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Research Article

Drug Resistance Pattern of Blood Culture Isolates in a Tertiary Care Hospital

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Abstract

Aim of the study: This study was carried out to find out In vitro susceptibility & resistance pattern of isolates were carried out by Kirby-Bauer disc diffusion method.
Material and Methods: - The present study was conducted in Department of Microbiology in Era's Lucknow Medical College and hospital. During the period of six months, 638 blood samples from septicemia suspected patients were received and processed routinely. Out of 638 blood culture, 90 (14.10%) were found positive for culture growth in which 38% were Gram's positive and 62% were Gram's negative. Out of 90 positive culture overall proportion of female (47) were found to be significantly higher them male (43).
Results: Among 90 total pathogenic cases, 47(52.22%) were females and 43 (47.78%) were males. Oyekale T Oluwalana *et al,* (2022) [26] also showed similar data i.e, 57% of males and 42.4% female. In our study, 37 samples belong to age group from newborn to 25 years age (41.11%), 24 belongs to group 26 to 50 years age (26.67%), while 29 from age group 51 to 90 years (32.22%) were suspected of septicaemia. *Staphylococcus aureus*, *coagulase-negative staphylococcus (CoNS*) and *Enterococcus species* showed 100 % sensitivity to Vancomycin, Teicoplanin & Linezolid and *Enterococcus species* showed 90% sensitivity to Gentamicin, 87% sensitivity to Linezolid, moderately sensitivity 67% to Gentamycin and 80% to Teicoplanin and low sensitivity showed by Ciprofloxacin, Amoxiclav, 100% Resistance showed to Cefoxitin in *staphylococcus aureus* and *coagulase-negative staphylococcus, enterococcus species.* showed high sensitivity to Vancomycin (98%), Teicoplanin (98%), Chloramphenicol (89%) and (100%) sensitivity to linezolid. High level gentamycin resistance was 44%.
Conclusion: Our study observed 100 % sensitivity to Tigecycline, Colistin and Piperacillin-Tazobactam in *Escherichia coli,* netilmicin was moderately sensitive to *klebsiella* and *Escherichia coli.* Higher resistance observed by Cefepime, Cefoperazone, Imipenem, Ceftriaxone.

Keywords: C*oagulase- Negative Staphylococcus (CoNS*), Antimicrobial sensitivity test, Bacteremia, Septicemia

1. Introduction

Numerous bacteria have been associated with causation of BSIs including Gram-negative bacteria: *Escherichia coli, Pseudomonas species, Klebsiella species, Serratia species, Salmonella species*. and *Enterobacter species* and *Gram-positive bacteria Staphylococcus species, Streptococcus species* and *Enterococcus species* [1,2].

Recent research, however, suggested an increase in BSIs brought on by multidrug-resistant bacteria, including members of the Enterobacteriaceae family such as Klebsiella species, Pseudomonas species, Acinetobacter species and Citrobacter species, the majority of which produce extended spectrum beta-lactamases (ESBLs), and some Gram-positive bacteria [3,4].

Coagulase-negative Staphylococcus (CoNS) is frequently isolated from blood cultures but it can be challenging to determine their relevance. Over the past few decades, CoNS bacteria, which were frequently neglected as culture contamination, have obtained more attention as actual pathogens [4].

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Coagulase-negative staphylococci (CoNS) are part of normal human skin flora [5]. Although these organisms have a low level of virulence, they can cause clinically significant infections of the bloodstream and other tissue sites. Immune compromised and the presence of prosthetic material (such an intravascular catheter) are risk factors for CoNS infection [6]. The length of hospitalisation, ICU stay, morbidity, and therapy-related expenses are all increased by CoNS-CRBSI and CoNS bacteraemia [7,8]. However, CoNS are considered as low-virulence microorganisms, and data on outcomes in terms of mortality due to these infections have been conflicting [8,9].

Current study conducted with the aim 'To study Bacteriological profile of blood culture & its drug resistance pattern in various wards of Era's Medical College and Hospital'. In this cross sectional study total 638 samples were collected included, out of 638 only 90 suspected were revealed to be septiceamic positive.

