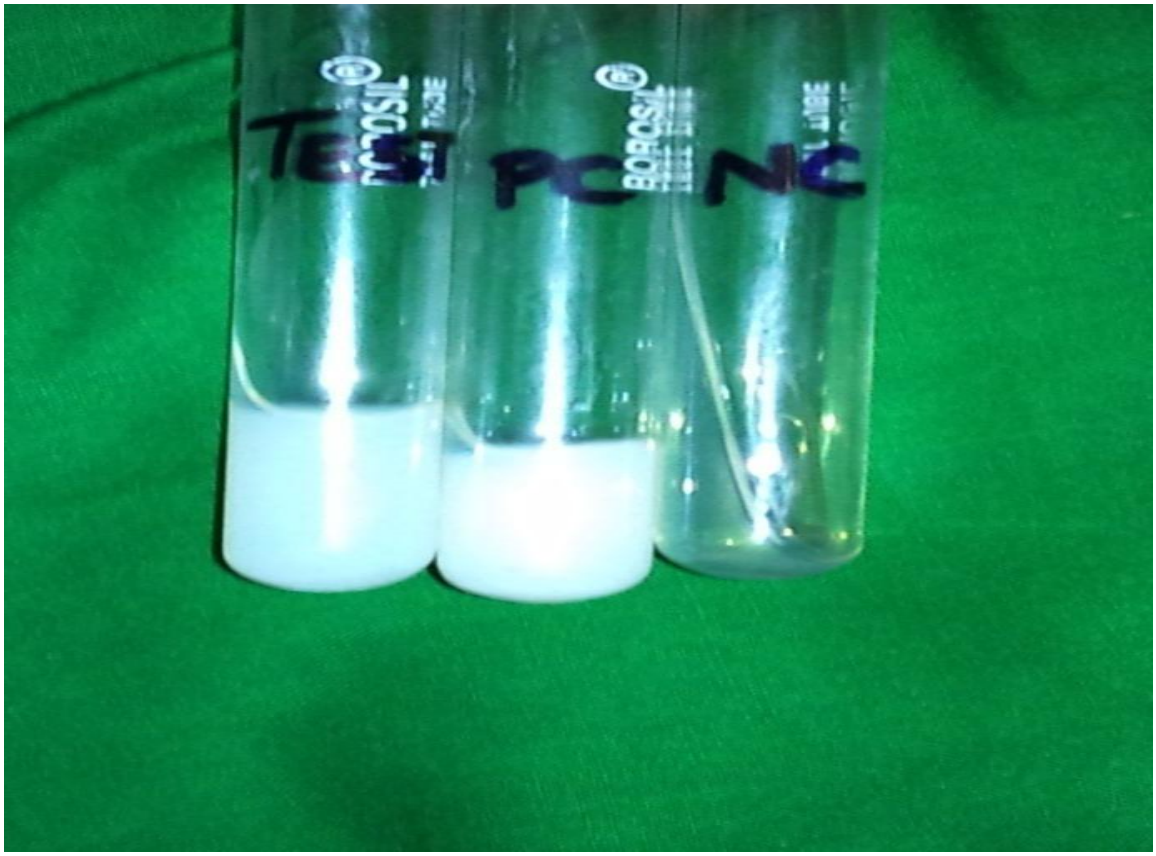

Fig.8. Showing Tube Coagulase Test

BILE ESCULIN TEST: Grop D streptococci can be seperated from non-Group D streptococci bythe bile esculin test. The Bile esculin slant was inoculated with bacterial colonies from an 24hrs old culture using a sterile loop and at incubated at 37^0c for 18-24hrs . Blackening of media indicates positive test.

Fig.9. Showing Bile Esculin Results

BIOCHEMICAL TEST FOR IDENTIFICATION OF GAM NEGATIVE BACTERIA:

CATALASE TEST:

Catalase test pocedure was same as done in gram positive organisms.

OXIDASE TEST :

This test is used to identify bacteria containing cytochrome oxidase. This test aids in differentiation among members of the genera neisseria and pseudomonas, which are oxidase positive and enterobacteriaceae, which are oxidase negative.

DRY FILTER PAPER METHOD:

Strip of whatman's No. 1 filter paper was removed. The colony to be tested was picked up with a platinum loop and smeared over the moist area. .

RESULTS :A positive reaction was indicated by an intense deep-purple color appearing within 5-10secs. Absence of colouration or colouration later than 60secs means negative reaction.

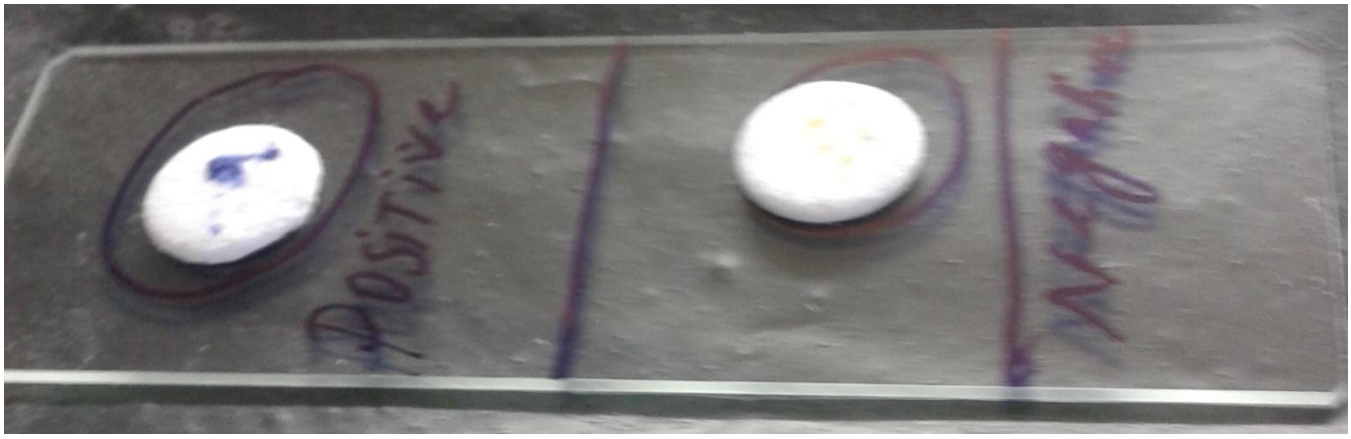

Fig.10. Showing Oxidase Test Results

TRIPLE SUGAR IRON AGAR: This medium is used for identification of gram negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.A heavy inoculumwas streaked over thesurface of the slope and stab into the butt of TSI agar. It was incubated at 37^0c for 24hrs.

RESULTS:

Slant/butt	color	Interpretation
K/A (alkaline/acidic)	red/yellowonly	glucose fermented
A/A (acidic/acidic)	yellow/yellowall	sugars fermented
K/K (alkaline/ alkaline)	red/red	no fermentation
NC/NC (no change	orange/orange	no fermentation
Black butt	black ppt	H_2S production
splitting or Bubbles	_	gas production

Fig.11. Showing TSI Results

INDOLE TEST:

Medium wasinoculated with bacterial colonies from an 24hrs old culture using a sterile loop and at incubated at 37^0c for 24hrs. 0.5ml kovac's reagent was added and shaken gently. Red color ring in the alcohol layer indicates a positive test.

