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Incidence and Prevalence of ESBL and MDR organisms isolated from blood cultures at a Tertiary Care Hospital.

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Abstract
Background: Highly resistant bacterial infections are associated with high mortality. The management of Blood Stream Infections is complicated in an era of antimicrobial resistance. The choice of antimicrobial therapy for blood stream infections is often empirical and based on knowledge of local antimicrobial activity profiles[4]. The objective of this study was to determine the pattern of blood isolates from the blood cultures at a tertiary care hospital and determine their antibiotic resistance.
Methods: A retrospective study was conducted in Department of Microbiology, Sunshine Hospital, Secunderabad, from January 01, 2019 to April 30th, 2019. Blood culture positive isolates were identified by BacT/Alert 3D, an automated blood culture system, while identification of samples and the AST was performed by Vitek 2 Compact.
Results: There were 746 blood samples of which 147 (19.7%) were identified to be culture positive. Gram negative isolates were 107, (72.78%) and Gram positive isolates were 40 (27%).Isolates from Critical areas were 120 (83.6%), while 27 (18.3%) were from Non-Critical areas. The most sensitive drugs were Carbapenems and Colistin while Amoxicillin was the most resistant drug.
Conclusion: The incidence of ESBL producers and Multi drug resistant bacterial infections was remarkably high in Critical areas of our institute[1]. The study emphasizes the need for periodic surveillance of antibiotic susceptibility to prevent further emergence and spread of resistant bacterial pathogens[4].

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I. Introduction

Infections caused by multidrug- resistant organisms are the principle threats to the critically ill patients of Intensive Care Units (ICU) [1]. Extensive and often inappropriate use of extended spectrum antibiotics is associated with the emergence and spread of MDR organisms[9]. Over the last half century the prevalence of Extended Spectrum Beta lactamases (ESBL), Vancomycin Resistant Enterococci (VRE), Methicillin Resistant Staphylococcus aureus (MRSA), Carbapenemase producing bacteria have become increasingly prevalent in hospitals[10]. One of the most commonly used and effective group of antibiotics Cephalosporins, exhibit resistance due to the production of ESBL[2]. ESBL is a resistance mechanism in which the beta lactam ring of the antibiotics such as Penicillins, Cephalosporins and Aztreonam is hydrolysed, inactivating the antibiotic[3]. Cefepime has shown to have greater, stable activity against ESBL compared to other extended spectrum Cephalosporins[3]. Some strains of S.aureus have developed resistance to antibiotic medications particularly Methicillin and drugs in its class; giving such strains the name MRSA. The Vancomycin Resistant Enterococcus (VRE) is attaining severity as MRSA. The majority of VRE are associated with species E. faecium and E. faecalis.

Rapid detection and identification of microorganisms in blood cultures and determination of their antimicrobial susceptibility pattern is very essential for administration of antimicrobial therapy.

This study was undertaken to investigate the bacterial strains isolated from blood culture, from both critical and non critical areas and also to study the antibiotic resistance patterns, the incidence of MDR and ESBL producing isolates from blood cultures.

1. The aim of this study is to analyse common pathogens isolated from blood cultures.
2. And also to determine the various AST pattern among the isolates.

II. Materials and Methods

Type and Place of Study: This was a retrospective study conducted in the Department of Clinical Microbiology, Sunshine Hospital, Secunderabad. The period of study extended from 1st January 2019 to 30th April 2019.

Study Population: Blood samples of patients were received in the Department of Microbiology, from various OPDs and wards of Sunshine Hospital, Secunderabad. Relevant patient data such as collection date, OPD ward, sex, culture results and antimicrobial sensitivity results were included during the study period.

Culture: Two or more sets of blood was collected in BacT Alert bottles and subjected to automation for detection of growth in blood culture. When it was flagged positive, it was inoculated into relevant media and processed. The blood culture broth was then sub cultured on different media like Blood agar and Mac Conkey agar and incubated aerobically at 37 degree Celsius overnight for bacterial isolation. Isolates were identified by Vitek MS.

Bacterial Identification and Antimicrobial Sensitivity Test: Appropriate biochemical tests were done on the culture isolates to identify the organisms based on colony morphology and results of Gram staining[11]. The biochemical tests performed involves Catalase, Tube Coagulase, Bile esculin tests for Gram positive cocci (gpc) and Catalase, Oxidase, Indole, Citrate, Urease and TSI tests for Gram negative bacilli (gnb). The antibiotic susceptibility test was performed by using Vitek-2 AST-N280, AST-N281 and AST-P628 cards. The results were interpreted according to CLSI guidelines[4].

