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RESEARCH ARTICLE

IDENTIFICATION OF MICRORGANISMS BY USING AUTOMATED MICROBIAL DETECTION SYSTEM IN ADULT PATIENTSSUSPECTED WITH SEPTICAEMIA WITH ITS SUPPLEMENTARY PARAMETERS

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Abstract

Septicaemia is a major cause of mortality in both developed and developing countries. Despite important progress in treatment and prevention of infectious diseases, they are considered as leading cause of death and disability. The purpose of this study was monitoring the spectrum of microorganisms that invade blood stream and to study role of different markers in septicaemia, such data are often used to determine empiric antibiotic therapy, to alert clinicians to emerging pathogens that may pose a threat to community and also help in diagnosing severity of septicaemia.

Aims and Objectives: To isolate and study the microorganisms from septicaemiccases, to study antimicrobial susceptibility of microorganisms isolated from septicemic casesandto study role of different markers in septicemic cases. Which help in diagnosing severity of septicaemia.

Methodology: The study was conducted from September 2015 to February 2017 in a tertiary care teaching hospital. 1993 samples were collected from patients clinically suspected patients of septicaemia. They were inoculated and then incubated, and gram staining was performed. Antibiotic resistance and susceptibility were measured by the disk diffusion method according to the Clinical and Laboratory standards institute (CSLI) guidelines

Result: Out of 1993 patients having, 695(34.87%) were culture positive. Among Gram negative isolates, Klebsiella spp.(14.96%)was commonest followed by Acinetobacter species (13.66%) ,E.coli (7.76%),Pseudomonas aeruginosa (6.76%) and salmonella spp.(1.29%). Among Gram positive organisms in our study CONS was the commonest (14.53%) followed by S. aureus (10.79%) and Enterococcus (6.18%), Streptococcus species (1.72%). It shows that colistin was found to be more sensitive for gram negative organisms followed by aminoglycosides and carbapenem group, while resistance was observed towards fluoroquinolone, 1st and 2nd generation cephalosporine groups. &Vancomycin, Teicoplanin and Linezolide were highly active drugs against Gram positive organisms.

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Conclusion: Septicaemia is an important cause of morbidity and mortality. The study conducted showed both Gram positive and Gram-negative bacteria were responsible for septicaemia. Most of the strains were multi drug resistant and leaving limited options for treatment. Thus, timely detection and knowledge of most likely pathogens causing septicaemia along with their antibiotic susceptibility pattern will help the clinicians in choosing appropriate antimicrobials for treatment which will reduce the major burden of septicaemia in critically ill patients and will also minimize the further emergence of resistance. Therefore, there should be an intensive surveillance, antibiotic policy formulations and preventive efforts for the effective management and prevention of drug resistance.

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Introduction:-

"Sepsis is a state caused by microbial invasion from a local infectious source into the bloodstream which leads to signs of systemic illness in remote organs," this was the first scientific definition of sepsis proposed by Dr.Schottmuller in 1914.Thus, bloodstream infection or bacteraemia was a condition to the diagnosis of sepsis and this definition did not change significantly over the years. Sepsis, septicaemia and bloodstream infections (bacteraemia) were considered to refer to the same clinical condition and in practice, the terms were often used interchangeably. Now, we know that less than one half of the patients who have signs and symptoms of sepsis have positive blood culture or other microbiological proof of an infectious focus. [8]

Septicaemia is a major cause of mortality in both developed and developing countries. Despite important progress in treatment and prevention of infectious diseases, they are considered as leading cause of death and disability. The isolation of a bacterium from patients suspected with septicaemia often indicates a serious infectious illness with potentially severe morbidity and mortality unless early appropriate therapeutic measures are initiated. The gold standard for diagnosis is a positive blood culture. [12]

Since early 1950's, there has been an increase in the incidence of bacteraemia caused by members of Enterobacteriaceae and other gram-negative bacilli. Multi drug resistance from gram negative bacilli like Salmonella, Klebsiella, Acinetobacter, Pseudomonas and Citrobacter species are gaining importance and have replaced Escherichia coli which was reported to be common in the past. [12]

In addition, Biomarkers can have an important place in this process because they can indicate the presence or absence or severity of sepsis. Biomarkers can add accuracy of any bacterial presence and they are useful to monitoring the evolution of infectious process.No single biomarker of bloodstream infections may be ideal, but many are helpful in terms of identifying bacterial infections in critically ill patients who need close monitoring so that the antibiotic therapy may be modified or stopped as soon as possible.[9]

The purpose of this study was monitoring the spectrum of microorganisms that invade blood stream and to study role of different markers in septicaemia, such data are often used to determine empiric antibiotic therapy, to alert clinicians to emerging pathogens that may pose a threat to community and also help in diagnosing severity of septicaemia.

Aims And Objectives:-

To isolate and study the microorganisms from septicemiccases,To study antimicrobial susceptibility of microorganisms isolated from septicemic casesandTo study role of different markers in septicemic cases. Which help in diagnosing severity of septicaemia.

Materials And Methods:-

The study was conducted from September 2015 to February 2017 in a tertiary care teaching hospital. 1993 samples were collected from patients clinically suspected patients of septicaemia.

Inclusion Criteria:
Any adult patients ≥18 years of age who presented to emergency outpatients, intensive care unit (ICU), ward of Medicine department and ward of surgical and gynaecology department during the study period with more than or equal to two of the systemic inflammatory response syndrome (SIRS) criteria.(temperature >38°C or <36°C,heart rate >90 beats/ minute,respiratory rate >20 breaths/minute,WBC>12,000 cells/mm3, <4000 cells/mm3 and had a suspected infection according to medical record.