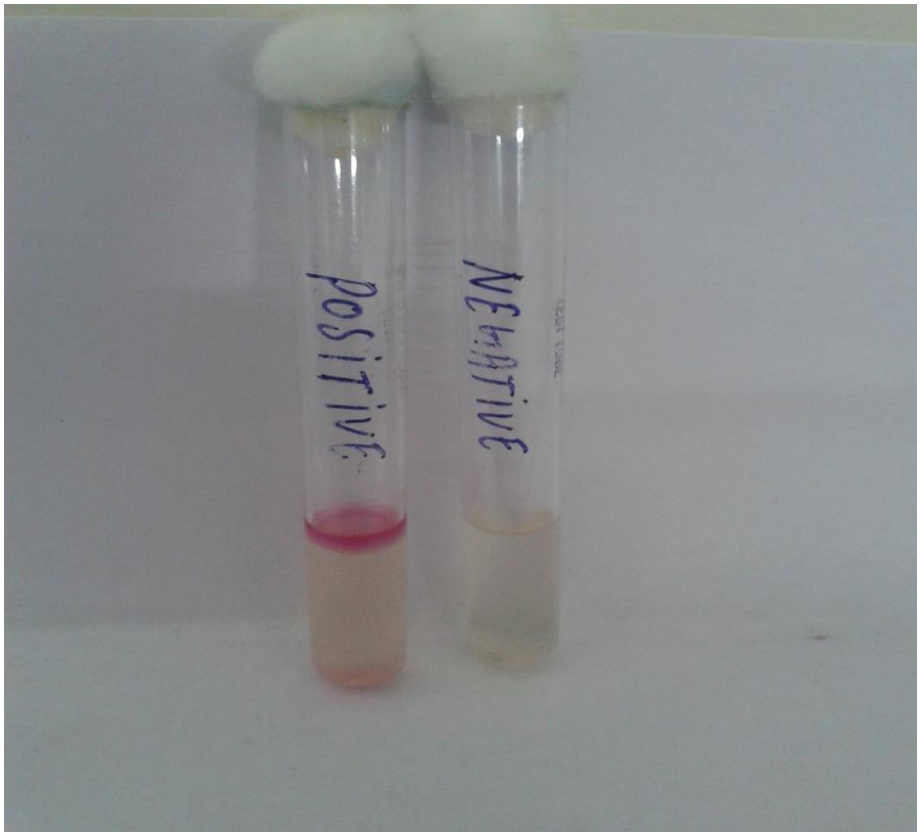

Fig.12. Showing Indole Test Results

SIMMON CITRATE AGAR:

The medium was inoculated with bacterial colonies from an 24hrs old culture using a sterile loop by making a streak onto the surface of the slant and incubated at 37^0c for

24hrs. in positive cases, color of the medium was changed to blue whileoriginal green color remained in negative cases.

Fig. 13. showing Simmon Citrate Agar Results

UREA AGAR BASE (CHRISTENSEN) : This test is used to distinguish urease producting organism proteus from other enteric bacteria. Heavy inoculation was done over the entire slope surface and incubation was done at 37^0c for 24hrs. in positive cases, color of the indicator was changed to pink.

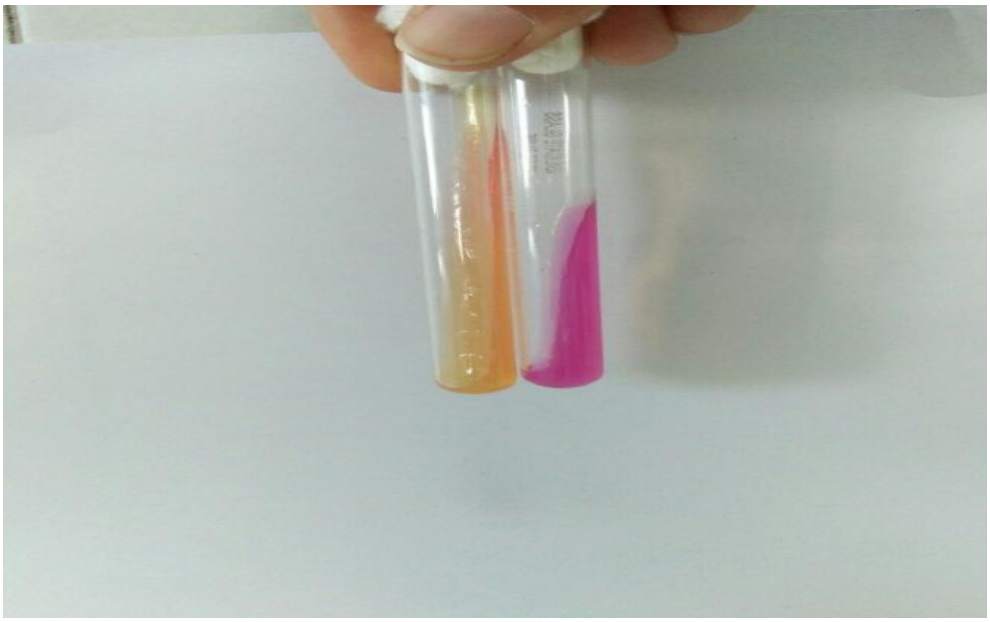

Fig.14. Showing Urease Test Results

PHENYLALANINE AGAR:

This medium tests the ability of an organism to produce the enzyme deaminase. This enzyme removes the amino group from the amino acid phenylalanine and releases the amine group as free ammonia. Heavy inoculation was done over the entire slope surface and incubation was done at 37^0c for 24hrs. a few drops of 10% solution of ferric chloride was allowed to run down over the growth on the slope. In positive cases, green color was developed in the fluid and in the slope due to the production of phenylpyruvic acid.

ANTIMICROBIAL SENSITIVITY TEST:

The antibiotic sensitivity test was performed by Kirby Bauer diffusion technique with commercially available disc (Himedia laboratories pvt. ltd mumbai) on Muller Hinton agar and results were interpreted according to the CLSI guidelines.

Mueller -Hinton Agar: This medium is used for determination of susceptibility of microorganism to antimicrobial agents.

KIRBY-BAUER METHOD:

This is a filter paper disc agar diffusion procedure, frequently used to determine the drug susceptibility of microorganisms isolated from infectious processes.this method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from the diffusion of the agent into the medium surrounding the disc.

PROCEDURE:

1. The colonies of organism from culture plates were passed into peptone water and incubated at 37^0c for 4-6hrs.
2. After 4hrs a cotton swab was dipped into inoculum and streaking with swab on Muller Hinton agar plate was done three times over the entire agar surface.
3. Surface of the agar was allowed to dry for 3-5mins .
4. Using a sterile foreceps antibiotic discs were applied to the MH plate. On a plate of 100mm diameter, seven discs may be applied, one in the centre and six in the periphery. Discs were diposit with centres at least 24 mm apart.
5. The plates were then incubated at 37^0c for 16-24hrs..
6. After incubation, the zone of complete growth inhibition around each discs were measured and the diameter of the disc was included in these measurements.
7. The interpretation of zone site into sensitive, intermediate and resistant were based on interpretation chart.

