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BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BLOOD CULTURE ISOLATES AMONG ICU PATIENTS IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Background: Bloodstream infections (BSI) are associated with significant morbidity and mortality. The condition can be life threatening in critically ill patients especially in intensive care units (ICUs) of the hospitals. The case fatality rate associated with BSIs is between 35-50%. Severe sepsis was more common in Indian ICUs with higher mortality rate as compared to western literature. This study was conducted to identify the bacteriological profile and their antibiotic susceptibility patterns from blood culture in a tertiary care hospital to guide clinicians to initiate empiric antibiotic therapy and to formulate antibiotic policy. **Material and methods:** A prospective experimental study was carried out at the Department of Microbiology, JLNMCH, Bhagalpur, Bihar on ICU, TICU, NICU, PICU patients over a period of 1 year from September 2020 to August 2021. **Results:** Age distribution showed that majority of the cases were between the ages 41-60 yrs followed by 21-40 yrs. It was seen that out of 370 patients, 226 (61.08%) were male and 144 (38.92%) were female. Gram-negative bacilli (isolates) were predominantly isolated from suspected septicemia patients as compared to gram-positive cocci (isolates). The antimicrobial sensitivity pattern of the organisms to different antimicrobials varied depending on the isolate. Overall, gram-negative organisms were found to show maximum susceptibility to imipenem (100%) and amikacin (90.90%). **Conclusion:** Presence of Methicilin resistant Staphylococcus aureus (MRSA) producing gram negative bacilli in IMCU setting leading to grave prognosis. So irrational use of antibiotics leads to emergence of resistant organisms.

KEYWORDS

Bloodstream infections, ICU, Gram positive, Gram negative, Antibiotics

INTRODUCTION:

The development of 'intensive care' as a medical discipline dates back to the mid of the 20th century. Sepsis remains the most important confounding factor in the morbidity and mortality of ICU patients. For combating sepsis appropriate antibiotic therapy can be initiated either in culture guided way or empirically [1]. Max Harry Weil is considered widely as the 'father of modern intensive care' – he established a four-bed 'shock ward' at Los Angeles County/University of Southern California Medical Center in the USA during the early 1960s [2]. Bloodstream infections (BSI) are associated with significant morbidity and mortality. The condition can be life threatening in critically ill patients especially in intensive care units (ICUs) of the hospitals. The case fatality rate associated with BSIs is between 35-50%. Bacteremia is the presence of viable bacteria in the circulating blood. The detection of bacteria in blood is always abnormal [3-5]. Detection of bacteremia by rapid and reliable method is by culturing blood. The blood should be collected aseptically before the administration of antibiotics [6]. Septicemia is a clinical term used to describe severe life-threatening bacteremia in which multiplying bacteria release toxins into the blood stream and trigger the production of cytokines, causing fever, chills, toxicity, tissue anoxia, reduced blood pressure and collapse. Septic shock is usually a complication of septicemia with Gram-negative bacilli, and less frequently, Gram-positive organisms and prompt treatment is essential [7]. In a multicentre study conducted in four intensive therapy units in India the incidence of severe sepsis was found to be 16.45% of all admissions, with ICU mortality were 12.08% of all admissions and that of severe sepsis was 59.26% [8-9]. For selecting empiric therapy, many factors should be considered including the kind of pathogen that is most probably the cause of the infection according to age, gender, risk factors and antibiotic susceptibility pattern. Thus, rapid detection and identification of clinically relevant microorganisms in blood cultures is very essential and determination of antimicrobial susceptibility pattern for rapid administration of antimicrobial therapy has been shown to reduce the morbidity and mortality associated with blood stream infections. Therefore, blood culture remains the mainstay of investigation of definitive diagnosis and management of blood stream infection in infants and children, despite recent advances in the molecular diagnosis of bacterial sepsis. Antibiotic sensitivity pattern to common pathogen has been changing from day to day and it is important to have latest information as this is essential in guiding local empirical choice of antibiotics. Hence, the result of the present study provides knowledge on the etiologic agents of pediatric septicemia and its rational use of antibiotics [10-12].