Exclusion Criteria:
Patients age < 18 years &Participants who presented with non-medical illness like trauma and surgical emergencies were excluded.

Sample collection:
Blood culture from peripheral vein was collected after proper hand washing and wearing gloves with proper sterilization of skin area with iodine and isopropyl alcohol. The blood culture bottles and laboratory request forms were labelled properly with patient's name age, sex, ward, hospital id number and site from which it was taken. These bottles and duly filled laboratory request forms were sent to laboratory immediately.Samples collected at night were stored at 37 c overnight and sent to the laboratory next day morning.Specimens were taken prior to start of next scheduled antibiotic dosage.

Sample Processing: - (1ST DAY FOLLOWUP)
All samples were cultured on Blood agar, Mac conkey agar, Chocolate agar and Nutrient agar as per the protocol of sample processing & incubated at 37°C for 24 hours in aerobic environment. Also smears were prepared from all the samples and they were subjected to gram stain, in order to see whether the organism is a gram positive or gram negative.

Further processing of sample: (2ND DAY FOLLOWUP)
Culture plates were observed for growth after 24 hours of incubation. If any visible growth was observed over the plates, then Colony smear was prepared and gram staining was carried out. Depending on the colony morphology and result of gram stain, a provisional identification of the organism was done. Different biochemical test is performed according to morphology and AST is performed.

Further processing of sample: (3rd DAY FOLLOWUP)
Identification of organism was done and antibiotic susceptibility was measured by the disk diffusion method according to the Clinical and Laboratory standards institute (CLSI) guidelines.

Quality Control:
Staphylococcus aureus ATCC 25923- Oxacillin susceptible
Staphylococcus aureus ATCC 43300- Oxacillin resistant
Staphylococcus aureus ATCC BAA966- D-Zone test Negative
Staphylococcus aureus ATCC BAA966- D-Zone test Positive
Klebsiella pneumonia ATCC 700603- ESBL positive
Escherichia coli ATCC J53RI (TEM ESBL) - ESBL positive
Escherichia coli ATCC 25922- ESBL Negative.

2) CRP (C-Reactive Protein):
Rapid latex agglutination slide test for the qualitative and semiquantitative determination of inflammatory diseases. Elevated CRP levels are usually observed in a variety of infections and inflammatory conditions. The CRP level measurement is useful in differential diagnosis of septicaemia and meningitis.

3)WBC (White Blood Cell) Count:
WBC were counted in automated cell counter(HORRIBA) normal range between (4-11) $\times 10^{3}$

4)PT (Prothrombin Time)& INR (International Normalized Ratio):[71]
PT INR were measuredin fully automated coagulometer (ACLTOP CTS 300) normal range ofProthrombin Time between 11-14 seconds and International Normalized Ratio between 0.8 to 1.2.

Observations And Results:-

Present study was conducted in Microbiology department of tertiary care hospital.1993 patients having septicaemia like clinical feature (during one year) were studied to find out incidence as well as microbiological profile.

(1) INCIDENCE: Out of 1993 patients having, 695(34.87%) were culture positive.

(2) SEX WISE DISTRIBUTION

Table 1:- Sex Wise Distribution.

	Male(No. Of cases)	**%**	**Female (No. Of cases)**	**%**	**Total cases (No. Of cases)**
Suspected case	1110	55.70	883	44.30	1993
Culture positive	395	56.83	300	43.17	695

(3) Microbial profile of septicemic patients

Table 2:- Microorganisms isolated from culture.

Microorganisms	Total no (n=695)	Total %(n=695)
Klebsiella spp.	104	14.96%
Coagulase Negative staphylococcus	101	14.53%
Acinetobacter spp.	95	13.67%
Staphylococcus aureus	75	10.79%
Escherichia coli	54	7.76%
Pseudomonas spp.	47	6.78%
Enterococuus spp.	43	6.19%
Candida spp	37	5.32%
Salmonella spp.	9	1.30%
Strepto spp.	12	1.72%
Bacillus subtilis	118	16.98%

5)Antibiotic Sensitivity Pattern.

Fig 1:- Antibiotic Sensitivity Pattern of Escherichia coli and Klebsiella pneumonia.