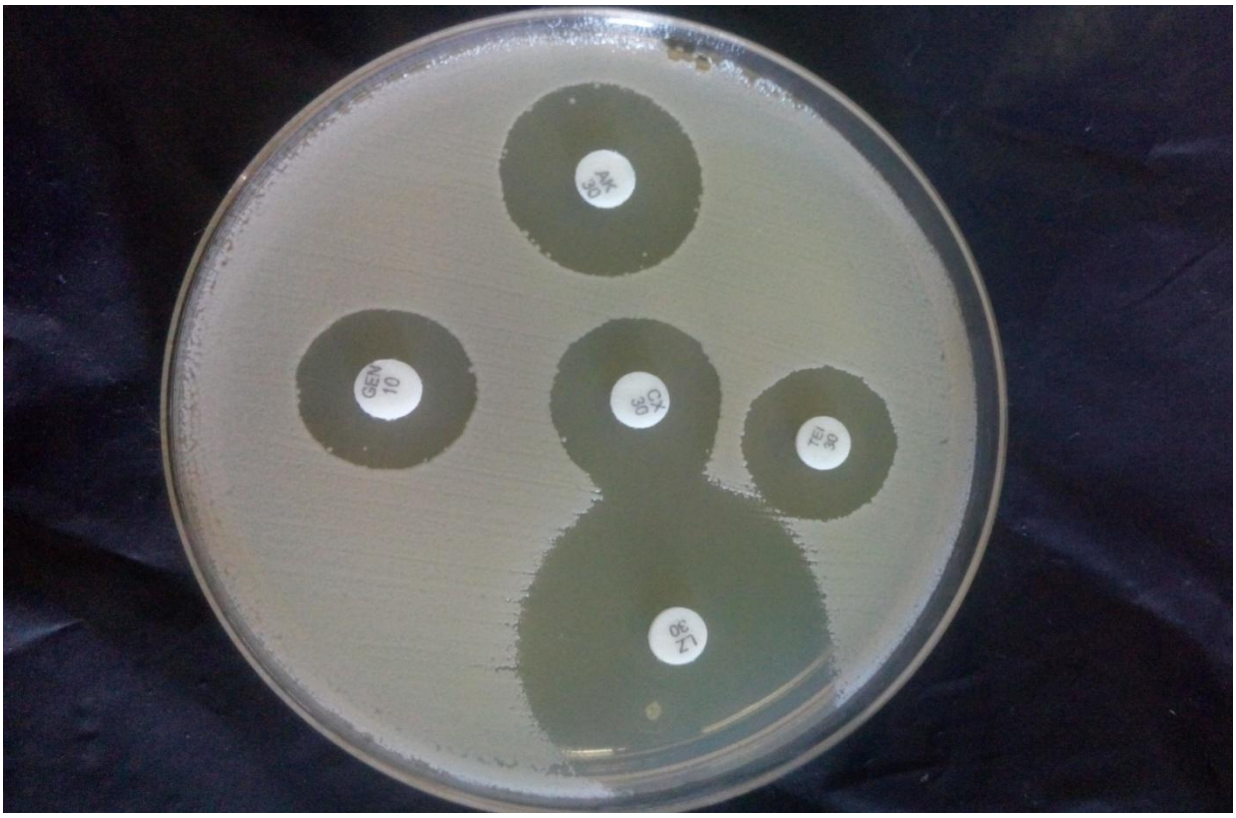

Fig.15. Showing Antibiotic susceptibility test Results of Gram positive organism on MH agar plate.

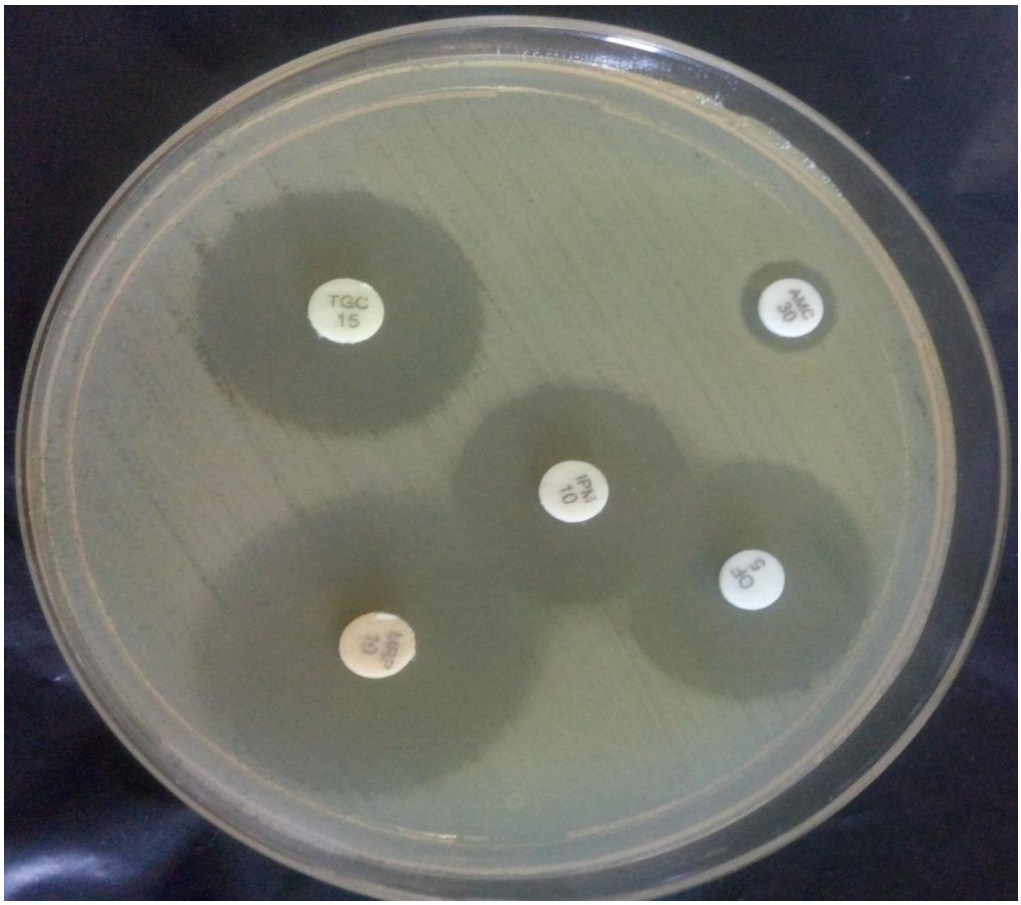

Fig.16. Showing Antibiotic Susceptibility Test Results of Gram negative organism on MH agar Plate.

TABLE 1. Various Antibiotic disc Used in Gram Positive and Gram Negative Organisms

Antimicrobial agents in gram positive org.	antimicrobial agents in gram negative org.
Amikacin(AK)	Amoxyclav(AMC)
Clindamycin(CD)	Ampicillin(AMP)
Co-trimoxazole(COT)	Aztreonam(AT)
Ciprofloxacin(CIP)	Amikacin(AK)
Cefoxitin(CX)	Cefixime(CFM)
Erythromycin(E)	Colistin(CL)
Gentamicin(G)	Ceftazidime(CAZ)
Linezolid(LZ)	Ceftriaxone(CTR)

Penicillin(P)	Cefepime(CPM)
Rifampicin(RIF)	Cefoperazone/tazobactam(CST)
Teicoplanin(TEI)	Cefuroxime (CXM)
Tetracycline(TR)	Cefotaxime(CTX)
Vancomycin(VA)	Gentamicin(G)
	Imipenem(IPM)
	Levofloxacin(LE)
	Ofloxacin(OF)
	Meropenem(MRP)
	Piperacillin/tazobactam(PIT)
	Tigecycline(TGC)
	Tobramycin(TOB)

RESULTS:

In this study, a total of hundred blood samples were processed for isolation and identification of causitive organisms of BSIs out of which 34% shows culture positivity as summarized in Table 2. male and females were found to be almost equally effected (1:1) TABLE 3 (Fig 17) with maximum effected age group was 61-70 Table 4 (Fig18). Gram positive organisms are the major cause of BSIs with S.aureus being the predominant cause followed by E.coli as summarized in Table 5 (Fig.20).

The antibiotic sensitivity pattern for gram positive organism S.aureus has shown the highest sensitivity towards Linezolid(100%), Glycopeptides(100%). Out of 12 Staphylococcus aureus isolates, 25% were identified as MRSA. .Further moderate resistance was observed for Ciprofloxacin,co-trimoxazole as summerized in TABLE 6 (Fig21). In case of E.coli Tigecycline was found to be 100% sensitive followed by amoxy/clav while ampicilin show 100% resistance as illustrated in TABLE 7(Fig 22) below. Enterococcus was resistance to most of the antibiotics used (Table 8).