III. Results:

During the period of study, a total number of 746 blood culture samples were analyzed; of which 147 were identified to be culture positive. Culture positivity was calculated to be 19.7 %. Among the total positive cultures 120 (83.6%) were from Critical areas (IMCU, NICU, HDU, MICU, E/R) and 27 (18.3%) were from Non-Critical areas. The frequency of isolation of Gram negative bacteria from total positive isolates (n=107, 72.78%) was found to be more than that of Gram positive bacteria (n=40, 27%). Escherichia coli was the predominant organism accounting for 39, (26.5%) of the total isolates (n=147) followed by Klebsiella pneumonia 31(21.08%). The other identified isolates were Staphylococcus aureus 20 (13.6%), Acinetobacter baumannii 9 (6.1%), Staphylococcus epidermidis 8 (5.44%), Bukholderia cepacia 8 (5.44%) and others 32 (21.7%). Table 01, depicts the distribution of various organisms isolated among critical and non- critical areas.

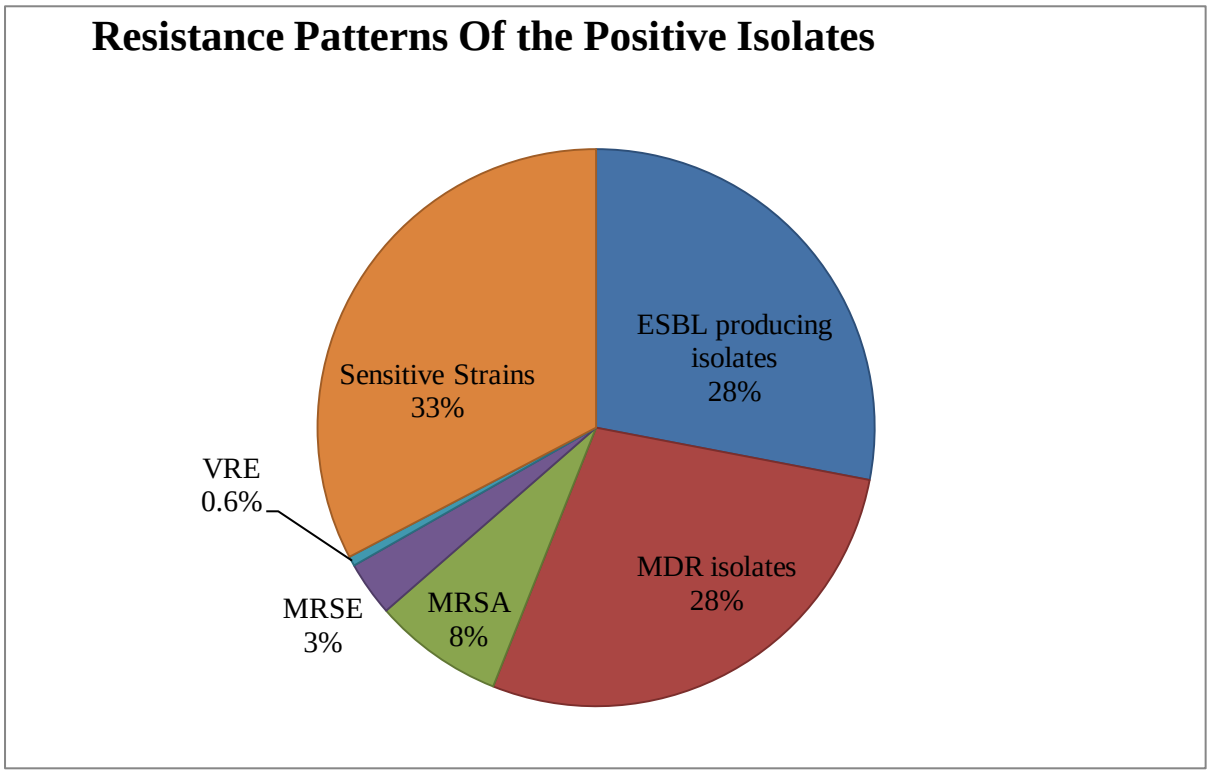
Table 01: Culture positive Isolates from Critical and Non-Critical Areas

Culture positive isolates	Isolates from Critical Areas	Isolates from Non-Critical Areas	Gram negative isolates	Gram positive isolates
19.7% (147)	83.6% (120/147)	18.3% (27/147)	72.78% (107/147)	27.21% (40/147)