2. Materials and Methods

The present study was conducted in Department of Microbiology in Era's Lucknow Medical College and hospital. During the period of six months, 638 blood samples from septicemia suspected patients were received and processed routinely. Out of 638 blood culture, 90 (14.10%) were found positive for culture growth in which 38% were Gram's positive and 62% were Gram's negative. Out of 90 positive culture overall proportion of female (47) were found to be significantly higher them male (43) fig-1 and table-1.

Table -1 Gender wise distribution

GENDER	NO OF PATIENTS	PERCENTAGE
MALE	43	47.78%
FEMALE	47	52.22%
TOTAL	90	100%

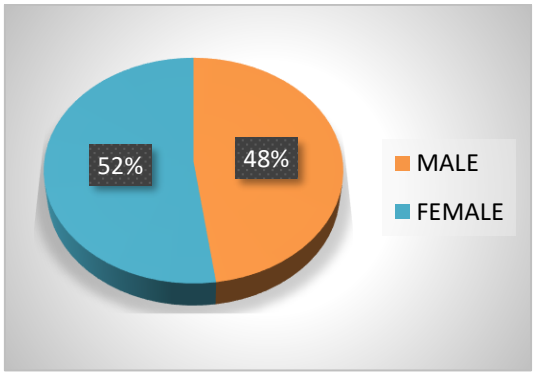

Fig-1 Gender wise distribution of patients

In this study among 90 culture positive samples, age wise distribution was done according to the age group, 0-25 years (41%), 51-90 years (32%) & 26-50 years (27%). In this study most commonly affected age group was 0-25 years (41%). Age wise grouping of patients are shown in table-2 and fig-2.

Table -2 Patients groups according to age

AGE GROUP	NO OF PATIENTS	PERCENTAGE
0-25 YEAR	37	41.11%
26-50 YEAR	24	26.67%
51-90 YEAR	29	32.22%
TOTAL	90	100%

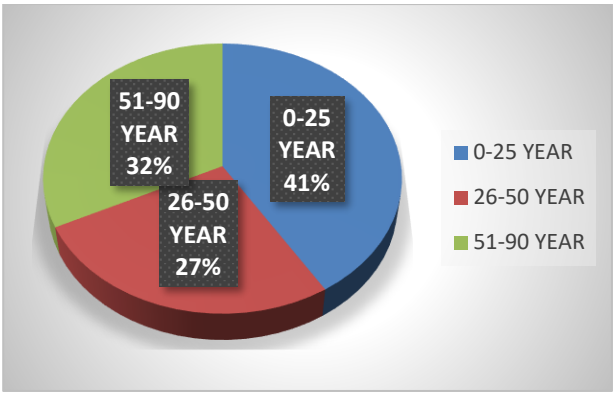

Fig -2 Showing patients groups according to age

2.1 Antimicrobial drug susceptibility

In vitro susceptibility & resistance pattern of isolates were carried out by Kirby-Bauer disc diffusion method according to CLSI guideline (2022). All the procedures in this study were performed inside safety cabinet to ensure no contamination by environment contaminants. Antibiotics used for gram- positive bacteria were Ciprofloxacin, Ampicillin, Gentamycin, Amikacin, Erythromycin, Cefotaxime, Tetracycline, Oxacillin, Vancomycin, Doxycycline, Clindamycin, Amoxy-clavulanic acid, Linezolid and for Grams negative bacteria antibiotics used are listed in table-3 were used for determination of resistance by disc diffusion test.

Table-3 Antibiotics used for gram-negative bacteria

Tigecycline	Colistin
Amikacin	Ampicillin-sulbactam
Amoxy-clavulanic acid	Carbenicillin
Cefoperazone + Sulbactam	Cefepime
Ceftriaxone	Cefixime
Gentamycin	Piperacillin-tazobactam
Ciprofloxacin	Meropenem / imipenem
Levofloxacin	Netilmicin
Doxycycline	Tobramycin

2.2 Disk diffusion test

Antibiotics sensitivity testing or antibiotics susceptibility testing was the measurement of the

susceptibility of bacteria to antibiotics. It was used because bacteria had resistance to some antibiotics. the kirby-Bauer test for antibiotics susceptibility (also called the disc diffusion test) was a standard that had been used for years. First developed in the 1950s.it is defined and by W. Kirby and A Bauer, then standardized by the world health organization in 1961. The test is used to determine the resistance or sensitivity of all (gram positive and gram negative) isolates were test for antimicrobial susceptibility pattern by Kirby-Bauer methods using commercially prepared disc of muller Hilton media laboratories