Common organism isolated from blood culture are Staphylococcus aureus, Escherichia coli, Coagulase Negative Staphylococci (CoNS), Enterococcus species, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Proteus species and β-hemolytic streptococci. CoNS have long been considered mainly as non pathogenic blood culture contaminant, however as a result of combination of increased use of intravascular devices and an increase in the number of hospitalized immune compromised patients, CoNS has emerged as a major cause of nosocomial blood stream infection [13]. The emergence of resistant bacteria makes it a requisite to know the prevailing antibiotic susceptibility pattern of the pathogens causing bloodstream infection. There is increase in antimicrobial resistance rates and a shift in organism distribution among important blood stream pathogen both in hospital and community settings. In hospital setting there has been a shift from a predominance of gram negative organism in the late 70's to the present day primacy of gram positive organism [13].

This study was conducted to identify the bacteriological profile and their antibiotic susceptibility patterns from blood culture in a tertiary care hospital to guide clinicians to initiate empiric antibiotic therapy and to formulate antibiotic policy.

OBJECTIVES:

(a) to know the occurrence of blood stream infection, (b) to isolate and identify the bacterial agent from the blood sample & (c) to determine the antibiotic susceptibility patterns of the isolates.

Material and methods:

Study Period:

This is a cross sectional study undertaken over a period of 1 year from September 2020 to August 2021.

Study Place: The study was carried out at the Department of Microbiology, JLNMCH, Bhagalpur, Bihar.

Study design: A prospective experimental study.

Study Centre: ICU, TICU, NICU, PICU of JLNMCH, Bhagalpur, Bihar.

Study population source: Patients admitted in Intensive Care Unit (MICU, TICU, NICU and PICU) fulfilling the inclusion criteria was enrolled in study.

Study Sample size (No of patients/subjects for study): P = % of growth in blood samples received from MICU/ TICU/ NICU/ PICU: = 31.1 %, L= allowable error = 5%, at 95% confidence interval, sample size

$$N = \frac{Z^2 1\text{-}\alpha/2 \times p \times q}{L^2} = \frac{(1.96)^2 \text{ X } 31.1 \text{ X } 68.9}{5^2} = 329.1$$

Sample size for the study taken 370.

INCLUSION CRITERIA:

Patients admitted in Intensive Care Unit (ICU, NICU, PICU, TICU) of a tertiary care hospital, Blood samples of clinically suspected Septicaemia.

EXCLUSION CRITERIA:

Samples other than above fore said or Patient admitted with Snake Bite, Poisoning cases, Suicidal / Homicidal cases.

Data entry and analysis:

All the data was tabulated, and then analyzed with appropriate statistical tools "MedCalc". "Chi – square Test" and "Fisher's exact test" was used for statistical significance test. Finally, the calculated value was compared with the tabulated value at particular degree of freedom and finds the level of significance. A "p-value" will be considered to be non-significant if > 0.05 and significant if <0.05.

Blood Sample Collection:

Blood samples was collected from patients with suspected BSI's (Bloodstream infection's), preferably before administration of antimicrobial therapy. 5 ml blood sample from each adult, 2 - 5ml from each child and 0.5 - 2ml from infants and neonates was collected aseptically using 70% alcohol and 2% tincture of iodine from a peripheral vein under strict aseptic precautions. The samples was collected in blood culture bottles containing Brain Heart infusion broth making a dilution of 1 in 10 to nullify the natural bacteriostatic/ bactericidal activity of blood. 50 ml of BHI in adults and 5 – 10 ml of BHI in pediatric cases was used and immediately the samples will be transported to the laboratory for further processing.

Culture:

The inoculated blood culture bottles will be incubated overnight at 37ºC aerobically and the first subculture will be done after 18-24 hours of incubation, the second on third day and final subculture on seventh day. Subcultures will be done on Blood agar, MacConkey agar and selective media if required as per the clinical diagnosis. The specific identification of bacterial pathogen will be done based on cultural colony characteristics, Gram's staining and biochemical test as per standard bacteriological techniques [13].

Antibiotic Sensitivity Test:

The antibiotic susceptibility testing of the isolates was done by Kirby-Bauer disk diffusion method on Mueller Hinton agar. Isolates was grown in peptone water by incubating at 37º C and turbidity will be matched with 0.5 McFarland standards. Then lawn culture was done on Mueller Hinton agar plate and commercial antibiotic disks was placed. The plates was incubated at 37º C overnight and on the next day the zones of inhibition was measured and susceptibility / resistance was interpreted according to CLSI guidelines 2017. A provisional report was issued after every subculture, and if after 7 days no growth is obtained the sample was reported as negative.