TABLE 2. Distribution of culture positivity in BSI

Total no of samples	No of positive culture	%
100	34	34 %

TABLE 3: Gender wise distribution of culture positivity in BSI

Sex	No of isolates
Male	18
Females	16

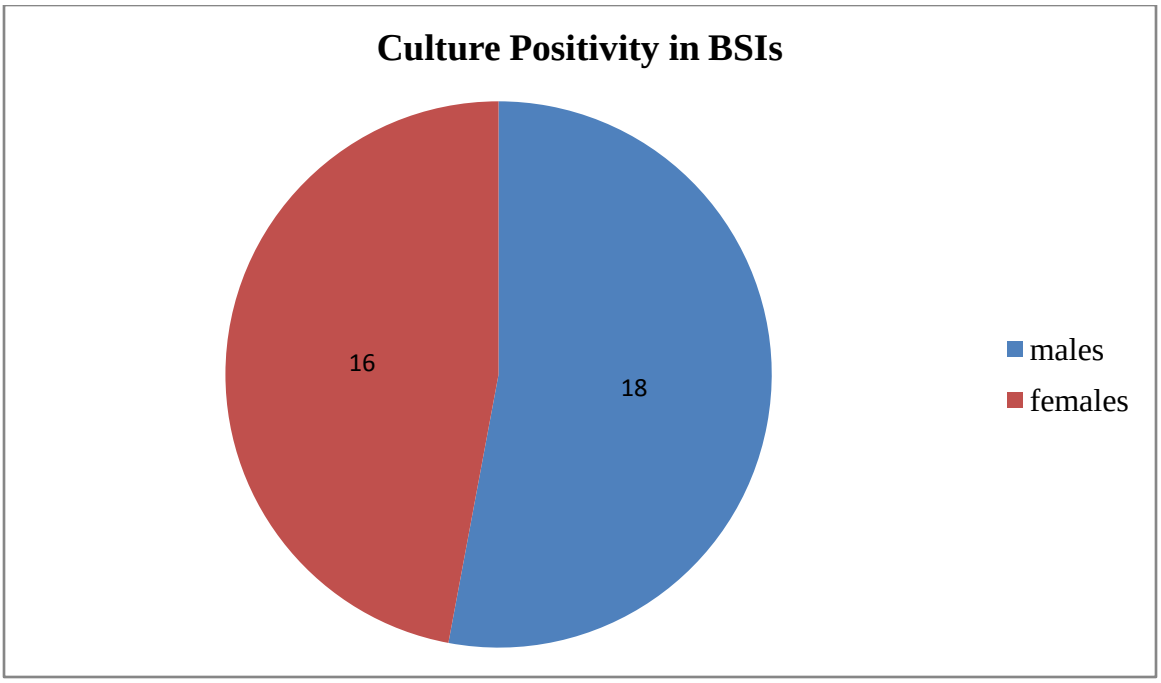

Fig.17. Showing Gender wise Culture Positivity in BSIs

TABLE 4. Age wise distribution of culture positive cases

Age	No of isolates
0-10	1
11-20	1
21-30	1
31-40	2
41-50	2
51-60	8
61-70	13
71-80	4
81-90	2
91-100	0
Total	34

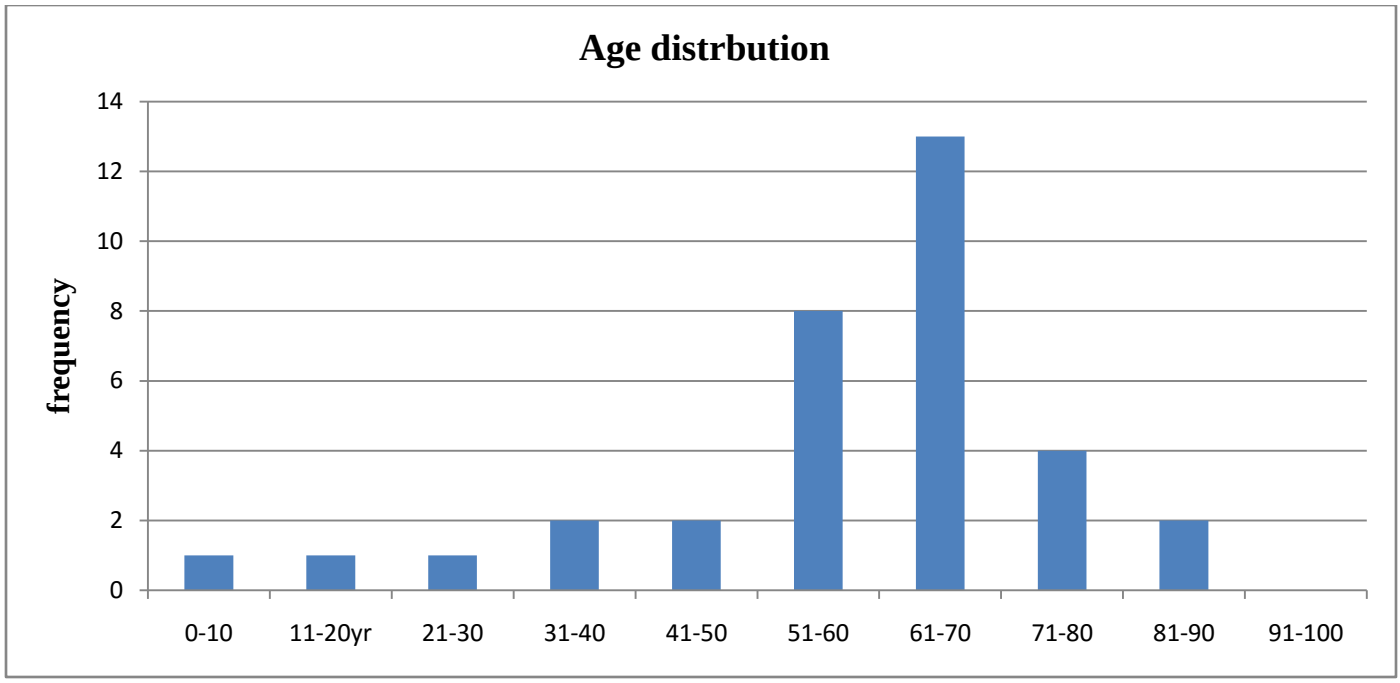

Fig.18. Showing Age wise Distribution of positive cases

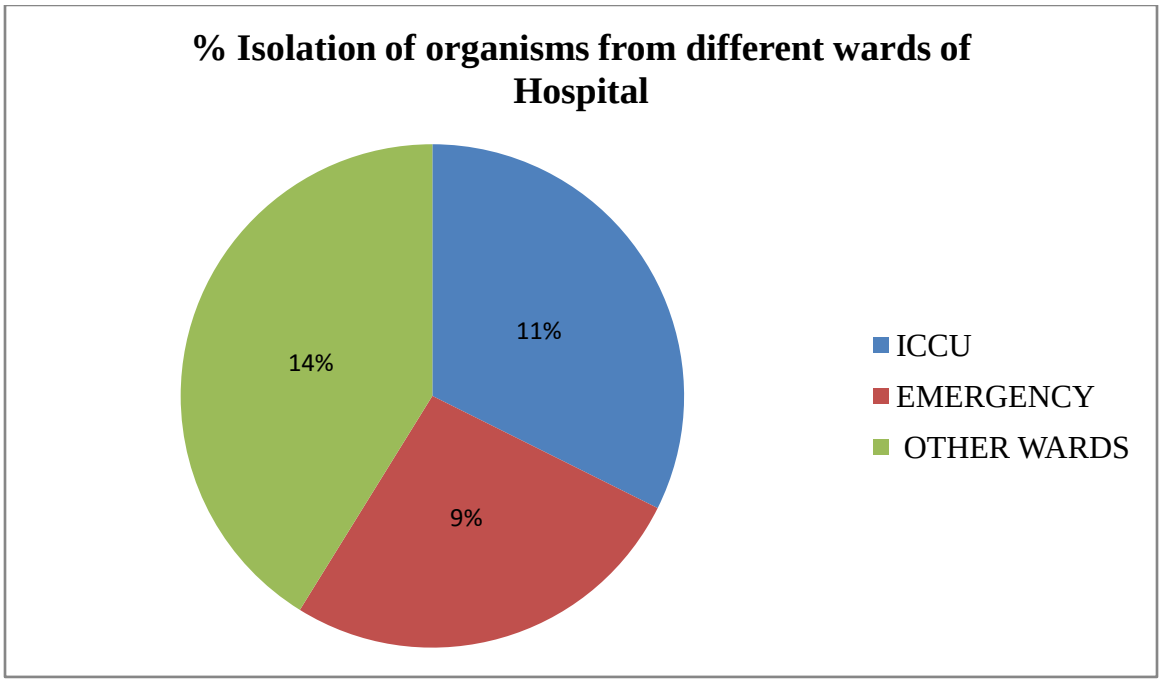

Fig.19. Showing % isolation of Organisms from different Wards of Hospital.