2.3 Procedure for disk diffusion (kirby-Bauer) Susceptibility Test media and reagent

1. Bacterial isolate from culture plate.
2. Normal saline
3. McFarland 0.5 standard for between comparing and adjusting the turbidity of the inoculum
4.Vortex mixture for suspension of the inoculum
5.View box for comparison of broth with standard
6.Muller Hinton agar plates (pH7.2 – 7.4) (depth of the medium kept approximately 4 mm)
7.Measuring scale for measuring the diameter of inhibitory zone.

Procedure

About 5 ml of peptone water broth in test tube was inoculated with bacteria to be tested

⇩

the turbidity of bacteria in peptone water with 0.5 McFarland.

⇩

For disc diffusion method, the turbidity of bacteria in peptone broth were equal to the turbidity of 0.5 McFarland.

⇩

After the preparation of inoculum. dispersed the inoculum uniformly in MHA by cotton swab, this was also known as carpet culture.

⇩

It was left for 10 min and then antibiotics disc was placed on the surface of MHA plate with the help of sterile forceps.

⇩

The plate was incubated for 24 hours at 37 ^{0} c.

⇩

Zone diameter around each disc were measure according to the CLSI guidelines (2022)

3. Results

Plates were observed 48hr after inoculation. All measurement were made with the naked eye while viewing the back of the Petri dish with reflected light against, non- reflecting background with the use of measuring scale. An Interpretation was done by observing the zone of inhibition (susceptible, intermediate susceptible or resistant) by reference to recent Clinical Laboratory Standard Institute 2021 published guidelines

Fig -3 AST pattern of gram-positive

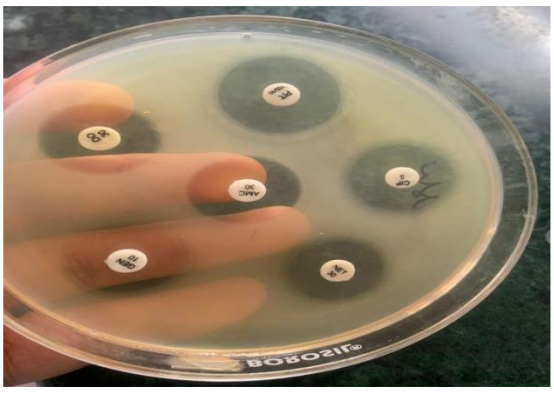

Fig -4 AST pattern of gram-negative

Table-4 Antibiotics susceptibility pattern of *cons* (n=25)

ANTIBIOTICS NAME	SENSITIVITY
Vancomycin	100%
Gentamycin	80%
Linezolid	100%
Amikacin	92%
Amoxy-clav	24%
Ciprofloxacin	60%
Cefoxitin	24%
Levofloxacin	52%
Teicoplanin	100%