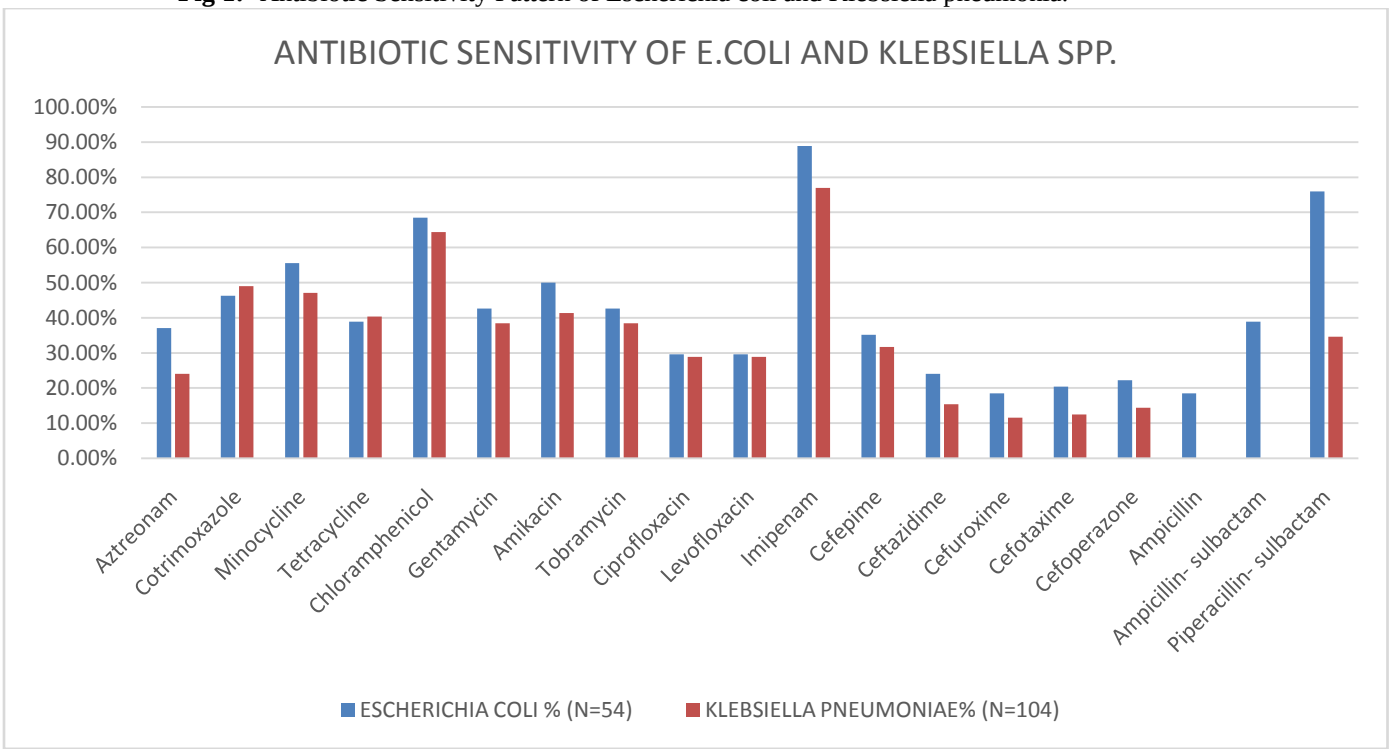


Fig 2:- Antibiotic Sensitivity Pattern of Pseudomonas Spp. and Acinetobacter Spp.

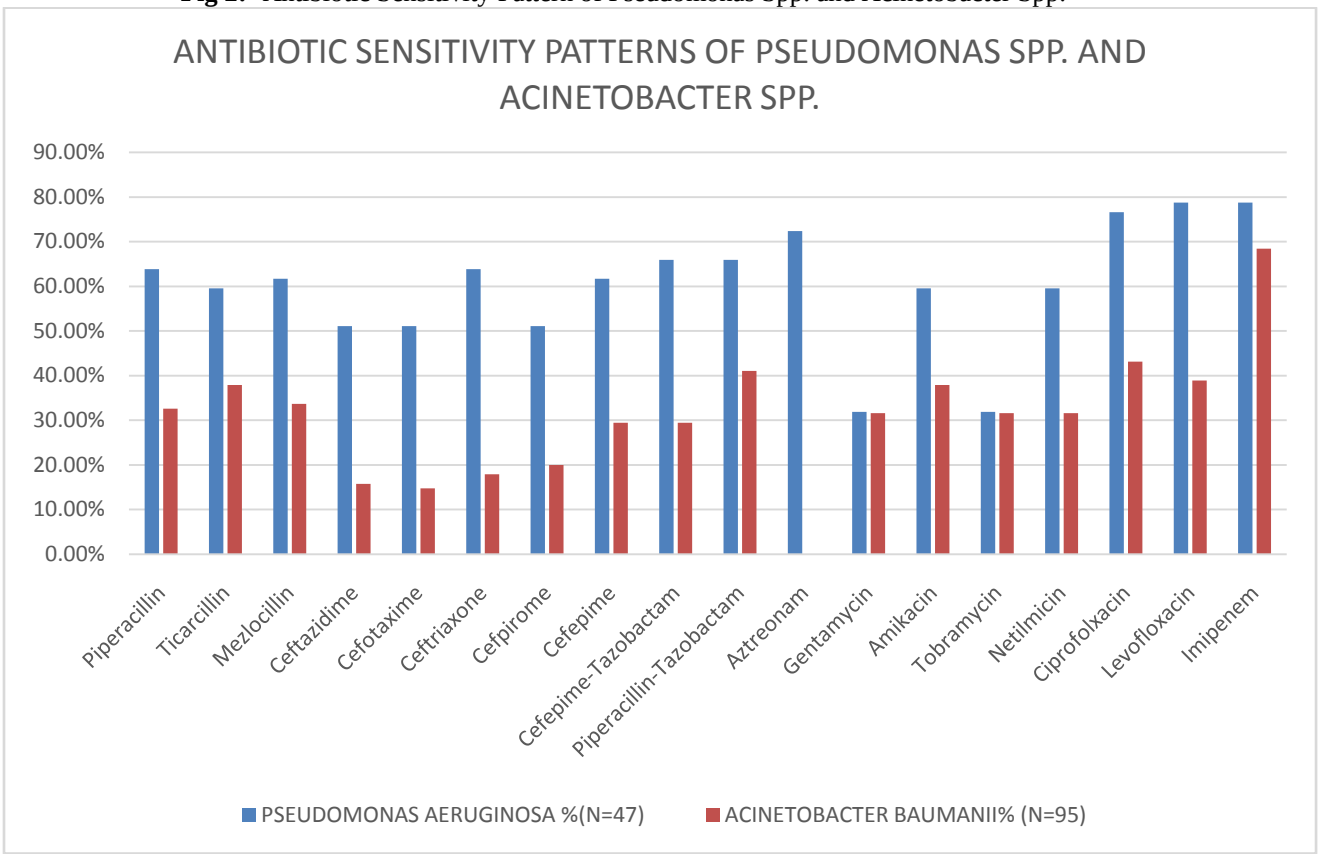


Fig 3:- Antibiotic Sensitivity Pattern of Salmonella SPP.