TABLE 5. Microbial profile among BSI

Organism	Percentage(%) of isolates
S.aureus	40
E.coli	36.67
Enterococcus spp.	10.00
Pseudomonas spp.	6.67
Klebsiella spp.	6.67
Acinetobacter spp.	6.67
Citrobacter spp.	3.33
B.cepacia	3.33

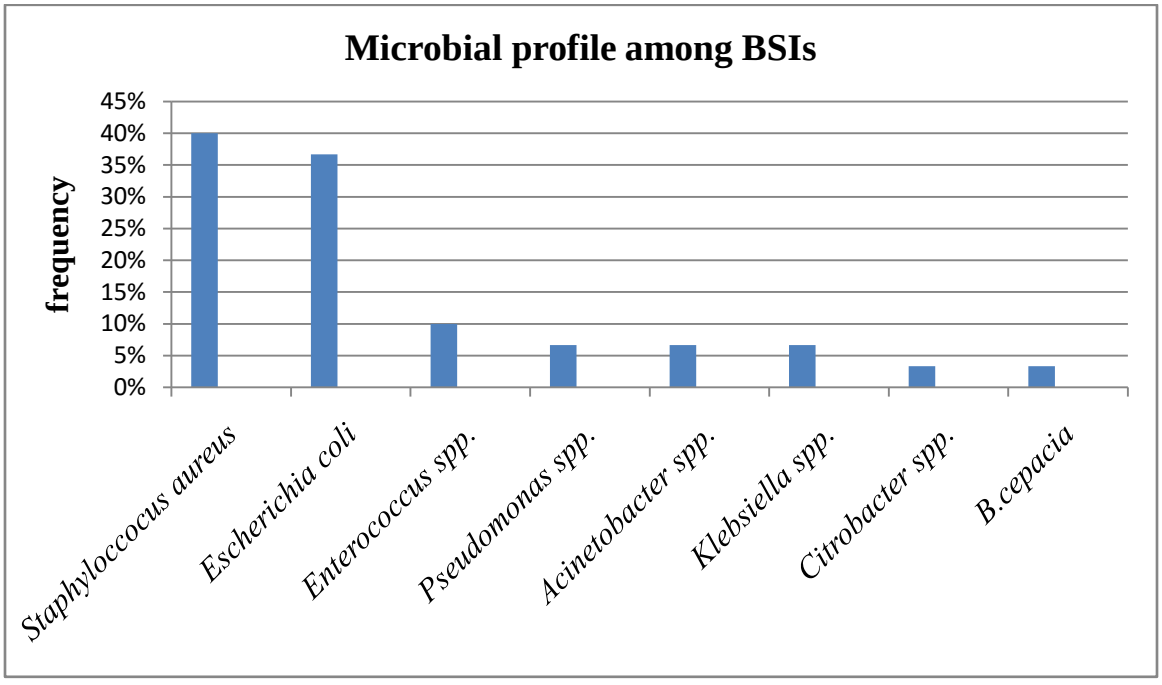

Fig.20. Showing the Microbial Profile among BSIs

TABLE6. Antibiotics Susceptibility pattern of gram positive organism (*S.aureus*) by Kirby Bauer disc method.

Antibiotics	% of Sensitivity
Amikacin(AK)	83.33

Clindamycin(CD)	83.33
Co-trimoxazole(COT)	50
Ciprofloxacin(CIP)	50
Cefoxitin(CX)	75
Erythromycin(E)	66.66
Gentamicin(G)	66.66
Linezolid(LZ)	100
Penicillin(P)	66.66
Rifampicin(RIF)	91.66
Teicoplanin(TEI)	100
Tetracycline(TRA)	66.66
Vancomycin(VA)	100

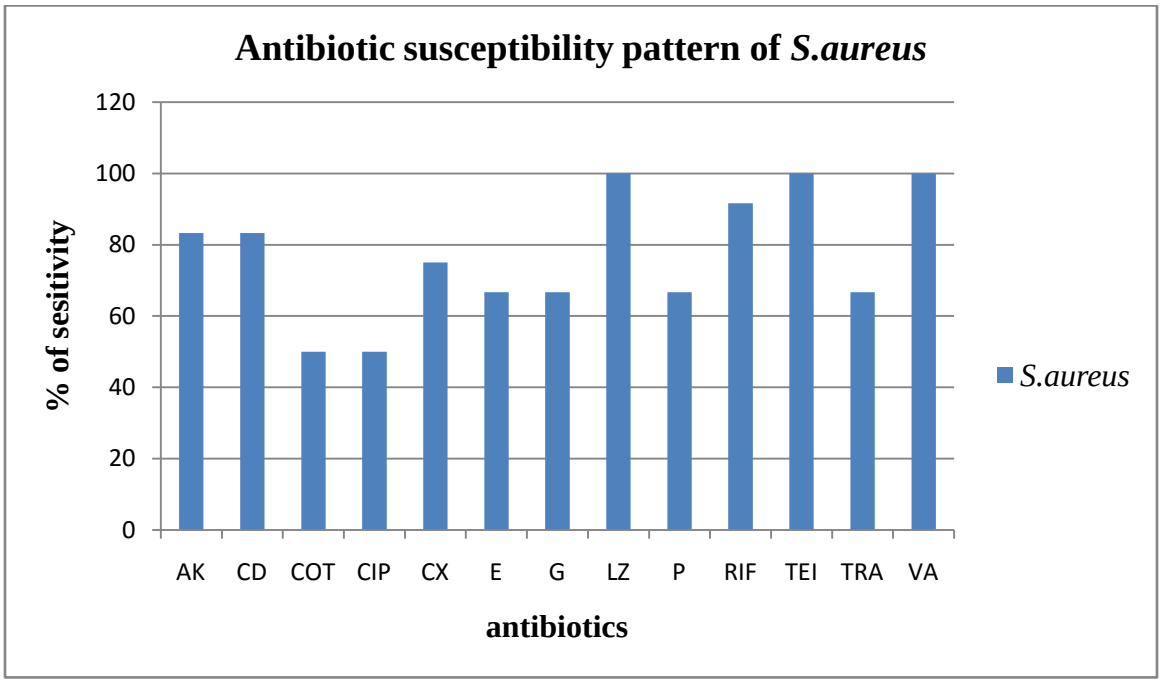

Fig.21. Showing Antibiotics susceptibility pattern of gram positive organism *S.aureus* by Kirby Bauer disc Method

TABLE7. Antibiotics Susceptibility Pattern of Gram Negative Organism (*E.coli*) by Kirby Bauer Disc Method

Antibiotics	%of sensitivity
Amoxyclav(AMC)	90
Ampicillin(AMP)	0
Amikacin(AK)	36
Cefixime(CFM)	45
Colistin(CL)	72
Ceftazidime(CAZ)	27
Cefoperazone/sulbactam (CSE)	90
Cefepime(CPM)	18
Cefoperazone/tazobactam(CST)	36
Cefuroxime (CXM)	90
Cefotaxime(CTX)	27
Gentamicin(G)	27
Imipenem(IPM)	63
Levofloxacin(LE)	18
Ofloxacin(OF)	18
Meropenem(MRP)	18
Piperacillin/tazobactam(PIT)	72
Tigecycline(TGC)	100