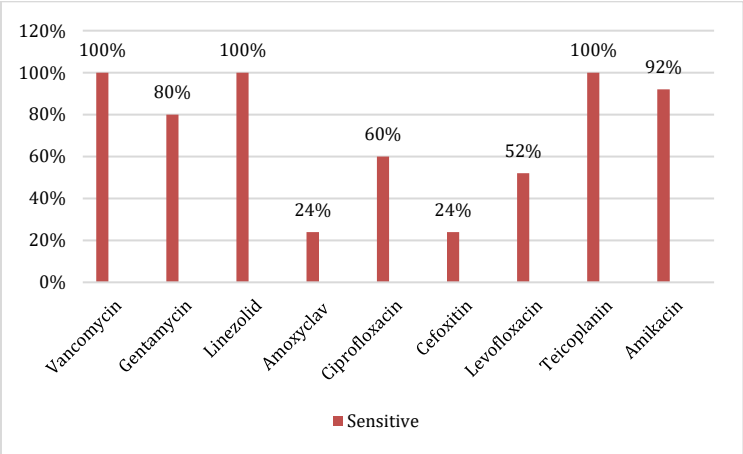

Fig-5 Antibiotics susceptibility pattern of *cons*

Table-5 Antibiotics susceptibility pattern of gram positive bacteria

Enterococcus species (n =06)		*Staphylococcus specie*(n=03)
ANTIBIOTICS	Sensitive 1	Sensitive 2
Vancomycin	100%	100%
Gentamycin (high level)	83%	-
Gentamycin	-	100%
Linezolid	100%	100%
Amikacin	66.67%	100%
Ampicillin	16.67%	-
Ciprofloxacin	33.33%	33.33%
Cefoxitin	-	0%
Levofloxacin	16.67%	-
Amoxy-clav	-	33.33%
Teicoplanin		100%

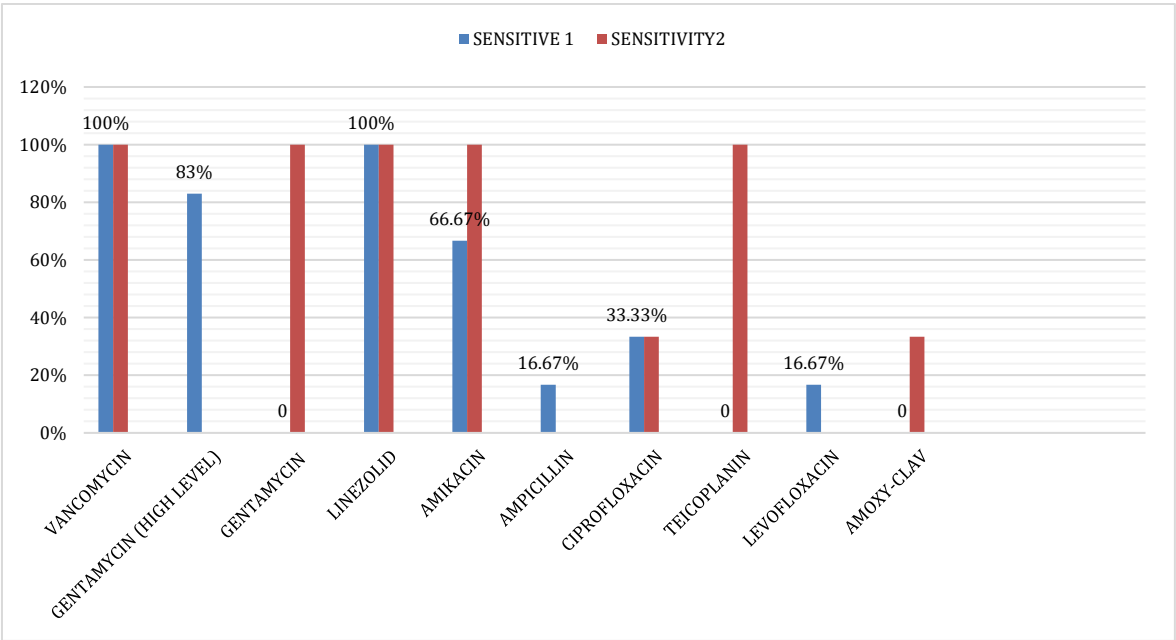

Fig-6 Among 638 sample, 90 (14.10%) samples were pathogenic. The gram-positive bacteria were 34 (37.78%) and gram-negative bacteria were 56(62.22%). Most sensitive antibiotic organism gram positive bacteria were Vancomycin, Teicoplanin, Linezolid and all were 100% sensitive to gram positive bacteria.

Table -6 Antibiotics sensitivity pattern on gram negative bacteria

ANTIBIOTICS	*Klebsiella spp.* (n=28)	*E. coli* (n=24)	*Pseudomonas Spp.* (n=03)	*Acinetobacter spp.* (n=01)
Tigecycline	100%	100%	-	-
Colistin	100%	100%	100%	100%
Cefepime	7.14%	4.16%	0%	0%
Amoxy-clav	17.85%	70.83%	-	0%
Cefoperazone+sulbactim	37.71%	80%	100%	0%
Ciprofloxacin	21.4%	12.5%	100%	0%
Meropenem/ impenem	7.14%	75%	!00%	0%
Netilmycin	67.85%	87.5%	100%	0%
Tobramycin	-	95.83%	100%	0%
Piperacillin-tazobactam	28.57%	100%	100%	0%
Doripenem	7.14%	75%	66.67%	0%
Ceftriaxone	0%	4.16%	0%	-