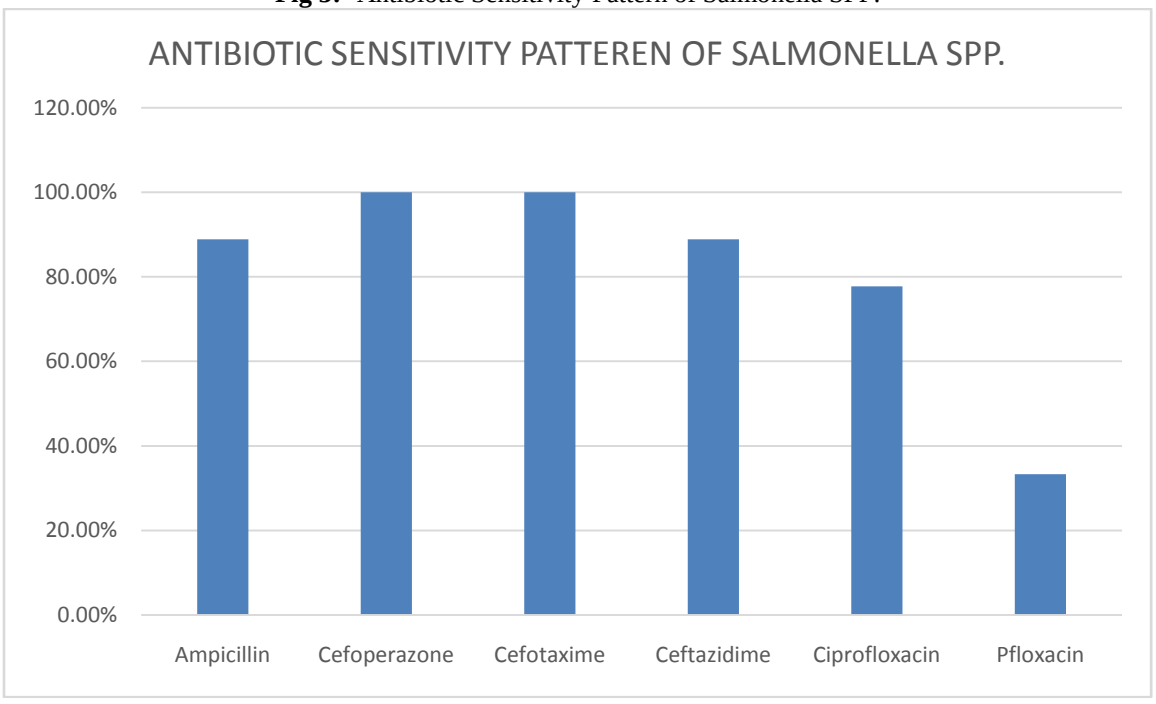


Fig 4:- Antibiotic Sensitivity pattern of Coagulase negative staphylococcus &Staphylococcus aureus.

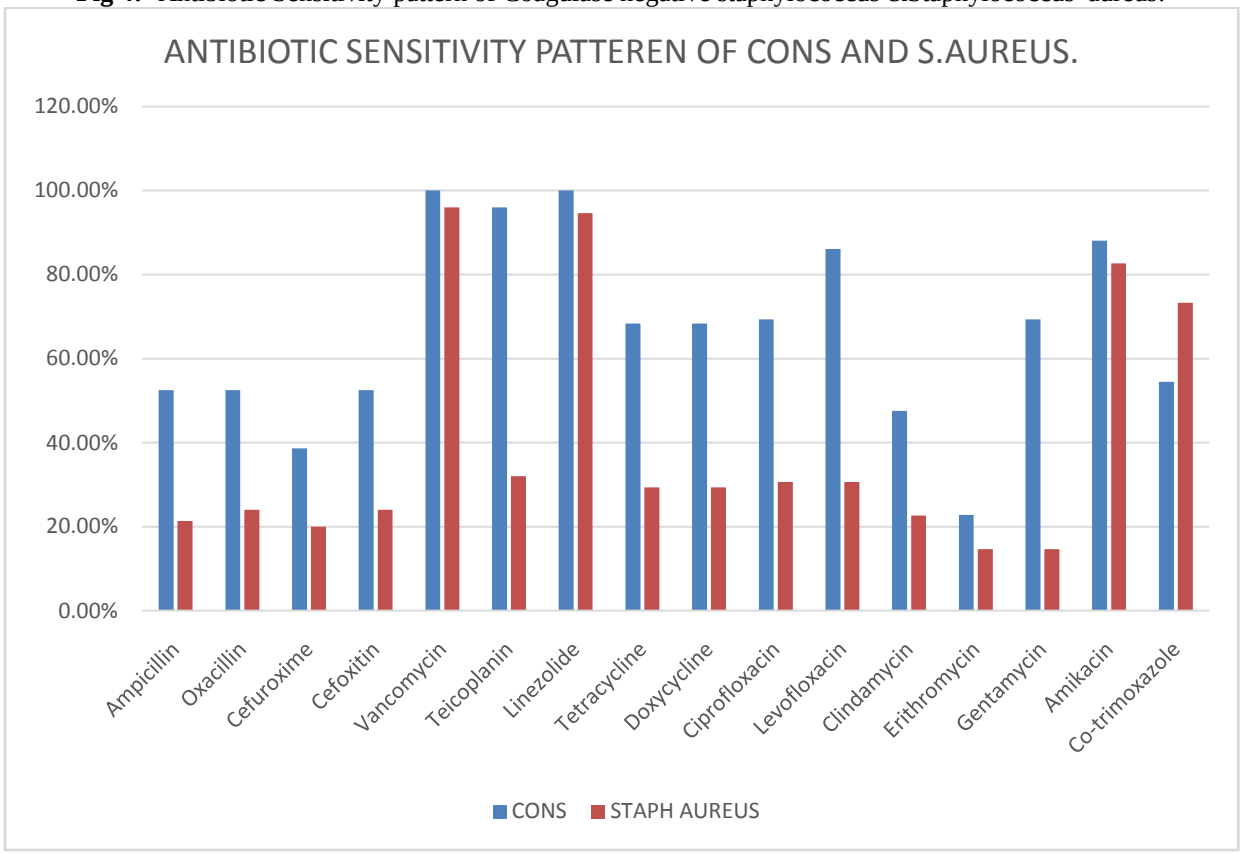


Fig 5:- Antibiotic Sensitivity Pattern of STREPTO SPP & ENTERO SPP.