Parameters:

Samples (Blood collected aseptically from ICU, TICU , NICU , PICU)→Overnight Incubation for 18 – 24 hours→ Inoculation on Blood agar and Mac-Conkey agar→ Culture Colony characteristic→ Gram staining→ Standard biochemical tests→ Isolate identified→ Antibiotic susceptibility pattern assessment.

RESULTS:

Blood culture samples were taken from 370 patients from MICU, TICU, NICU and PICU of a tertiary care centre, suspecting of having septicemia in present study.

Table 1: Age distribution of patients studied

Age in years	No. of patients	Percentage (%)
<20	07	01.90
21-40	110	29.73
41-60	139	37.57
61-80	89	24.05
81-100	25	06.75
Total	370	100
Sex	No. of patients	Percentage (%)
Male	226	61.08
Female	144	38.92
Total	370	100

Age distribution depicted in Table no. 1 showed that majority of the cases were between the ages 41-60 yrs followed by 21-40 yrs. It was seen that out of 370 patients, 226 (61.08%) were male and 144 (38.92%) were female.

Table No. 2: Showing distribution of blood culture positivity

Age in years	Blood Culture Positive	Blood Culture Negative
<20	04 (57.14%)	03 (42.86%)
21-40	48 (43.63%)	62 (56.37%)
41-60	65 (46.76%)	74 (53.24%)
61-80	41 (46.06%)	48 (53.94%)
81-100	10 (40.00%)	15 (60.00%)
Total	168 (45.40%)	202 (54.60%)

Table No. 3: Bacterial isolates (gram positive and gram-negative isolates) in suspected septicemia patients

Isolates	Total isolates (n = 168)	Percentage (%)
Gram positive cocci	64	38.05%
Gram negative bacilli	104	61.95%

Gram-negative bacilli (isolates) were predominantly isolated from suspected septicemia patients as compared to gram-positive cocci (isolates).

Table No. 4: Frequency of bacterial isolates causing septicemia

Sr. No.	Organism	Frequency	%
A	**Gram Positive Isolates**	64	38.05
1	Staphylococcus. aureus	57	33.92
2	Coagulase Negative Staphylococcus aureus	05	02.97
3	Enterococcus species	02	01.19
B	**Gram Negative Isolates**	104	61.95
1	Klebsiella pneumoniae	44	26.19
2	Escherichia coli	38	22.61
3	Acinetobacter baumannii	10	05.95
4	Pseudomonas aeruginosa	07	04.17
5	Citrobacter species	04	02.38
6	Salmonella typhi	01	00.59
	Total	168	100.00

Table 4-showed 168 organisms were isolated. Klebsiella pneumoniae (26.19%) was the most common isolate amongst gram-negative isolates followed by Staphylococcus aureus (33.92%) among gram-positive isolates.

Table No. 5: Antibiotic Susceptibility Pattern of Gram Negative isolates

S/N	Drugs	**K.pneumoniae (n=44) S* (%)**		**E.coli *(n=(38)* S* (%)**		**A. baumannii (n=10) S* (%)**		**P.aeruginosa (n=07) S* (%)**		**Citrobacter spp (n=04) S* (%)**		**Salmonella typhi (n=1) S* (%)**	
1	Ampicillin	00	00	00	00	00	00	00	00	00	00	00	00
2	Gentamycin	35	79.54	15	75	04	40	05	71.4	01	33	01	100
3	Amikacin	40	90.90	16	80	06	60	06	85.7	02	66.6	01	100
4	Ciprofloxacin	32	72.72	08	40	03	30	02	28.5	01	33	00	00
5	Ceftazidime –Clavulanic acid	15	34.09	03	15	01	10	04	57	01	33	00	00
6	Piperacillintazobactum	30	68.18	01	5	07	70	04	57	01	33	00	00
7	Cefotaxime	10	22.72	00	00	03	30	00	00	01	33	00	00
8	Ceftazidime	10	22.72	00	00	03	30	03	42.8	01	33	01	100
9	Tetracycline	05	11.36	01	5	02	20	01	14	01	33	01	100
10	Imipenem	44	100	19	95	07	70	04	57	03	100	01	100

***S = Sensitive**

The antimicrobial sensitivity pattern of the organisms to different antimicrobials varied depending on the isolate. Overall, gram-negative organisms were found to show maximum susceptibility to imipenem (100%) and amikacin (90.90%).