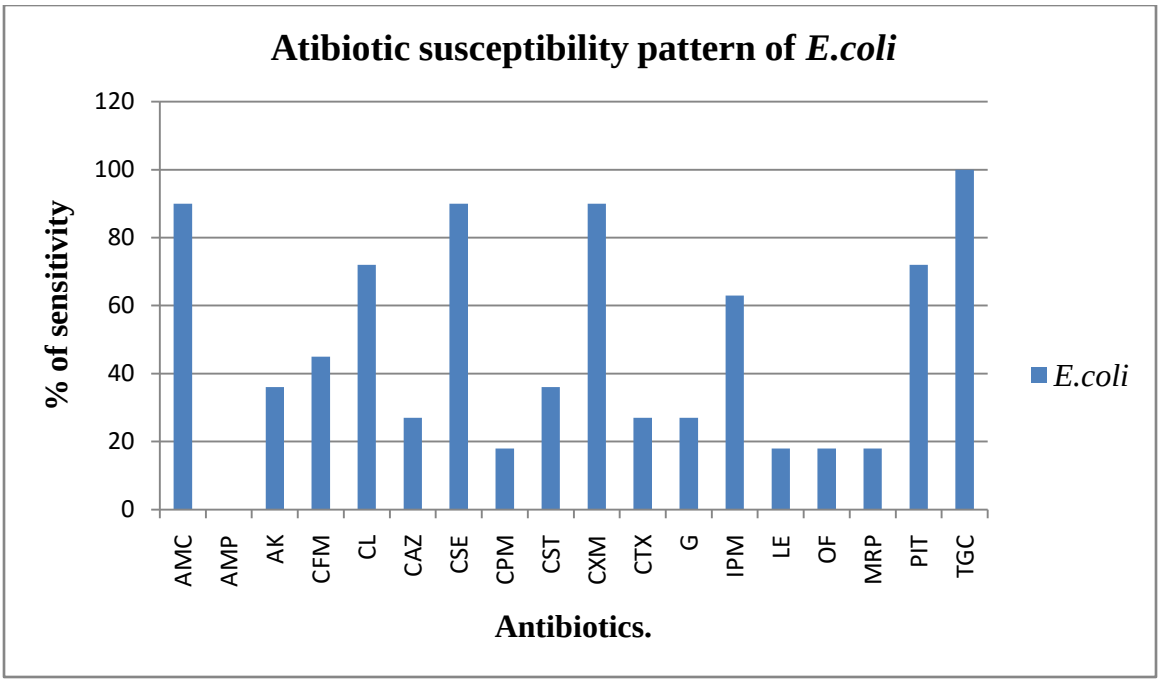

Fig.22. Showing Antibiotics susceptibility pattern of gram negative organism *E.coli* by Kirby Bauer disc Method.

TABLE 8: Antibiotics susceptibility pattern of gram positive organism *Enterococcus spp*. by Kirby Bauer disc Method.

Antibiotics	% of sensitivity
Amikacin(AK)	0
Clindamycin(CD)	0
Co-trimoxazole(COT)	0
Ciprofloxacin(CIP)	33
Cefoxitin(CX)	0
Erythromycin(E)	0
Gentamicin(HLG)	0
Linezolid(LZ)	0
Penicillin(P)	66
Rifampicin(RIF)	33
Teicoplanin(TEI)	`100
Tetracycline(TRA)	100

Vancomycin(VA)	100

DISCUSSION:

In the current study,out of total 100 samples, 34% showed culture positivity. The rate of BSIs in our study was comparative to another study by Bicudo D et al[20] where prevalence of BSIs was 28%. Whereas another study done by Anita C et al.[19] showed only 14.5% prevalence of BSIs. In our study, Males and Females have been found out to be almost equally affected (1:1); {Males (53%) and Females (47%)} which was comparable to another study by Anita C et al[19] which also documented similar rates of infection in males (56.2%) and females (43.8%). This denotes that BSIs do not affect any particular gender more significantly than the other. Though there are few studies which contradict this finding (Vanitha Rani N.et.al) [21]

Another finding of our study was that the maximum affected age group was 61-70 years. This age group might hav shown more infection rate as these patients usually have longer stays in hospital, because of weak immune system and other chronic ailments leading to delayed recovery. More use of intavascular devices as well as high frequency antibiotic treatment weakened their immune system which results in higher infection in this age group. According to a study done by Erik L. Munson et.al[15] 40-59yrs of age shows 32%and 60-79years of age groups shows 28% incidence of BSI which was in similar to our study where maximum affected age group was found to be between 50- 70 years.The distribution and relative rank of these species varied with geographical area and environment of critical care units.

The range of microorganisms that invade the bloodstream has been studied by several researchers. In current study, gram posittive organisms are major cause of bloodstream infections then gram negaitive organisms with *S.aureus* being the predominant isolate in BSI followed by *E. coli, Enterococcus spp., Pseudmonas spp., Klebsiella spp.* This study was favoured by a study done by Beekmann et al,[5]acc to which out of 100 positive samples 68.18% show *S.aureus* growth.and Karam El-Din et al[7], in which out of 52 cases 25.1% show *S.aureus.* Acc to studies by weinstein et.al[14],Mark H Reacher[11] *S. aureus* was among the top five causes of bacteraemia in every age group,whereas,coagulase negative *staphylococci,S.pneumoniae,and Enterococcus* species ranked lower then *Staphylococcus spp.* .Several studies in USA (65 and 25%), Iran(72 and 28%) and UK (66.2 and 31.3%) have shown higher prevalence of gram-positive and lower prevalence of gram negative organisms respectively.[22-24] These all studies were in favour of present study.while studies conducted in Iran (42.3 and 42.3%) and Saudi Arabia (62.2 and

33.8%) had higher no of gram neg organisms.[25-26] Only one-one case of B.cepacia and citrobacter were isolated from blood culture in the present study. The main reason behind the more positive cases of *S.aureus* was use of intravascular devices with intravenous catheters being the major cause. Moreover staph now become multi drug resistant so they were present in high no in hospitals wards and get attached to the surfaces of intravenous devices (hub of catheter) and reached sterile blood stream leading to infection.

In case of antibiotic susceptibility, *S.aureus* was sensitive to most of the antibiotics tested with 100% sensitivity to Linezolid, Glycopeptide(Teicoplanin,Vancomycin). Cefoxitin was 25% resistant with 50% resistance to Ciprofloxacin,Co-trimoxazole while other antibiotics used were intermediate.(Erythromycin,Penicillin,Trimethoprim,Clindamycin,Rifampicin,Aminoglycerides(Gentamicin)). According to a study by Prakash KP et.al.,[17] *Staphylococcus* species (*S. aureus*) were more commonly resistant to Trimethoprim/Sulphamethoxazole (35.3%), Penicillin (25.9%), Erythromycin (19.6% and Gentamicin (15.5%). Only one (2.4%) of the *S. aureus* isolates was resistant to Methicillin/Oxacillin (MRSA). this study favour the pesent study. In case of *E.coli* in the present study, Tigecycline was 100% sensitive followed by Amoxy/clav(90%), Cefoperazone/sulbactam(90%) while Ampicilin show 100% resistance followed by Cefepime(82%),Quinolones(LE,OF(82%),Carbapenems(MRP,IPM),Aminoglycosides(G,AK), Ceftazidine(73%) and the remaining drugs respectively. Gram negative were resistant to Ampicillin (59.7%),Gentamicin(63.6%,Imipenem(28.6%)) Acc to a study done by prakash et.al(17) and Anita C et al.[19] where gram negative organisms show higher resistance toward Amikacin(68.75%), Ceftazidime (59.4%)and least resistance towards Imipenem(18.8%)which favours current study.

According to a study done by Mureithi Maryanne Wanjiru et.al.,[27] *E.coli* were moderate resistancetoAmpicillin,cefuroxime and highly sensitive to Gentamycin,Ceftazidine,Ciprofloxacin and *S.aureus* was highly sensitive to Ciprofloxacin and Cotrimoxazole which is in opposition of our study .