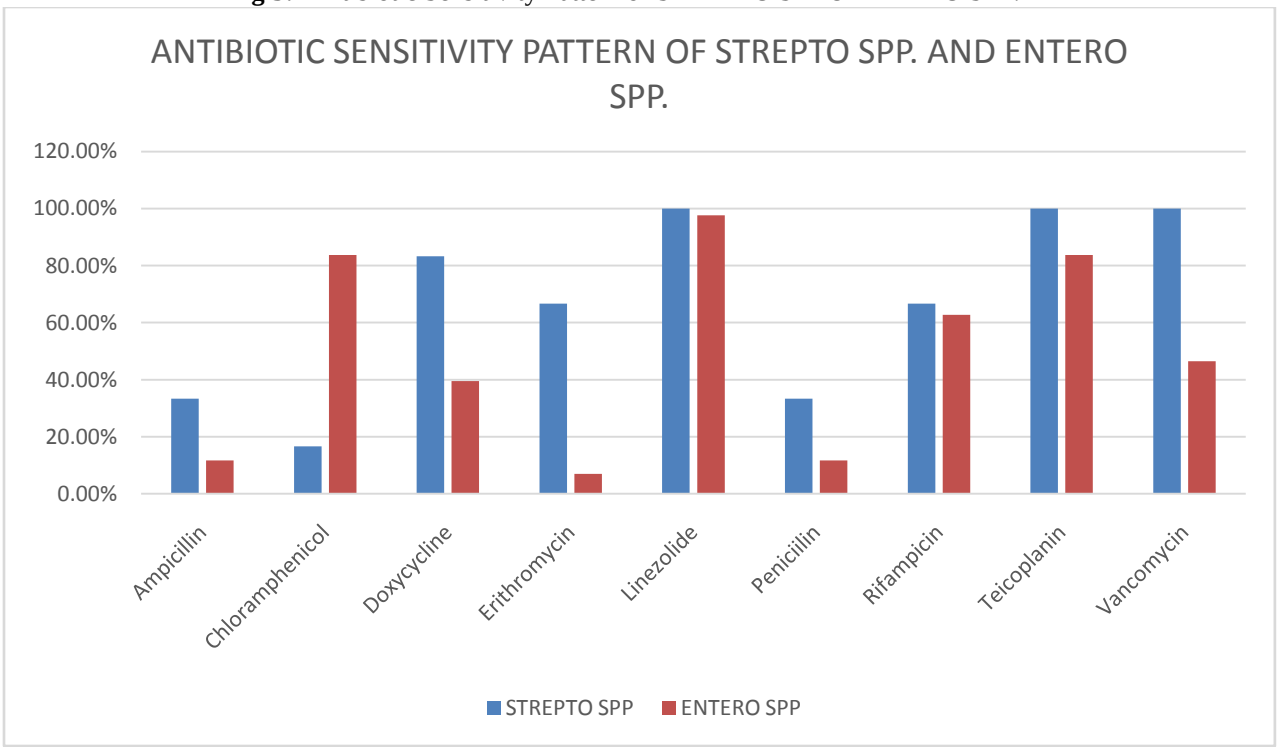
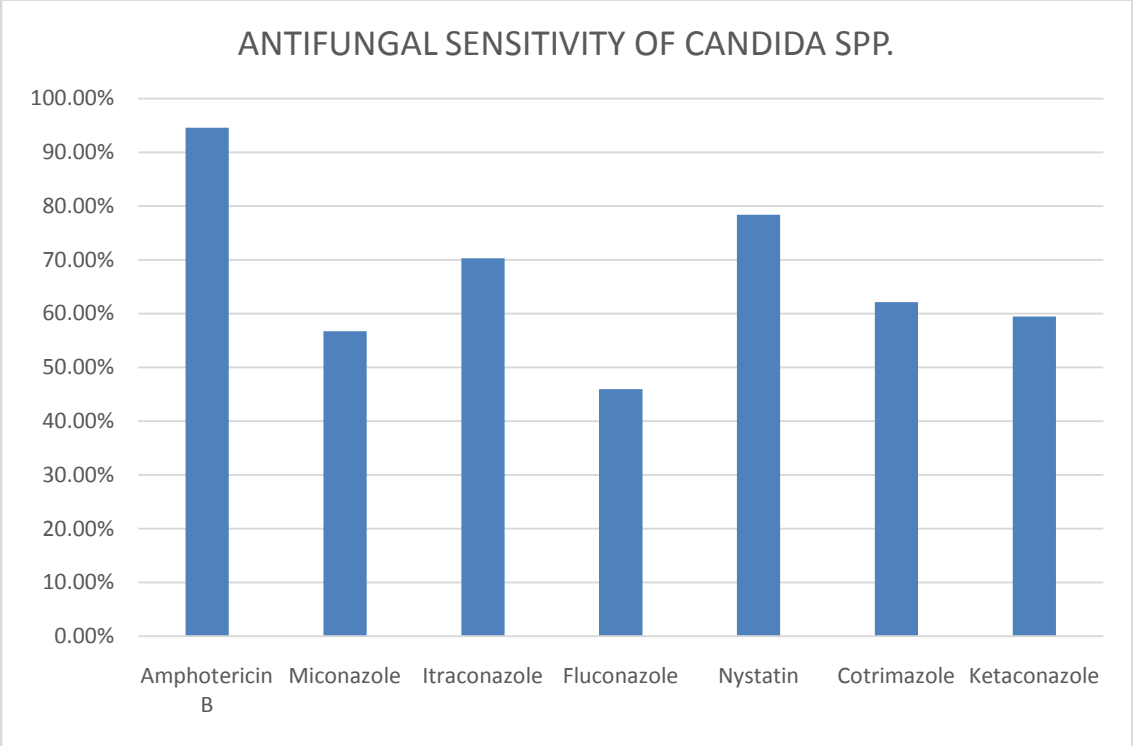


