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Increasing trend of antimicrobial resistance among Gram Negative organisms isolated from blood samples with special reference to ESBL and MBL producers, A study from a tertiary care center in the rural areas of Wayanad

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Abstract:
Background*:Antimicrobial resistance (AMR) is a threat to the modern world. Enterobacteriaceae, a family encompassing many clinically important bacterial species, exhibits rising levels of AMR. Infection with either extended spectrum b-lactamase-producing Enterobacteriaceae (ESBL-E) or carbapenemase-producing Enterobacteriaceae (CPE) is associated with increased mortality rates, time to effective therapy, length of stay and overall healthcare costs.* ***Materials and Methods:****The study was conducted in Department of Microbiology, DM WIMS Medical College, Wayanad during a period of one year. The isolates were identified on the basis of conventional microbiological procedures.* ***Results****: The magnitude of ESBL producing GNB was 29.4 % and that of MBL was 8.9%.* ***Conclusion****The study concludes the increase in number of ESBL and MBL producers and effective measures should be taken to reduce mortality among patients due to this rising disaster.*
Key Words: *ESBL, MBL, Blood isolates, Enterobacteriaceae*

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I. Introduction

Modern medical care has greatly been influenced by the development and introduction of antimicrobials in the 20th century. Antimicrobial resistance (AMR) is a threat to the modern world(1). Enterobacteriaceae, a family encompassing many clinically important bacterial species, exhibits rising levels of AMR. Infection with either extended spectrum b-lactamase-producing Enterobacteriaceae (ESBL-E) or carbapenemase-producing Enterobacteriaceae (CPE) is associated with increased mortality rates, time to effective therapy, length of stay and overall healthcare costs(2). The impact of the continued spread of AMR could have repercussions in multiple sectors. In the healthcare sector itself, patient deaths resulting from AMR are projected to reach 10 million annually by 2050, but AMR will also cause losses in the trillions to global economic output (3). ESBL-E and CPE have spread globally (4,5), and technologies such as whole-genome sequencing (WGS) are providing detailed insights into their evolution and dissemination. The World Health Organization has recently published a global priority pathogens list to focus attention on the most significantly resistant pathogens. Enterobacteriaceae resistant to third-generation cephalosporins (which includes ESBL-E) and Enterobacteriaceae resistant to carbapenems (CRE) are included within the critical category of this list(6).

The World Health Organization (WHO) has published a global priority pathogens list of antibiotic-resistant bacteria, in order to increase the significance of and galvanize research into new treatments for particular antibiotic-resistant pathogens. Of critical importance on this list are carbapenem-resistant *Acinetobacterbaumanii*,*Pseudomonas aeruginosa*and *Enterobacteriaceae*. Pathogens of this nature cause high morbidity and mortality and increased healthcare costs. Carbapenem resistant *Enterobacteriaceae*encompasses a number of genera, many of which harbour acquired multidrug resistance plasmids, which can carry and transmitantimicrobial-resistance genes on an intra- and interspecies level(6). This complicates surveillance, outbreak investigations and actions by infection control professionals. The spread of multidrug resistance is a globally important problem, with several countries currently reporting endemicity of highly resistant pathogens such as carbapenem-resistant *Klebsiellapneumoniae*.

II. Material And Methods

The study was conducted in Department of Microbiology, DM WIMS Medical College, Wayanad. Samples were obtained between June 2018 and June 2019. The isolates were identified on the basis of conventional microbiological procedures .The identified isolates were subjected to Antimicrobial susceptibility Testing by Disc Diffusion Method. Antibiotic susceptibility of the isolates was determined by Kirby Bauer Disk

Diffusion method. They include ceftriaxone, cefotaxime, ceftazidime, gentamicin, ampicillin, tobramycin, amikacin, netilmicin, amoxyclav,aztreonam, cefotaxime, ciprofloxacin, imipenem,imipenem EDTA, meropenam, meropenam EDTA, cefuroxime,tetracycline, tigecycline, cefaperazone- sulbactum, co-trimoxazole, piperacillin-tazobactum and chloramphenicol. The results were recorded and interpreted according to the standard guidelines. This was a laboratory based study which has no directly involvement with the concerned patients. The specimen sources and patient information such as sex, age and setting, were carefully recorded from laboratory request forms.