TABLE 02: COMMON ORGANISMS ISOLATED FROM BLOOD CULTURES

Identified Isolates from total 147 blood samples	Number and Percentage
E.Coli	39 (26.5%)
K.pnuemoniae	31 (21%)
S.aureus	20 (13.6%)
A.baumannii	09 (6.12%)
Burkholderia cepacia	08 (5.4%)
S. Epidermidis	08 (5.4%)
Others(E.faecalis, S.typhi, S.pneumoniae, S.pyogenes, P.aeruginosa, Serratia marcescens Chryoseobacterium indolgenes,)	32 (21.7%)

A total of 44 (30%) isolates among the 147 isolates were positive for ESBL production. The study identified 44 (30%) isolates that met the criteria for classification of MDR strains. Among gram positive isolates MRSA 12 (8.1%), MRSE 5 (3.4%) and VRE 01 (0.6%) were identified. The remaining 41 isolates of 147 were susceptible to most of the antibiotics.



ESBL PHENOTYPE: In total 44/47 strains were flagged positive for ESBL phenotype (30%), of which 41 (93.1%) were from critical areas and 3 (6.8%) were from non-critical areas. These ESBL producing strains were distributed as follows: 32/44 (72.7%) were E.coli, 9/44 (20.4%) were K. Pneumonia, 2/44 (4.5%) Serratia marcescens, 1/44 (2.27) Enterobacter aerogenes.
21/44 ESBL producing strains carried genes from the CTX-M group (47.7%), 2/44 carried AmpC genes, 3/44 (6.8%) carried both AmpC and CTX-M and for 14 (40.9%) the ESBL gene was unknown.

All 32 ESBL producing isolates were sensitive to Imipenem, Meropenem, Ertapenem and Colistin. 93.75% of E.coli isolates were sensitive to Amikacin and Tigecycline, followed by 87.5% sensitivity to Cefaperazone/Sulbactam combination. The least sensitive was Ampicillin (100%) resistant[12]. Furthermore, 93.7% and 87.5% of E.coli were resistant to Levofloxacin, Cefuroxime and Cefuroxime axetil respectively. All of the 9 ESBL producing K.pneumoniae isolates were sensitive to Piperacillin/Tazobactam, Ticarcillin/ Clavulanic acid, Aztreonam, Ertapenem, Doripenem, Amikacin and Tiigecycline (100% sensitivity). All of the isolates were resistant to Ampicillin. One strain of ESBL producing K.pneumoniae was found to be resistant to Colistin.

Table 03: ESBL isolates and their Genotype

Isolates	Number and Percentage
Total ESBL producers	44
CTX-M Type	21
Ampc Type	02
Ampc+CTX-M	03
Unrecognised phenotype	18