Fig 6:- Antifungal Sensitivity for Candida SPP.



6.Incidence Of Carbapenamase Resistance:

In present study 158 Gram negative bacilli were isolated, out ofthem 30 (18.98%) gram negative bacilli wereCarbapenamase producing.

7.**Incidence Of Metallo Beta Lactmase Resistance:**

In present study 142 non-fermenterwere isolated, out of them 39(27.46%)non-fermenterwere Metallobetalactmase producing.

8.Incidence Of Mrsa And Mrs.

In present study 75 S.aureuswere isolated, out of them 30(40%) S.aureus are MRSAand 101 CONS were isolated out of them 25(24.75%) were MRS.

9.Comparsion Of Result Of Blood Culture Positive And Other Factor. (Table 3)

PARAMETER	INCREASES VALUE
BT > 38 ^{o}C	41.72% (290/695)
WBC > 12,000/µl	61.15%(425/695)
CRP (0.6 mg/dl)	64.74%(450/695)
PT (>13.5)	52.80%(367/695)
INR (>1.1)	61.58%(428/695)

Discussion:-

Globally, the burden of sepsis on health care is substantial.[2] Blood culture remains the gold standard of diagnosis to isolate the etiologic agents for sepsis. The present study provides information on the distribution of bacterial isolates causing septicaemia along with their antibiotic susceptibility pattern that plays a crucial role in effective management of septicaemic cases.

The blood culture positivity rate in present study was 34.87%. Pathogen isolation rate was found to be high in medical ICUs (43.02%) followed by ward patients. [17] Single microorganism was found in all case of septicaemia similar to other studies. [17]

The isolation rate of this study is comparable with the rates reported from other developing countries as shown in table.

Table 4:- A comparative study of culture positivity in various study.

Culture positivity	Different study
20.02%	Arora and Dev et al, Amritsar, India[4]
19.3%	Ayobola et al, Malaysia[5]
34.5%	Present study.
56%	Sharma et al, Bijapur India[25]
47.5%	Roy et al, North India[24]

Bacteraemia was seen in 658 (94.67%) of patients whereas fungaemia was seen in 37(5.32%) cases. [17] Such differences in prevalence of blood culture isolates across countries could be due to the difference in blood culture system, the study design, geographical location, nature of patient population, epidemiological difference of the etiological agents, and differences in the infection control policies between nations.

Table 5:- Comparative study of sex wise distribution.

	Males	Females
Present study	56.83%	43.13%
Jyoti.P.Sonawane et al, Navi Mumbai, India[17]	64.6%	35.39%

In the present study males are affected more 56.83% than females 43.13%. This could be explained on the basis of genetic factors. The usual predominance of male suggested the possibility of a sex-linked factor in host susceptibility. A gene located on X- chromosome is involved with the function of thymus or with synthesis of immunoglobulins has been postulated. Females have double dose of genes affecting these factors and thus might possess greater resistance to infection.[17]

Table 6:- Comparative study of prevalence of different isolates.

Isolates	Gram negative	Gram positive
Present study	44.60%	33.23%
Mehta et al,northindia[20]	80.96%	18.0%
Garg et al, Varanasi [13]	66.1%	32.7%
Gupta et al, Ludhiana[12]	50.6%	49.3%

This variation of etiologic agents from country to country might be due to geographical locations, epidemiological variation/difference in etiologic agents. The other factors might also be due to nature of patient population, limited sample size and span of study time.

Among Gram negative isolates, Klebsiella spp.(14.96%)was commonest followed by Acinetobacter species (13.66%) ,E.coli (7.76%),Pseudomonas aeruginosa (6.76%) and salmonella spp.(1.29%).[17]The high occurrence of non-lactosefermenters especially Pseudomonas spp. &Acinetobacter spp., which has emerged as important nosocomial pathogens, is of concern because both of these bacteria are associated with a high degree of resistance to antibiotics and are associated with high morbidity and mortality.

In present study, S.typhi was isolated in 1.29% of total cases which was lower than Wasihun, Wlekidan 2015[3]who reported about 5.6% S.typhi. But the other studies reported an increasing incidence of Salmonella species7.70% [17]. This difference might be due to patient population in which most of the critically ill patients/septic patients were often younger with a higher prevalence of human immunodeficiency virus (HIV) infection. In the other case the variation of etiological agents of sepsis may reflect the changing demography of sepsis in developing countries, which might be related to the geographical variations.