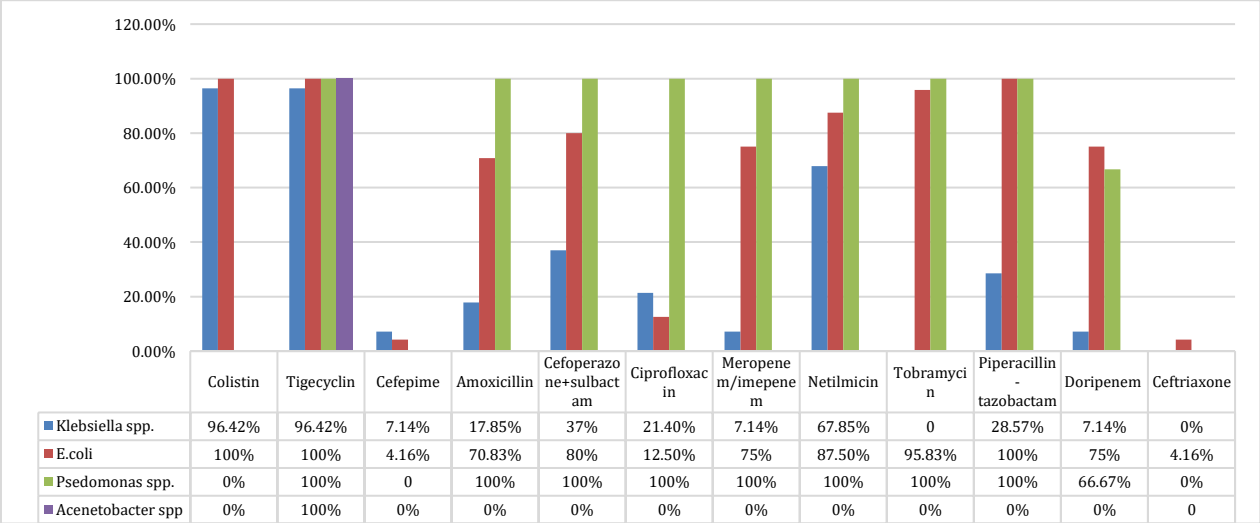

Fig-7 Among 638 sample, 90 (14.10%) samples were pathogenic. The gram-positive bacteria were 34 (37.78%) and gram-negative bacteria were 56 (62.22%). Most sensitive antibiotic among gram negative bacteria, *Klebsiella species* & *Escherichia coli* were found 100% sensitive to Tigecycline and Colistin.

4. Discussion

Bloodstream infection (BSIs) is a challenging problem and may be life-threatening, therefore its timely detection, identification, and antimicrobial susceptibility testing of BSIs pathogens is the most important task of diagnostic microbiology laboratory [11,12]. Each hour of delay in therapy initiation is associated with an average 8% decrease in survival rate [13].

In the present study, the culture positivity was 14.10%. This rate of isolation was consistent with many studies from India [14-16]. and abroad [17-18]. Higher culture positivity is reported by some authors [19-21]. The prevalence rate of BSIs in our study was 14.10%. the similar prevalence rate of 13% BSIs were observed by Nasrin *et al,* [22]. in hospital in Uttara Adhunik Medical College, Dhaka, Bangladesh. In contrast, the high prevalence rate of 17.18%, 19.67%, 22.3% and 28.26% were reported by Meshram P. *et al,* [23-25]. Variation in culture positivity rates could be due to difference in geographical location, nature of population, epidemiological difference of the etiological agents, also factors such as volume or number of blood culture samples. The low rate of isolation in our study could be due to the fact that most of the patients would have already received antibiotic treatment at peripheral health centre before being referred to our tertiary care hospital.

In this study, among 90 total pathogenic cases, 47(52.22%) were females and 43 (47.78%) were males. Oyekale T Oluwalana *et al,* (2022) [26] also showed similar data i.e, 57% of males and 42.4% female. In our study, 37 samples belong to age group from newborn to 25 years age (41.11%), 24 belongs to group 26 to 50 years age (26.67%), while 29 from age group 51 to 90 years (32.22%) were suspected of septicaemia. The similar data was observed in another study in which positivity was found in the age group of less than one month (23%) and greater than one years (28%). Attack rate was very high among infants, particularly low birth weight newborn experienced

high risk. It might be possible because Sepsis incidence and its related mortality decreases after the 1st year of life and thereafter increases with increasing age [27-29].