It is critical to do properly identification as well as accurate reporting of identified organism and antibiotic sensitivity pattern. Antibiotic sensitivity identification errors may occur. so surveillance programs are necessary to identify changes in the spectrum of microbial pathogens, risk factors causing them and to monitor trends in antimicrobial resistance patterns and to

implement appropriate measures in nosocomial and community-acquired BSIs. Low cases of MRSA in the present study may be due to improve hand hygiene among healthcare workers and low usage of antibiotics in the hospital. The low yield of the blood cultures as observed could have resulted from a number of reasons. blood cultures produce best results when performed in the first week of infection. This requires keen diagnosis by the clinician and early presentation by the patients to the hospital. The use of antibiotic prior to hospital visit has been shown to have a bactericidal effect on the bacteria by preventing their growth in the culture medium. Another reason may be due to reliance on a single blood and cultures beause It was not possible to perform multiple cultures due to resource constraints. The high levels of resistance of the blood isolates to antibiotis could have been attributed to the increasing high usage of orally administered drugs and the resistance patterns observed could have been due to self medication with antibiotics by peoples.

CONCLUSION:

BSIs are among the most severe manifestations of bacterial disease with Gram-positive bacteria being the predominant cause of BSIs. Patients can present to hospital with a bloodstream infection or may develop as a result of healthcare interventions. Increasing antimicrobial resistance among blood pathogens is a matter of great concern to start empirical antibiotic treatment and to monitor continuously the sensitivity pattern of isolates so as to make a rational use of antibiotics. strict aseptic condition should be maintained during blood collection and injecting it into blood culture bottles in order to avoid unnecessary antibiotic therapy. surveillance programs (national,regional or at the hospital level) are necessary to identify changes in the spectrum of microbial pathogens, risk factors causing them and to monitor trends in antimicrobial resistance patterns and to implement appropriate measures in hospital acquired BSIs. There is a need for optimizing the use of antimicrobial therapies as well as development of new antibiotics and vaccines.

REFERENCES

1. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. In: Olmsted RN, ed. APIC infectioncontrol and applied epidemiology: principles and practice. St. Louis:Mosby, 1996:A1–20.
2. Centers for Disease Control and Prevention. Guidelines for the prevention of intravascular catheter–related infections. MMWR RecommRep 2002; 51(RR-10):19
3. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. Clin Microbiol Rev 1997; 10:444–65.
4. Archibald, L.K., Pallangyo, K., Kazembe, P., Reller L.B. Blood Culture contamination in Tanzania, Malawi and The United States: A Microbiological Tale of three Cities. J. Clin Microbiol, 2006; 44: 4425.
5. S. E. Beekmann et.al. Effects of Rapid Detection of Bloodstream Infections on Length of Hospitalization and Hospital Charges. Journal of clinical microbiology, July 2003, p. 3119–3125 vol 41, no.7.
6. Larry G. Reimer et.al., Update on Detection of Bacteremia and Fungemia. Clinical Microbiology Reviews, July 1997, p. 444–465 Vol. 10, No. 3
7. Al-Zahraa A. Karam El-Din et.al,. Prevalence of microbial pathogens in blood cultures: an etiological and histopathological study. journal of Taibah Universty for Science (JTUSCI) 3: 23-32 (2010).
8. Harald Seifert Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, German. The Clinical Importance of Microbiological Findings in the Diagnosis and Management of Bloodstream Infections Clinical Infectious Diseases *CID 2009:48 (Suppl 4)*
9. Bhatta D R et.al., Bacteriological Profile of Blood Stream Infections among febrile patients attending a tertiary care centre of western nepal. Asian Journal of Medical Science, Volume-4(2013).
10. Arunaloke Chakrabarti Prashant Sood et.al., Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med,dec 2014.

11. Mark H Reacher et.al., Bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998: trend analysis. BMJ volume 320 22 January 2000 www.bmj.com.
12. Soraya S. Andrade et.al, Advances in the Microbiological Diagnosis of Sepsis. Shock, Vol. 30, Supplement 1, pp. 41Y46, 2008.
13. Sakina Bhattiwala et.al., "A study of Common Isolates in Blood Cultures from Tertiary Care Pediatric Hospital". Volume 4, Issue 10, 2002-2007
14. Melvin P. *Weinstein* et.al. The clinical significance of positive blood cultures in the 1990s: A *prospective* comprehensive evalution of the Microbiology, epidemiology, and Outcome of Bacteremia and Fungemia in Adults in the 1990, Clinical Infectious Diseases 1997, 24 April.
15. Erik L.munson et.al. Detection and Treatment of Bloodstream Infection: Laboratory.ReportingandAntimicrobialManagement,JournalofClinicalMicrobiology,Jan. 2003,p.495-497
16. Marcio.M. Loureiro et al. Study of Multi-Drug Resistant Microorganisms isolated from Blood Cultures of Hospitalized Newborns in Rio de Janeiro city, Brazil. Brazilian Journal of Microbiology (2002) 33:73-78
17. Prakash KD et.al, Bloodstream Bacterial Pathogens and their Antibiotic Resistance Pattern Dhahira Region, Oman. Oman Medical Journal (2011) vol.26,N0.4: 240-247
18. Alo, M. N et al, Presence of extended spectrum β-lactamase (ESBL) E. coli and K. pneumoniae isolated from blood cultures of hospitalized patients Adv. Appl. Sci. Res., 2012, 3(2):821-825.
19. Anita C et al., Hospital acquired blood stream infections and resistogram pattern of Gram negative organisms in a tertiary care hospital of Kanchipuram. Advances in Biomedicine and Pharmacy Vol. 3 (1) 2016
20. Bicudo D, Batista R, Furtado GH, Sola A and Medeiros EA.., "Risk factors for catheter-related bloodstream infection: a prospective multicenter study in Brazilian intensive care units", Braz. J. Infect. Dis., 15:328-331, 2011.
21. Vanitha Rani N., "A retrospective study on blood stream infections and antibiotic susceptibility patterns in a tertiary care teaching hospital", Int. J. Pharm. Sci., 4:543-548,2012.

22. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004 Aug;39(3):309-317.
23. Mamishi S, Pourakbari B, Ashtiani MH, Hashemi FB. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996-2000. Int J Antimicrob Agents 2005 Nov;26(5):373-379. .
24. Gray JW. A 7-year study of bloodstream infections in an English children's hospital. Eur J Pediatr 2004 Sep;163(9):530-535.
25. Rahbar M, Gra-Agaji R, Hashemi S. Nosocomial blood stream infections in Imam Khomeini Hospital, Urmia, Islamic Republic of Iran, 1999-2001. East Mediterr Health J 2005 May;11(3):478-484.
26. Elbashier AM, Malik AG, Knot AP. Blood stream infections: microorganisms, risk factors and mortality rate in Qatif Central Hospital. Ann Saudi Med 1998 Mar-Apr;18(2):176-180.
27. Mureithi Maryanne Wanjiru, Isolation and Characterization of Bacteria Pathogens in Blood and Stool Samples among patients presenting with typhoid fever symptoms in Alupe, Busia County ,November, 2013.

Appendix I

A. LIST OF MATERIALS

1. Equipment

- Autoclave.
- Bunsen burner.
- Incubator.
- Microscope.
- Refrigerator.
- Weighing machine.

2. Glass wares

- Petri plates.
- Test tubes.
- Slides.
- Conical flask.
- Cover slips

3. Others

- Cottons.
- Scissors.
- Forceps.
- Inoculating loops
- Face mask.
- Gloves

APPENDIX-II

B. GRAM STAIN

1. PRINCIPLE:

When the bacteria is stained with primary stain crystal violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by the alcohol. The cell wall of gram positive baccteria have a thick layer of protein- sugar complexes called peptidoglycan and low lipid content. Decolorizing the cell causes this thick cell wall to dehydrate and shrink, which closes the pores in the cell wall and prevents the stain from exiting the cell. So the acetone cannot remove the crystal violet -iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears purple in color.

In case of gram negative becteria, cell wall also take up theCV-Iodine complex. But gram negative bacteria have thin layer of peptidoglycan and thick outer layer of lipids, CV-Iodine complex get washed off. When the are exposed to decolorizer , acetone dissolves the lipids in the cell walls, which allows the CV-Iodine complex to leach out of the cells. On staining with ccounterstain, the cell takes the color of the safranin and appears pink in color.