Procedure methodology
Detection of ESBL
Phenotypic confirmatory test

Test organisms were inoculated into Mueller-Hinton agar as lawn culture. The ceftazidime (30 g) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 g discs) were placed. An increase of 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

Detection of MBL:
Imipenem EDTA combined disc test

Two (10 g) imipenem discs were placed on a plate inoculated with the test organism, and 10 l of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of 7 mm was interpreted as a positive result for MBL production.

III. Result

Out of the total 1871 samples obtained and processed, a total of 158 (8%) grew an organism. Gram negative organisms constituted 5% (78/158) of all the isolates.The distribution of ESBL and MBL organisms shown in Table:1. Among this Enterobacteriaceae were the most commonly isolated Gram negative organisms, constituting58% (46/78) of all Gram negative bacilli. The susceptibility pattern of ESBL isolates is given in Table:2. In this, the carbapenem group of drugs proved to be highly sensitive with Imipenem 79.4% and Meropenem (83.3%).The sensitivity to Ampicillin was more significantly associated with ESBL isolates. When we compared MBL producers with non-MBL producers, we came to a conclusion that betalactam/ beta lactamase inhibitor combination like ceftazidime / clavulanic acid was more sensitive in MBL producers.

Table:1 ESBL and MBL mediated resistance in organism

Organism	No. of isolates	ESBL	MBL
E. coli	27	17	1
Acinetobacter sp.	18	1	3
Klebsiella pneumonia	8	1	2
Pseudomonas aeruginosa	6	1	0
Enterobacterspp	4	1	0
Other Gram Negative Non-fermenters	8	1	1
Salmonellaspp.	4	0	0
Citrobacterspp	3	1	0
Total	78	23	7

Table:2 Antibiotic susceptibility pattern of ESBL producers

Antibiotic	Number (%)
Ampicillin	21(26.9%)
Amoxyclav	37(47.4%)
Gentamycin	18(23%)
Ciprofloxacin	9(11.5%)
Cotrimoxazole	22(28.2%)
Aztreonam	38(48.7%)
Netilmicin	24(30.7%)
Amikacin	18(23%)
Cefotaxime	27(34.6%)
PiperacillinTazobactam	23(29.4%)
Imipenem	62(79.4%)
Meropenem	65(83.3%)
Ceftazidime	13(16.6%)
CeftazidimeClavulanic acid	42(53.8%)
CefoperazoneSulbactam	30(38.4%)

Cefuroxime	12(15.3%)
Ceftriaxone	34(43.5%)
Cefixime	29(37.1%)

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IV. Discussion

Septicaemia has a high morbidity and mortality worldwide. Blood culture plays a pivotal role in timely identification of the pathogens and this will in turn lead to appropriate treatment modality and a better understanding of drug resistance pattern. Physical signs and symptoms, have limited specificity in diagnosis(7,8). Growing resistance to conventional and even the latest antibiotics is also a matter of serious concern. Among the organisms isolated during this study, Gram Negative outnumbered the Gram positives. The most frequently isolated organism was E.coli among the Enterobacteriaceae and Pseudomonas among the Gram negative non- fermenters(8). Minimal resistance to carbapenems was observed during the course of the study. The misuse of antibiotics can be visualized in the form of the deterioration in sensitivity to the second and third generation cephalosporins. This is a matter of great concern. Establishment of an appropriate and rational antibiotic policy is therefore essential to control this growing danger.ESBLs and MBLs producing organisms are a serious threat today. This is creating a very serious therapeutic difficulty which intern affects the outcome for the patients. In the present study the magnitude of ESBL producing GNB was 29.4 % and that of MBL was 8.9%. This is similar to the studies by Nepalet al. in 2017(2) . In our study E.coli (34.6%) outnumbered other Gram Negative organisms which is similar to the studies by Nepal et al and Raut et al. Each study emphasizes the need to implement strong infection control strategies and adopt proper antibiotic policy to avoid a crisis in

the near future. The most important factor which is considered as the leading cause for the emergence of ESBL production is the selective pressure due to the use of 3rd generation Cephalosporins.

V. Conclusion

This study has demonstrated how significant is regular antibiotic susceptibility testing of blood culture. The current study also highlightsthe fact that the prevalence of ESBLs and MBLs is on a rise since last one decade among bloodculture isolates. Screening ofbeta lactamases would help in infection control to a large extend. Hence an easy, economical and cost effective method like the one used in this study could help in screening for beta lactamases and this will inturn help in reducing mortality rates.The rise in the number of ESBLs and MBLs necessitates the strengthening of clinical bacteriology research and the need to amplify the capacity of laboratory professionals for the surveillance of antibiotic resistance.

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