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Original Research Article

Prevalence of various Beta-lactamases in Enterobacteriaceae in a tertiary care hospital in South India: A Cross-sectional study

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ABSTRACT

Introduction: Antimicrobial resistance among bacteria has constantly increased over the years with emergence of novel resistance mechanism among these versatile organisms. Identifying the resistance pattern of the isolates both regionally and globally is essential for the institution of appropriate antimicrobial therapy. This study was conducted to assess the prevalence of beta-Lactamase enzymes among species of Enterobacteriaceae.

Materials and Methods: 312 consecutive isolates belonging to the family of Enterobacteriaceae were included in the study conducted over a period of one year. After preliminary antibiotic susceptibility testing using standard guidelines, production of various beta-Lactamase enzymes was assessed by phenotypic methods.

Results: 22.76% of the isolates were found to be Extended-Spectrum Beta-Lactamase producers, 37.82% were AmpC Beta-Lactamase producers and 7.37% of the isolates produced the Carbapenemase enzyme.

Conclusion: Our study estimates the prevalence of various beta-Lactamase enzymes in isolates of Enterobacteriaceae in a tertiary care centre. The increase in production of these enzymes among bacteria necessitates the implementation of strict adherence to antibiotic policy and infection control measures.

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1. Introduction

Antimicrobial resistance, especially in Gram negative bacilli is a growing public health concern. Treatment options for the multi-drug resistant strains are limited due to paucity of newer antimicrobials in the pipeline. Infections with antimicrobial resistant strains lead to longer duration of hospital stay, increased cost of health care and contribute to higher rates of morbidity and mortality.¹ The burden of antimicrobial resistance is higher in low- and middle-income countries like India due to unregulated and indiscriminate use of antibiotics.

Extended spectrum beta lactamases (ESBL) are a group of enzymes that are capable of hydrolysing Penicillins,

first, second and third generation cephalosporins and monobactams like Aztreonam but are inhibited by beta lactam inhibitors like Clavulanic acid. They do not have any effect on Cephamycins and Carbapenems.²

Bush-Jacoby-Medeiros functional classification divides beta lactamases into three groups based on their substrate and inhibitor profiles. Amp C beta lactamases are placed in group 1, Extended-Spectrum Beta lactamases in group 2 and Metallo-beta-lactamases in group 3. Ambler's classification identifies 4 types of beta-lactamases based on amino acid sequence wherein types A, C and D are serine beta-lactamases and type B is a metallo-beta-lactamase containing Zinc.³

Around 1300 types of beta-lactamases and 200 types of Extended spectrum beta-lactamases have been identified.^{2,3}

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ESBL producing Gram negative bacilli are frequently multi-drug resistant as the plasmids coding for ESBL production may also carry genes coding for resistance to other classes of antibiotics like quinolones, aminoglycosides and trimethoprim-sulfamethoxazole.⁴

Amp C production may be chromosomal or plasmid-mediated.³ “ESCPM” organisms which include *Enterobacter cloacae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Citrobacter freundii*, *Providencia* species and *Morganella morganii*, intrinsically produce Amp C enzyme on exposure to beta lactams.³ In isolates that co-produce AmpC and Extended Spectrum beta lactamase, the AmpC beta lactamase resists inhibition by clavulanic acid and hence these isolates give false negative results in tests detecting ESBLs.²

Carbapenems are the antibiotics of choice for the treatment of severe infections caused by Extended spectrum beta-lactamase producing bacteria.⁵ They are broad-spectrum antibiotics that inhibit the enzyme transpeptidase thus preventing peptide cross linking during peptidoglycan synthesis. Carbapenem resistant Enterobacteriaceae show resistance to one or all of the Carbapenem antibiotics like Imipenem, Meropenem, Doripenem or Ertapenem.⁶ Enterobacteriaceae can acquire resistance to carbapenems by production of Carbapenemase enzyme, hyperproduction of Amp C or by production of Extended spectrum beta lactamase along with porin mutations or by efflux pumps.³ Carbapenemases are plasmid-mediated and readily transferred among bacteria.⁷ The most common type of carbapenemase found in India is NDM-1.⁵

Determining the accurate burden of antimicrobial resistance in India is difficult due to absence of an obligatory antimicrobial resistance reporting system and the non-uniform testing methods and modalities used by different laboratories with varying sensitivity and specificity.¹ Ongoing surveillance of the distribution and antimicrobial resistance pattern of the isolates in a hospital facilitates the selection of appropriate empiric therapy and also strengthens the infection control practices by prompt identification of resistant isolates. The present study intends to estimate the prevalence of various types of Extended Spectrum beta lactamases among isolates of Enterobacteriaceae in our hospital.