2. PREPARATION OF SMEAR:

1. A clean glass slide was taken and a drop of 0.9% NaCl(normal saline) wasplaced on it.
2. Using sterile technique, a loopful of clinical specimen or bacterial suspension was placed,mixed with normal saline and spread on the slide to make thin smear
3. The smear was allowed to dry in the air or the smear was fixed to the slide by gentle heating over a flame.

3. REAGENTS:

1. Primary stain: crystal violet – 1g crystal violet in 50ml of absolute alcohol
2. Mordant: gram's iodine – 0.6g iodine crystals+1.2g of pottasium iodide in 80ml of dis.water.
3. Decolorizing agent : acetone

4. Counterstain: safranin - 1g in 100ml of dis.water.

Appendix-III

C. PRINCIPLE, COMPOSITION AND PREPARATION OF DIFFERENT TYPES OF CULTURE MEDIA

1. BLOOD AGAR

PRINCIPLE: Blood agar are enriched medium used to culture fastidious microbes or bacteria such as streptococci that have specific nutritional needs and do not grow well on ordinary media. It also permits demonstration of the hemolytic properties of some microorganisms such as alpha, beta and gamma hemolysis.

Alpha hemolysis means incomplete lysis of red blood cells, results in a greenish hallow around the bacterial growth. Beta hemolyis means complete lysis of red blood cells, results in a clear zone surrounding the colonies. Gamma hemolysis means no lysis of red blood cells, results in no significant change in the appearance of medium surroundind the colonies.

COMPOSITION

NUTRIENT AGAR:

	INGREDIENTS	**gm/lt**
1.	Peptic digest of animal tissue	5.00
2.	Sodium chloride	5.00
3.	Beef extract	1.50
4.	Yeast extract	1.50
5.	Agar	15.00

METHOD:

1. 28.0 gms of media was dissolved in 1000ml of d.water. it was heated to boli to disslove the medium completely. Media was sterilized by autoclaving at 15lbs pressure (121^0c) for 15mins.

2. After autoclaving,nutrient agar was melted and cooled to 50^0c.
3. Aseptically 5-10% sterile blood was added into the medium. Then it was mixed and poured into the petridishes.

2. MACCONKEY AGAR :

PRINCIPLE:

The inhibitory action of crysal violet on the growth of gram positive organisms allow the isolation of gram negative bacteria. Incorporation of the carbohydrate lactose, bile salts and the pH indicator neutral red permits the differentiation of enteric bacteria on the basis of their ability to ferment lactose. Bacterial colonies that can ferment lactose (E.coli) turn the medium red due to response of the pH indicator to the acidic environment created by the fermentation. Organism that do not ferment lactose (salmonella) do not cause a color change.

COMPOSITION:

INGREDIENTS	**gm/lt**
Peptic digest of anilal tissue	17.00
Proteose peptone	3.00
Lactose	10.00
NaCl	5.00
Neutral red	0.03
Bile salts	1.50
Agar	15.00

Final pHwas adjust the to 7.1+/-0.2. (25^0c)

METHOD:

51.53gms of media was dissolved in 1000ml of d.waterandwas heated to boiling to dissolve the medium completely. It was sterlize by autoclaving at 15lb pressure 121^0c for 15mins. Then it was cool to 45-50^0c. then it was mixed and poured into the petridishes.

3. Mueller -Hinton Agar:

PRINCIPLE:

The use of suitable medium for testing the susceptibity of microorganisms to sulfonamides and trimethoprim is essential. Antagonism to sulfonamide activity is demonstrated by para aminobenzoic acid and its analogs. Reduced activity oftrimethoprim resulting in smaller inhibition zones and innerzonal colonies, is demonstrated on MH agar medium possessing high levels of thymidine. Both PABA and thymidine content in medium are reduced to minimum,thus reducing the inactivation of sulfonamides and trimethoprim when the media is used for testing the susceptibility of microorganism to these antimicrobics.

Composition

INGREDIENTS	**gm/lt**
1. Beef, infusion from	300.00
2. Casein acid hydrolysate	17.50
3. Starch	1.50
4. Agar	17.00

Final pH was adjust to 7.3 +/-0.1

METHOD:

38.0g of media was dissolved in 1000ml of d.H_20 and was heated to boiling to dissolve the medium completely. It was sterlize by autoclaving at 15lbs pressure 121^0c for 15mins. Then it was cool to 45-50^0c. mixed and was poured into sterile petriplates.

Appendix-IV

D. Biochemical tests used in the study

1. CATALASE TEST:

The breakdown of hydrogen peroxide into oxygen and water is mediated by the enzyme catalase. When a small amount of an organism that produce catalase enzyme is introduced into hydrogen peroxide, rapid elaboration of bubbles of oxygen, the gaseous product of the enzyme's activity is produced.

2. COAGULASE TEST:

coagulase test helps to measure coagulase.coagulase is extracellular enzyme produced by S.aureus but not by other species. The substrate is a coagulase reacting factor in plasma, which forms a thrombin like substance, which then polymerises fibrinogen to form a fibrin clot, the reaction is independent of ca^{++}. The enzyme acts within host tissues to convert fibrinogen to fibrin, the fibrin mesh thus formed is believed to surround the bacterial cells thus protect the organisms from non-specific host resistance mechanism such as phagocytosis.

3. **BILE ESCULIN TEST:** This test is based upon the ability of an organism to hydrlyse esculin.this medium contain oxgall to inhibit the growth f gram positive organisms other then enterococci and group D streptococci. When an organism hydrolyses the glycoside esculin to form esculetin and dextrose, the esculetin reacts with the ferric citrate to produce adark black phenolic iron complex, which turns the media black.

4. **OXIDASE TEST :**

This test depends on the presence in bacteria of certain oxidases that will catalayse the transport of electrons between the electron donors in the bacteria and a redox dye – tetramethyl-p-phenylene-diamine. Cyochrome oxidase catalyses the oxidation of a reduced cytochrome by molecular oxygen, resulting in the formation of H_2O or H_2O_2.. The dye is reduced to a deep purple color.

5. TRIPLE SUGAR IRON AGAR:

This medium can determine the ability of an organism to utilize specific carbohydrates incorporated in medium, with or without the gas production,along with the determination of hydrogen sulfide production. This medium contains three sugars in varying conc: glucose(1), lactose(10)and sucrose(10) and the pH indicator phenol red. If sugar fermentation occurs, glucose will be initially used and the butt of the tube will be acidic yellow. After glucose utilization the may continue to ferment the remaining sugars. This cause the entire tube acidic. Some bacteria are unable to utilize any sugars and will breakdown the peptone present. Peptone utilization causes the alkaline shift red in the medium that causes a color change from orange to red. Hydrogen sulphide production causes the blackening of medium which changes ferrous sulfate to ferrous sulfide. splitting of medium or presence of bubbles in the butt of the tube can determine gas production.

6. INDOLE TEST:

This test is used todetermine the ability of an oranism to split amino acid tryptophan to form indole, which accumulates in the medium. Conversion of tryptophan into metabolic product is mediated by the enzyme tryptophanase. Indole is then tested for by a colorimetric reaction with p-dimethylaminobenzaldehyde.

7. SIMMON CITRATE AGAR:

This method is used to test the ability of organisms to utilize citrate as carbon source. This medium contain sodium citrate as the source of carbon and ammonium dihydrogen phosphate as nitrogen source. Organisms which can utilize citrate as their sole carbon source use the enzyme citrase to transport citrateinto the cells and also covert ammonium dihydrogen phosphate to ammonium and ammonium hydroxide, which creaes an alkaline environment in the medium.at pH 7.5 or above the indicator in the medium bromothymol blue turns royal blue. At neutral pH it remains green.

8. UREA AGAR BASE (CHRISTENSEN) :

Urea is the product of decarboxylation of amino acids. Organisms having a urease enzyme,cause the hydrolysis of urea and produces ammonia and $c0_2$. The formation of ammonia alkalinizes the

medium,and the pH sifts is detected by the color change of phenol red from light orange at pH 6.8 to pink at pH 8.1.