Table No. 6: Antibiotic Susceptibility Pattern of Gram Positive isolates

Sr. No.	Drugs	S. aureus (n=57) Sensitive	(%)	CONS (n=07) Sensitive	(%)
1	Penicillin	08	14.03	00	00
2	Ampicillin	08	14.03	00	00
3	Erythromycin	30	52.63	00	00
4	Clindamycin	28	49.12	00	00
5	Cefoxitin	18	31.57	01	14.28
6	Linezolid	46	80.70	05	71.42
7	Vancomycin	48	84.21	06	85.71
8	Gentamycin	30	52.63	01	14.28
9	Amikacin	36	63.15	04	57.14
10	Ciprofloxacin	36	63.15	03	42.85

Table No. 7: Staphylococcus aureus showing Methicillin Resistance

Isolates	Methicillin Sensitive Staphylococcus aureus	Methicillin Resistant Staphylococcus aureus	Total
Staphylococcus aureus	42 (73.68%)	15 (26.31%)	57 (100%)

It was seen in Table 7 that Methicillin Sensitive Staphylococcus aureus was seen in 42 (73.68%) as compared to Methicillin Resistant Staphylococcus aureus which was found to 21 (26.31%) where (n=57).

Table No. 8: Gram-negative isolates showing MBL Production (n = 17)

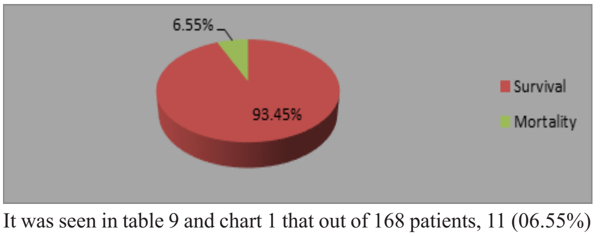
Isolates	MBL Producers	Non MBL Producers	Total
Pseudomonas aeruginosa	03	04	07
Acinetobacter baumannii	03	07	10
Total with Percentage	6 (35.29%)	11 (64.71%)	17 (100%)

It was seen in table 8 that MBL production was seen in 6 (35.29%) as compared to Non MBL producers which was found to be 11 (64.71%) where (n=17).

Table No. 9: Blood culture positivity and mortality

Outcome	Number Of Blood culture Positive Patients	Percentage (%)
Survival	157	93.45%
Mortality	11	06.55%
Total	168	100%

Chart No. 1: Outcome of patients with positive blood culture.



It was seen in table 9 and chart 1 that out of 168 patients, 11 (06.55%) did not survived but 157 (93.45%) patients had survived.

Table No. 10: Correlation between bacterial isolates and mortality.

Bacterial isolates	Mortality	Percentage (%)
Klebsiella pneumoniae	05	45.45%
Staphylococcus aureus (MRSA)	03	27.27%
Pseudomonas aeruginosa	02	18.18%
Citrobacter species	01	09.10%
Total	11	100%

Chart No. 2: Bacterial isolates associated with Mortality.

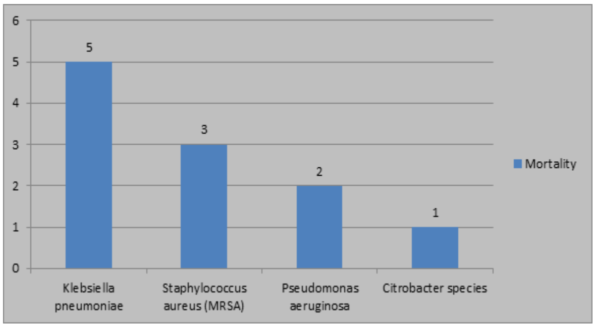


Table 10 and chart 2 showed Klebsiella pneumoniae was the predominant organism isolated from 05 (45.45%) cases followed by Staphylococcus aureus 03 cases (27.27%) which were responsible for mortality among patients.