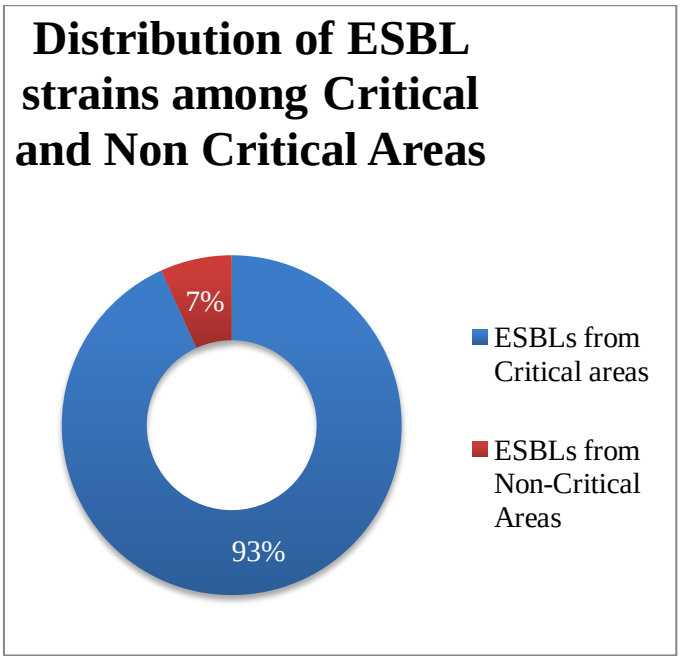


TABLE 04 : Antibiogram of ESBL isolates from CRITICAL AREAS

ANTIBIOTICS TESTED	E.Coli		K.pneumoniae	
	Sensitive %	Resistance%	Sensitive%	Resistance%
1.Ampicillin	0	100%	0	100%
2.β-lactamase inhibitors:				
Amoxicillin/Clavulanic acid	46.6%	53.3%	37.5%	62.5%
Pieracillin/Tazobactam	86.6%	13.7%	100%	0
Ticarcillin/Clavulanic acid	78.3%	21.4%	100%	0
3.Cephalosporins:				
• Cefuroxime	13%	83%	37.5%	62.5%
• Cefuroxime axetil	13%	83%	25%	75%
• Ceftriaxone	21.4%	85.71%	37.5%	62.5%
• Cefperazone/Sulbactam	86.5%	13.7%	100%	0
• Ceftazidime	66.6%	28.5%	100%	0
• Cefepime	86.2%	13.79%	88.8%	11.1%
4.Carbapenems:				
• Ertapenem	100%	0%	100%	0
• Imipenem	100%	0%	100%	0
• Meropenem	100%	0%	100%	0
• Doripenem	100%	0%	100%	0
5.Aztreonam	28.5%	71.1%	100%	0
6.Aminoglycosides				
• Amikacin	93.1%	7.4%	100%	0
• Gentamicin	85.1%	20.6%	77.7%	22.2%
7.Quinolones				
• Nalidixic acid	20%	80%	50%	50%
• Ciprofloxacin	24%	79%	44.4%	55.5%
• Levofloxacin	7.1%	92.8%	100%	0
8.Tetracycline:				
• Minocycline	85.7%	14.2%	100%	0
• Tigecycline	96.6%	4.8%	100%	0
9.Nitrofurantoin	60%	40%	75%	75%
10.Colistin	100%	0	88.8%	11.1%
11.Trimethoprim/sulfameth-oxazole	58.6%	41.3%	88.8%	11.1%

TABLE 05: ANTIBIOGROM OF ESBL PRODUCERS FROM NON-CRITICAL AREAS
(Only ESBL producing E.coli was isolated from Non-Critical Areas)

ANTIBIOTICS TESTED	SENSITIVE	RESISTANT
1.Ampicillin	0%	100%
2.β-lactamase inhibitors:		
• **Amoxicillin/Clavulanic acid**	0%	100%
• **Pieracillin/Tazobactam**	66.6%	33.3%
• **Ticarcillin/Clavulanic acid**	50%	50%
3.Cephalosporins:		
• **Cefuroxime**	0%	100%
• **Cefuroxime axetil**	0%	100%
• **Ceftriaxone**	0%	100%
• **Cefperazone/Sulbactam**	100%	0%
• **Ceftazidime**	100%	0%
• **Cefepime**	66.6%	33.3%
4.Carbapenems:		
• **Ertapenem**	100%	0%
• **Imipenem**	100%	0%
• **Meropenem**	100%	0%
• **Doripenem**	100%	0%
5.Aztreonam	100%	0%
6.Aminoglycosides		
• **Amikacin**	100%	0%
• **Gentamicin**	100%	0%
7.Quinolones		
• **Nalidixic acid**	0%	100%
• **Ciprofloxacin**	0%	100%
• **Levofloxacin**	0%	100%
8.Tetracycline:		
• **Minocycline**	100%	0%
• **Tigecycline**	100%	0%
9.Nitrofurantoin	100%	0%
10.Colistin	100%	0%
11.Trimethoprim/sulfameth-oxazole	100%	0%

MULTI DRUG RESISTANCE: Among the 147 total positive cultures, 44 isolates were multi drug resistant. MDR was observed more among the isolates from Critical areas 38, (86.3%) while 6 (13.6%) of isolates were from Non-Critical areas. Multi drug resistance was assessed highest in gram negative organisms. Among 44 MDR isolates only one strain was identified as gram positive. The most common isolate with MDR phenotype was K. Pneumonia, 21/44 (47.7%), which were K.pneumoniae producing Carbapenamase. An MDR phenotype occurred in 13.6% Burkholderia cepacia, 11.36% of E.coli, 11.31% of Acineobacter baumannii, 2.27% of Chryseobacterium indolgenes, Raoultella ornithinolytica, Psuedomonas aeruginosa and Enterobacter cloace. 79% of MDR isolates were Amikacin/Gentamicin resistant.