Among Gram positive organisms in our study CONS was the commonest (14.53%) followed by S. aureus (10.79%) and Enterococcus (6.18%), Streptococcus species (1.72%).CONS predominance was observed in and in other study most common organism is S.aureus followed by CONS but the rate of isolation is similar that is 14.6%[5,] 13.86%[20], 6.9%[6] and 8.3% [13].This difference might be due to difference in blood culturing system and the content of culture media .The significance of CONS when isolated from blood culture should be considered. In most studies CONS were considered contaminant [15,26]. They have long been dismissed as culture contaminants, but now they are potentially important pathogens and their increasing incidence has been recognized. In recent years, CONS become an important nosocomial pathogen and health-care related infections partly as results of the increasing use of medical devices such as long-term indwelling catheters, vascular grafts, and prosthetic heart valves and joints [22].

In present study bacillus species isolated was 16.83%. Which was more than 3% standard published by American society of microbiologist [27]. So various strategies have been implemented to decrease blood culture contamination rate e.g. training staff with regard to aseptic collection technique.

In the present study, among the antibiotics used for susceptibility testing for Gram negative isolates, imipenem, polymyxin-B and colistin was very effective against Enterobacteriaceae, whereas for P. aeruginosa isolated were sensitive to amikacin, piperacillin and Cefepime-Tazobactam and ciprofloxacin similar to study by Mehta et al where cefoperazone+ sulbactum(82.66%), ciprofloxacin (65.17%) and amikacin (62.50%) showed higher activity.[20]Most of the Gram negative isolates were multidrug resistant. Other studies have also reported similar multidrug resistance in Gram negative isolates.[19]**.** Ampicillin and cephalosporine was the least effective drug in all the isolates. An increased ampicillin resistance has also been reported by Guha et al [14] and Khatua et al. [18]

In the present study, vancomycin was highly active drug against Gram positive organisms with 100% sensitivity. With increasing resistance to ampicillin (63.09%) Gentamicin (58.01%), Ciprofloxacin (50.01%) and Erythromycin (81.28%) clindamycin (64. 90%).Similarly in other studies vancomycin was highly effective drug against Gram positive bacteria[11,13,21]. However, this should not be expected that vancomycin activity continues for a long time as, there have been reports of vancomycin resistant S. aureus (VRSA) from studies [23,1.] Among S. aureus strains isolated from blood culture (40%) were MRSA (Cefoxitin disc used) and (24.75%) were MRS CONS were resistant. Our finding was comparable with other studies reported in India in which (42.4 %) of S. aures was MRSA [34]and in Nigeria (24.7 %) [15]

The study identified Gram negative bacteria as the predominant organisms causing blood stream infections and most of them were found to be multi-drug resistant. Carbapenems, Piperacillin Tazobactum combination and amikacin were found to be most effective for Gramnegative isolates whereas Vancomycin, Teicoplanin and Linezolid were found to be most effective for Gram-positive isolates.

The high frequency of MDR might be a reflection of inappropriate use of antimicrobials, lack of laboratory diagnostic tests, unavailability of updated guideline for the selection of antibiotics.The increase in the prevalence of Multidrug-resistant bacteria emphasize the immediate need for rational use of antibiotics, formulation of antibiotic policy, and implementation of infection control practices for the effective management and prevention of drug resistance.

Table 7:- comparative study of prevalence of Candida.

	Albicans species (%)	Most common Non-Albicans isolates.
Present study	15%	Candida paraspsilosis
Banerjee et al, (Kerala, India)[7]	27.5%	Candia glabarta
Chander J et al, (Chandigarh, India, India)[10]	29.4%	Candida tropicalis

This might be due to regional variation. Emerging nonalbicans Candida spp. showed increased virulence, increased mortality and resistance to common antifungal drugs like fluconazole and ketoconazole[3]. Hence, species identification is very important as C.kruzei and C.glabrata are inherent resistant to fluconazole and voriconazole.

In present study we observed body temperature increases in 41.72% individual WBC in 61.15% ,CRP in 64.74%, PT:52.80% and INR:61.58%,similar finding was observed in Yi-Ling Chan, et al. CRP, an acute-phase protein, has been widely used clinically for many years as a diagnostic tool for infection identification.[28] CRP may be particularly helpful when the SIRS diagnostic criteria are less reliable, for example in the presence of other disease processes that affect heart rate, WBC count, or BT. In contrast to most acute-phase proteins for which there are wide plasma level variations (which depend on synthesis, consumption, and catabolic rates), the plasma half-life of CRP is constant under almost all conditions. Its plasma level is determined exclusively by its rate of synthesis, which reflects the presence and extent of disease activity. Morever this factor (other than CRP)help in diagnosing DIC (Disseminated Intravascular Coagulation) which was morbid condition that require continuous observation. Sensitivity, specificity, positive and negative predictive values and test effectiveness all followed this same pattern of being highest for CRP followed by leukocyte count and PT (Prothrombin Time), INR(International Normalized Ratio) and body temperature being lowest.[29]

Conclusion:-

Septicaemia is an important cause of morbidity and mortality.The study conducted showed both Gram positive and Gram-negative bacteria were responsible for septicaemia. Most of the strains were multi drug resistant and leaving limited options for treatment. Thus, timely detection and knowledge of most likely pathogens causing septicaemia along with their antibiotic susceptibility pattern will help the clinicians in choosing appropriate antimicrobials for treatment which will reduce the major burden of septicaemiain critically ill patients and will also minimize the further emergence of resistance. Therefore, there should be an intensive surveillance, antibiotic policy formulations and preventive efforts for the effective management and prevention of drug resistance. In addition, the hematologic organ system is a central player in the clinical manifestation of Sepsis, identification of haematological organ dysfunction in a patient with two or more criteria and infection indicates that the diagnosis is sever sepsis. Knowledge of the hematologicmanifestation of sepsis can improve diagnosis and therapy of patients with this common and frequently fatal disorder.