DISCUSSION:

Similar to our study Syal K et al [14], stated Majority of the patients (41.33%) belonged to 31-50 year age group but in contrast to our study NICU& PICU contains 212 cases but MICU & TICU contains 88 cases in which most prevalent age group was 20-60 yrs 68 cases. Similarly Gupta S et al [15], Incidence of growth positive cases was found to be more among male patients 64 (65.3%) than in female patients 34 (34.6%) which was comparable to present study. With respect to blood culture positivity, Gupta S et al [15], Total 500 samples were taken in this study out of which study showed that there were 19.6% (98/500) blood culture positive cases. Also Subha. M et al [16], Out of 1101 blood cultures, 151 (13.7%) gave positive result.

Gupta S et al [15]	19.60%
Ebrahim-Saraie HS et al [17]	15.10%
Dash M et al [18]	17.20%
Subha. M et al [16]	13.70%
Sangwan J et al [19]	57.60%
Present study	45.40%

Organisms isolated by Blood Culture in Septicaemic Patients n=168 by present study compared by Nazir A et al [20], The most frequently identified Gram-positive bacteria were coagulase-negative staphylococci (CoNS) 208, (67.9%), Staphylococcus aureus 75 (24.5%) {out of which 62 (20.2%) were methicillin resistant and 13 (4.2%) were methicillin sensitive} and Enterococci species 23 (7.5%). The Gram-negative bacteria that were identified were Acinetobacter species 89 (63.1%), Escherichia coli 19 (13.4%), Klebsiella pneumoniae 15 (10.6%) Salmonella typhi 9 (6.3%), Pseudomonas aeruginosa 9 (6.3%). Also Jhajhria A et al [21], Out of 75 positive cultures, 39 (52%) were gram-positive, 32 (42.7%) were gram-negative and 2 (5.3%) were Candida spp.

Very similar to our study, Nazir A et al [20], The results of antibiotic drug sensitivity of gram-positive bacteria showed that both CoNS and Staphylococcus aureus were highly sensitive to vancomycin (100%) and linezolid (100%). Also Sangwan J et al [19], The most predominant bacteria isolated from all the ICUs was Staphylococcus aureus followed by CONS, Enterobacteriaceae and Pseudomonas. Non albicans Candida and Candida albicans were mainly isolated only from patients of NICU and PICU.

Nazir A et al [20], Antimicrobial resistance levels for the gram-negative organisms most commonly causing blood stream infections were relatively high. Among Gram negative bacterial isolates, Acinetobacter species, Escherichia coli and Klebsiella pneumoniae were dominant species in descending order. Third generation cephalosporins showed a very weak activity against them (<30%) and carbapenem resistance was detected in 83% isolates of Acinetobacter spp. 56% cases of Escherichia coli and 65% of Klebsiella pneumonia. were multidrug resistant (MDR). For highly resistant bacteria, especially Klebsiella species and Acinetobacter species, even ß-lactam +ß-lactam inhibitor combinations and carbapenems were not effective. Amikacin was slightly better. However, all of these strains were sensitive to Colistin. The most frequently identified Gram-positive bacteria were coagulase-negative *staphylococci* (CoNS) 208 (67.9%), *Staphylococcus aureus* 75 (24.5%) {out of which 62 (20.2%) were methicillin resistant and 13 (4.2%) were methicillin sensitive} and *Enterococci* species 23 (7.5%) in the same study.

CONCLUSION:

The most common source of infections are invasive procedures like Central Venus Catheterization (CVC) in critical care setting followed by genitourinary sources in Hospital acquired blood stream infections. Presence of Methicilin resistant Staphylococcus aureus (MRSA) producing gram negative bacilli in IMCU setting leading to grave prognosis. So irrational use of antibiotics leads to emergence of resistant organisms. To reduce the morbidity and mortality in IMCU setting following interventions are followed.

1. Adherence to guidelines on insertion and care of Central Venous Catheterization.
2. Use of antimicrobial or antiseptic impregnated Catheters
3. Improved hand hygiene.