2. Materials and Methods

This prospective cross-sectional study was conducted in the department of Microbiology at Shridevi Institute of Medical Sciences & Research Hospital, Tumkur for a period of one year from January 2019 to December 2019. Isolates of Enterobacteriaceae were identified using standard Microbiological techniques.⁸ Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method and the susceptibility to antibiotics were assessed based on Clinical Laboratory Standards Institute

guidelines.⁹ All the antibiotic discs were procured from Himedia Laboratories Pvt. Ltd.

Isolates of Enterobacteriaceae showing a zone diameter of ≤ 22 mm for Ceftazidime or ≤ 27 mm for Cefotaxime were presumptively identified as ESBL producers and were included in the study.⁹ Isolates of *Salmonella* and *Shigella* species were not included.

ESBL production was confirmed by Double Disc Synergy Test (DDST) and Phenotypic Confirmatory Disc Diffusion test (PCDDT).⁹ An extension of zone of inhibition of Ceftazidime or Cefotaxime towards disc of Amoxyclav in DDST was considered positive and an increase in zone diameter of ≥ 5 mm for Ceftazidime and Clavulanic acid in comparison to disc containing only Ceftazidime or an increase in zone diameter ≥ 5 mm for Cefotaxime and Clavulanic acid in comparison to disc containing only Cefotaxime in PCDDT was considered positive. Figure 1 shows a positive Phenotypic Confirmatory Disk Diffusion test. Himedia ESBL Identification kit I and III was used for PCDDT.

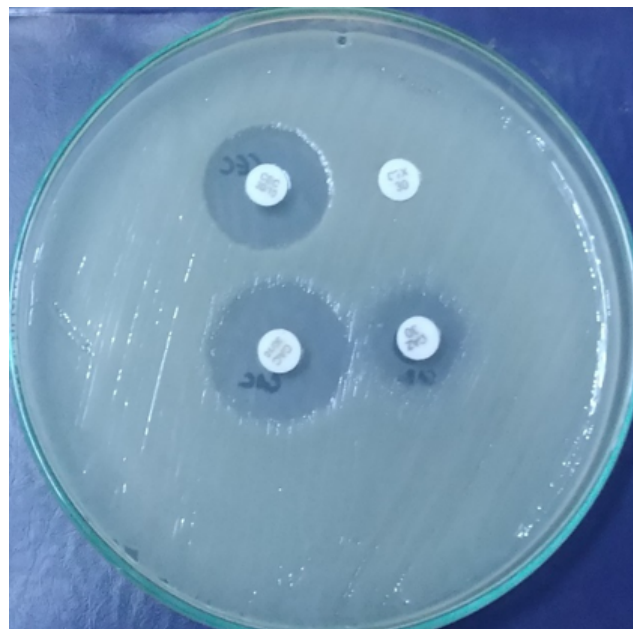


Fig. 1: Phenotypic Confirmatory Disk Diffusion Test positive

Isolates that were resistant to third generation cephalosporins and Cefoxitin were subjected to Amp C disk test to detect plasmid-mediated Amp C production. Lawn of *Escherichia coli* ATCC 25922 was made. Cefoxitin disc was placed close to filter paper disc containing 4-5 colonies of isolate. After overnight incubation, an indentation of zone of inhibition for Cefoxitin was considered as plasmid-mediated Amp C positive.¹⁰ Figure 2 shows a positive AmpC disk test.