Staphylococcus aureus, *coagulase- negative staphylococcus (CoNS*) and *Enterococcus species* showed 100 % sensitivity to Vancomycin, Teicoplanin & Linezolid and *Enterococcus species* showed 90% sensitivity to Gentamicin (high level), this observation was very much similar to Yousuf M. *et al,* (2020) [30]. who also showed 100% sensitivity to these drugs for all gram- positive. Other studies, Palewar M. *et al,* 2020 [31], showed 87% sensitivity to Linezolid, moderately sensitivity 67% to Gentamycin and 80% to Teicoplanin and low sensitivity showed by Ciprofloxacin, Amoxiclav, 100% Resistance showed to Cefoxitin in *staphylococcus aureus* and *coagulase-negative staphylococcus, enterococcus species.* showed high sensitivity to Vancomycin (98%), Teicoplanin (98%), Chloramphenicol (89%) and (100%) sensitivity to linezolid. High level gentamycin resistance was 44%. The difference just because of the low sample size and due to short study period.

Our study observed 100 % sensitivity to Tigecycline, Colistin and Piperacillin-Tazobactam in *Escherichia coli,* netilmicin was moderately sensitive to *klebsiella* and *Escherichia coli.* Higher resistance observed by Cefepime, Cefoperazone, Imipenem, Ceftriaxone. Similar finding was observed by Palewar M *et al,* 2020 [31], in which Gram-negative bacteria showed high susceptibility to Meropenem (72%) and Colistin (100%), Aminoglycosides such as Amikacin (63%), Gentamicin (60%), Chloramphenicol (60%), and Piperacillin-Tazobactam (64%) and low sensitivity to Ampicillin (9.18%), Cephalosporins such as Cefotaxime (37%), Cefepime (23%), Ciprofloxacin (35%) and Aztreonam (30%). *Pseudomonas* showed least resistance to Carbapenems, Piperacillin-Tazobactam, and Aminoglycosides. In present study Colistin, Cefeperazone, Ciprofloxacin, Meropenem, Piperacillin-Tazobactam showed 100% sensitivity to *Pseudomonas species* and higher resistance showed by Ceftriaxone and Cefepime.

Conclusions

- The current study was conducted on patients suffering from, Bloodstream infection, admitted in Era's Medical College and Hospital, Lucknow. In this study total 638, blood samples included, out of which 638 only 90 suspected were revealed to be septiceamia positive.
- Blood-stream infections (BSIs) are a major public health problem, which leads, to high morbidity and mortality. Timely diagnosis and appropriate treatment was helpful in reducing morbidity and mortality.
- Among 638 sample, 90 (14.10%) were found to be pathogenic. The gram-positive bacteria were 34 (37.78%) and gram-negative bacteria were 56(62.22%). In this study most commonly identified pathogenic organism were gram-negative bacteria.
- In our study *Klebsiella species* & *E. coli* among gram-negative bacteria, *CoNS* & *Staphylococcus aureus* among gram-positive were the predominant blood borne pathogens in all age group and both sexes.
- In this study females showed high positivity rate of 47 (52.22%).
- Most commonly affected age group was 0-25 years (41%). So attack rate was very high among infants, particularly low birth weight newborn.
- In this study gram-positive bacteria showed high sensitivity to Vancomycin, Linezolid and Teicoplanin (100%).Gram-negative bacteria showed high sensitivity to Tigecycline and Colistin (100%).
- Both gram-positive and gram-negative bacteria were found to be responsible for blood stream infections, and mostly were Multi-Drug Resistant (MDR).
- Regular monitoring of data regarding the prevalence of microorganisms and its resistance pattern would benefit the current prescribed antimicrobial regimens.
- In also helps in rational use of antibiotics, proper antibiotic policy and implementation of infection control practices for the active treatment and inhibition of drug resistance.

Conflict of interest: Author declares that there is no conflict of interest.

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