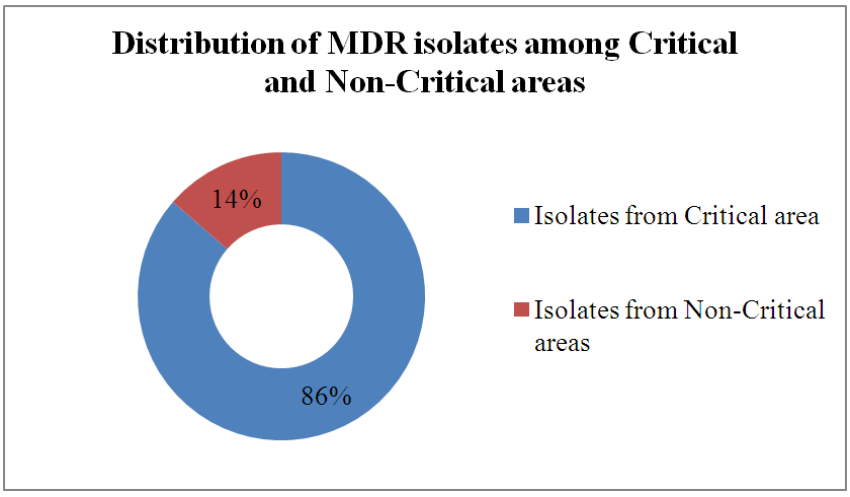


Table 06: Comparison among Aminoglycosides sensitivity with that of Colistin among MDR organisms / KPC

Antibiotics	Sensitive	Resistant
Amikacin/Gentamicin	9(20.45%)	35(79%)
Colistin	29(65.9%)	15(34.0%)

IV. Discussion:

Antimicrobial resistance of pathogens responsible for a majority of infections continue to increase throughout health care system[13]. Infections by ESBL producing organisms have emerged as a major problem in the failure of therapy with broad spectrum antibiotics; while infections with MDR pathogens are associated with higher morbidity and mortality, making it imperative to identify MDR isolates and assess their susceptibility patterns, to help and guide proper treatment[2, 13]. In this retrospective study an attempt was made to provide information on the distribution of bacterial isolates in blood samples, along with their antibiotic susceptibility pattern as it plays a crucial role in effective management of septicemic cases[4].

The results of our study demonstrated that 147 (19.7%) out of 746 blood samples screened positive for the presence of pathogenic bacteria. These results are comparable to other studies from India. Studies by Arora et al (20.2%) and Dr. Asifa Nazir (25.3%) in North India have shown comparable results[4].

In the present study the highest rate of prevalence was from critical areas (83.6%) while (18.3%) was from non-critical areas.

The rate of gram negative bacteria (n=107,72.78%) was more than that of gram positive bacteria (n=40,27%)[6]. It is in contrast to the study made by Dr. Asifa Nazir in Kashmir (2018), where gram positive bacteria (54.2%) were found to be more. This indicates that infections by gram negative isolates constitute a significant threat to septicaemia in our local geographical area[4].

We found that 44 out of 147 isolated strains (30%) were ESBL producers of which 32 (72.7%) were E.coli and 9 (20%) were K.pneumoniae. This is in contrast to several global and regional studies, where K.pneumoniae was the most frequent ESBL producing organism[10]. However it was in accordance with a study conducted in Bhubaneshwar (2015), which showed higher prevalence of ESBL producing E.coli. The Canadian National Intensive Care Unit was the first to document the ESBL producing E.coli are becoming more common than ESBL producing K. Pneumonia[2]. This study demonstrated that CTX-M genotype (21/44, 47.7%) was the predominant ESBL genotype among our isolates, which is in accordance to CANWARD surveillance study[5].

Critical areas are the most common areas affected by ESBL production in hospitals. This may be due to the increased use of β-lactam antibiotics which are being routinely prescribed to them. In our study about 93.1% of ESBL producers were from critical areas[7].

We observed that a majority of ESBL producers were susceptible to Carbapenems (100%), followed by Amikacin (93.1%) and Piperacillin/Tazobactam (84%)[8]. Complete resistance was seen against Ampicillin (100%). Co-resistance to Cefuroxime (87.5%) and Ciprofloxacin (78.1%) has also been observed[7]. Failure to control ESBL producing organisms leads to excessive use of Carbapenems and the potential emergence of Carbapenem-resistant pathogens.[7]

High prevalence of multi drug resistance was observed in the GNB among the blood isolates. From the total 44 MDR isolates, only one gram positive strain was identified.

It appears Colistin and Trimethoprim/Sulfamethoxazole as the last resort antibiotics[6]. About 29/44 (65%) isolates were sensitive to Colistin, while 24/44 (54.4%) isolates were sensitive to Trimethoprim/Sulfamethoxazole. Currently resistance to Colistin is relatively rare, but its increase use can develop the risk of Colistin resistant strains.

V. Conclusion:

The result from this study suggests that multiple blood cultures and automation helps in rapid diagnosis of septicaemia cases. The study revealed that E.coli was predominant organism followed by Klebsiella pnuemoniae at our Institute. Irrational or inappropriate use of antibiotics can create havoc in healthcare systems producing ESBLs, KPCs, and MDR strains. This study emphasizes that appropriate diagnosis and timely intervention can effectively manage septicaemias with decreased morbidity and mortality.

Acknowledgments
Conflict of interest – nil

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