Bibliography:-

1) Alam MS, Pillai PK, Kapur P, Pillai KK (2011) Resistant patterns of bacteria isolated from bloodstream infections at a university hospital in Delhi. J Pharm Bioallied Sci 3: 525-530
2) Anbumani N, Kalyani J, Mallika M. Distribution and antimicrobial susceptibility of bacteria isolated from blood cultures of hospitalized patients in a tertiary care hospital. Indian J Practising Doctor 2008; 5(2):1-7.
3) Araya G.Wasihun, Letemichael N. Wlekidan. Bacteriological profile and antimicrobial patterns of blood culture isolates among febrile patients in Mekelle Hospital, Northern Ethiopia. SpringerPlus (2015)4 : 314
4) Arora U, Devi P. Bacterial profile of blood stream infections and antibiotic resistance pattern of isolates. J K Science 2007; 9: 186-90.
5) AyobolaED, Sochi EO and Ovuokeroye O. Study of prevalence andantimicrobial susceptibility of blood culture bacterial isolates. Malaysian Journal of Microbiology2011; 7(2) : 78-82
6) BaratiM, Taher MT, Abasi R, Zadeh MM, Barati M, Shamshiri AR. Bacteriological profile and antimicrobial resistance of blood culture isolates. Iranian Journal of Clinical Infectious Diseases2009;4(2):87-95
7) Barnini Banerjee, Saldanha Dominic R.M, Srikala Baliga Clinico-microbiological study of candidemia in a tertiary care hospital of southern part of India. Volume 7 Number 1 (February 2015) 55-61
8) Cesar Henriquez-Camacho and JuanLosa.Biomarkers for Sepsis. Hindawi Publishing Corporation BioMed Research International Volume 2014, Article ID 547818, 6 pages
9) CharalamposPierrakos and Jean-Louis Vincent.Sepsis biomarkers: a review. Pierrakos and Vincent Critical Care 2010, 14:R15.
10) Chander j,singlaN,sindhuSK,gombar s. Epidemiolgy of candida blood stream infections: experience of tertiary care cenre in north india. J infect dev ctries 2013:7(9):670-675.
11) Chopra S, Mahajan G, Kaur J, Sheevani, Chopra M, et al. (2012) Septicemia due to MDR isolates in ICU: a therapeutic challenge. Asian J Pharm Hea Sci 2: 476-480.
12) Deepinderchhina and Veenugupta. bacteriological profile and antimicrobial susceptibility pattern of blood isolates from a tertiary care hospital in north india.IJPRBS, 2013; Volume 2(2): 24-35.
13) Garg A, Anupurba S, Garg J, Goyal RK, Sen MR. Bacteriological profile and antimicrobial resistance of blood culture isolates from a university hospital. JIACM 2007; 8(2):139-43.
14) Guha DK, Jaspal D, Dass KMS, Guha RA, Khatri RL, Srikumar R. Outcome of neonatal septicemia. A clinical bacteriological profile. Indian J Pediatr 1978; 15: 423-27.
15) Jacob ST, Moore CC, Banura P, Pinkerton R, Meya D, et al. (2009) Severe sepsis in two Ugandan hospitals: a prospective observational study of management and outcomes in a predominantly HIV-1 infected population. PLoS One 4: e7782.
16) Jerome o. klein& Michael marcy, bacterial infection chep:17 in infectious diseases by Remington &klein. Philadelphia: 1976:747-891
17) Jyoti P.Sonawane1Bacteriological Profile and Antimicrobial Susceptibility of Blood Culture Isolates from Tertiary Care Hospital, Navi MumbaiJyoti P.Sonawane et al JMSCR Volume 04 Issue 10 October
18) Khatua SP, Dass AK, Chaterjee BD, Khatuas, Ghosh B, Saha A. Neonatal septicaemia. Indian J Pediatr 1986; 53 :50914
19) kumars,Rizvi m,,vksharma,changing face of septicaemia and increasing drug resistance in blood isolates indian journal of pathology and microbilolgy 2004:47:441-6
20) Marie cheesbrough,District Laboratory Practice in topical countries,2nd Ed.2005,151-162.

21) Mehta M, Dutta P, Gupta V (2005) Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in north India. Jpn J Infect Dis 58: 174-176.
22) Piette A, Verschraegen G (2009) Role of coagulase-negative staphylococci in human disease. Vet Microbiol 134: 45-54
23) Pradipta IS, Sandiana AT, Halimah E, Diantini A, Lestari K, et al. (2013) Microbial and Resistance Profile in Isolate from Adult Sepsis Patients: An Observational Study at an Indonesian Private Hospital during 2009-2012. Intern Journ of PharmacScienRevi and Resear 19: 24-29.
24) Roy I, Jain A, Kumar M , Agarwal SK. Bacteriology of neonatal septicemia in a tertiary care hospital of northern India. IJMM 2002; 20:156-9.
25) Sharma PP, Halder D, Dutta AK. Bacteriological profile of neonatal septicemia. Indian Paediatr 1987; 24:10117
26) Ssekitoleko R, Pinkerton R, Muhindo R, Bhagani S, Moore CC (2011) Aggregate evaluable organ dysfunction predicts in-hospital mortality from sepsis in Uganda. Am J Trop Med Hyg 85: 697-702.
27) Washington II JA, Cumitech IA. Blood cultures II. In: Reller LB, Murray PR, MacLowry JO, editors. American Society for Microbiology; 1982.
28) Who guideline on drawing blood: best practice in phlebotomy.
29) Yi-Ling Chan,Hao-Chin Liao, Pei-Kuei Tsay1,; Shy-Shin Chang, Jih-Chang ChenShiumn-Jen Liaw, C-Reactive Protein as an Indicator of Bacterial Infection of Adult Patients in the Emergency Department .Chang Gung Med J Vol. 25 No. 7 July 2002.