REFERENCES:

1. Kelly FE, Fong K, Hirsch N, Nolan JP. Intensive care medicine is 60 years old: the history and future of the intensive care unit. Clinical medicine. 2014 Aug 1;14(4):376-9.
2. Tang W, Sun S. Max Harry (Hal) Weil–a leader, mentor, friend, and wonderful colleague. Resuscitation. 2011 Dec 1;82(12):1481-2.
3. Leibovici L, Samra Z, Konigsberger H, Drucker M, Ashkenazi S, Pitlik SD. Long-term survival following bacteremia or fungemia. Jama. 1995 Sep 13;274(10):807-12.
4. Prakash Simkhada SR, Lamichhane S, Subedi S, Shrestha UT. Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture Isolates from Patients Visiting Janamaitri Hospital, Balaju, Kathmandu, Nepal. Global Journal of Medical Research. 2016 May 10.
5. Mahon CR. Lehman DC &Manuselis G. Textbook of Diagnostic Microbiology.
6. William JH and Max S. Bacteremia, septicemia and endocarditis. In: William JH, Max Sussman. Topley and Wilsons Microbiology and Microbial Infections, Vol 3. 11th eds., London; 1998, pp. 178-187.
7. Cheesbrough M. District laboratory practice in tropical countries. Cambridge university press; 2006 Mar 2.
8. Todi S, Chatterjee S, Sahu S, Bhattacharyya M. Epidemiology of severe sepsis in India: an update. Critical Care. 2010 Mar;14(1):P382.
9. Buttery JP. Blood cultures in newborns and children: optimising an everyday test. Archives of Disease in Childhood-Fetal and Neonatal Edition. 2002 Jul 1;87(1):F25-8.
10. Murty DS, Gyaneshwari M. Blood cultures in paediatric patients: A study of clinical impact. Indian journal of medical microbiology. 2007 Jul 1;25(3):220.
11. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. Journal of clinical microbiology. 2001 Apr 1;39(4):1553-8.
12. Mehta M, Dutta P, Gupta V. Antimicrobial susceptibility pattern of blood isolates from a teaching hospital in North India. Japanese journal of infectious diseases. 2005 Jun 1;58(3):174.
13. Jhajhria A, Yadav AK, Parihar G, Gupta PS. Bacteriological profile and antimicrobial susceptibility of blood culture in a tertiary care hospital Ajmer. infection.;6:8.
14. Syal K, Singh D, Thakur A, Goyal A. Micro-Organism Profile and Antibiotic Susceptibility Patterns in General ICU of Tertiary Care Hospital Situated in Hills, Journal of Intensive and Critical Care ISSN 2471-8505.
15. Gupta S, Jain D, Durlabhji P. A study of blood culture isolates and their antimicrobial susceptibility pattern in a tertiary care hospital of jaipur, Rajasthan. International Journal of Current Research and Review. 2013 Jun 1;5(11):49.
16. Subha. M, Vijayalakshmi A, Rajeswari J. Bacteriological profile and antimicrobial susceptibility pattern of blood culture isolates from a tertiary care teaching hospital, south india, Volume-7 | Issue-2 | February-2018 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 93.98.
17. Ebrahim-Saraie HS, Motamedifar M, Mansury D, Halaji M, Hashemizadeh Z, Ali-Mohammadi Y. Bacterial etiology and antibacterial susceptibility patterns of pediatric bloodstream infections: a two year study from Nemazee Hospital, Shiraz, Iran. Journal of Comprehensive Pediatrics. 2016;7(1).
18. Dash M, Panda RK, Mohapatra D, Paty BP, Sarangi G, Chayani N. Bacteriological profile and antimicrobial resistance patterns of bloodstream infections in a tertiary care hospital, Eastern India. International Journal of Health & Allied Sciences. 2016 Oct 1;5(4):210.
19. Sangwan J, Mane P, Vohra P, Lathwal S, Malik AK. Bacteriologic Profile and Antimicrobial Resistance of Blood Culture Isolates of Septicemic Patients from Various Intensive Care Units in a Teaching Tertiary Care Institute of Haryana, India, Int.J.Curr.Microbiol.App.Sci (2016) 5(7): 599-608.
20. Asifa Nazir, Ifshana Sana, Bushra Yousuf Peerzada, Study of prevalence and antimicrobial susceptibility pattern of blood culture isolates from a tertiary care hospital of North India 2018 ;6(12):4046-4052.
21. Jhajhria A, Yadav AK, Parihar G, Gupta PS. Bacteriological profile and antimicrobial susceptibility of blood culture in a tertiary care hospital Ajmer. infection.;6:8.