Isolates that were sensitive to third generation cephalosporins and Cefoxitin were subjected to Disk

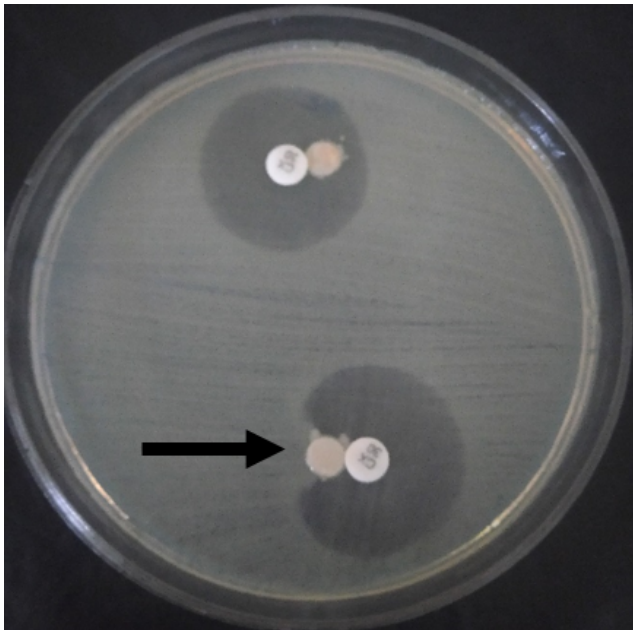


Fig. 2: AmpC Disk Test positive

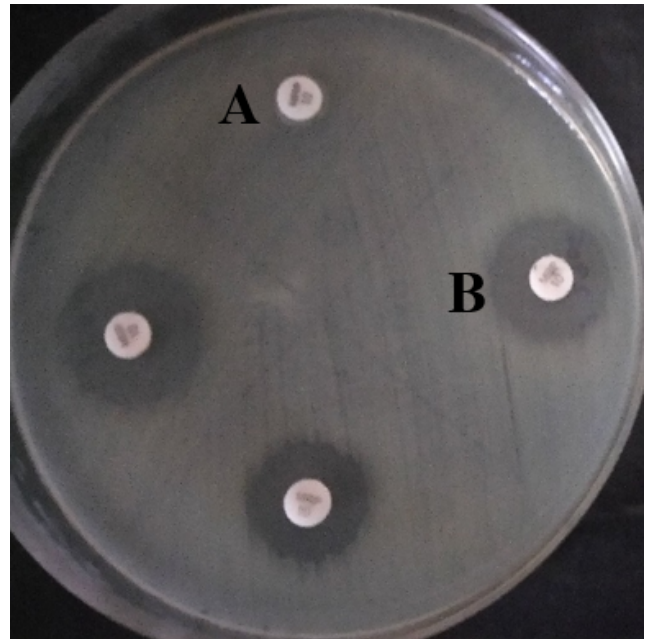


Fig. 4: mCIM & eCIM: A – mCIM positive, B – eCIM positive

Antagonism test to detect inducible Amp C production. Inducible AmpC was identified by blunting of Ceftazidime or Cefotaxime zone towards inducer (Imipenem) and sensitivity to Cefepime.¹¹ Figure 3 shows a positive Disk Antagonism test.



Fig. 3: Inducible AmpC

All isolates resistant to either Meropenem or Imipenem were further tested for Carbapenemase production using Modified Carbapenem Inactivation Method (mCIM). Isolates that were positive by mCIM were further tested by EDTA-modified Carbapenem Inactivation Method (eCIM) to differentiate between Serine and metallo-beta-lactamase. Isolates of Enterobacteriaceae giving a negative result by mCIM test were considered negative for Carbapenemase enzyme. Isolates positive by mCIM and negative by eCIM were considered Serine-β-Lactamase producers and isolates positive for both mCIM and eCIM were considered as

Metallo-β-Lactamase producers.⁹ Figure 4 shows an isolate producing Metallo-Beta-lactamase enzyme demonstrating a positive mCIM and eCIM test.

Escherichia coli ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 was used for quality control.

3. Results

312 consecutive isolates of Enterobacteriaceae were obtained from various clinical samples during the study period. Figure 5 shows the sample-wise distribution of the various isolates.

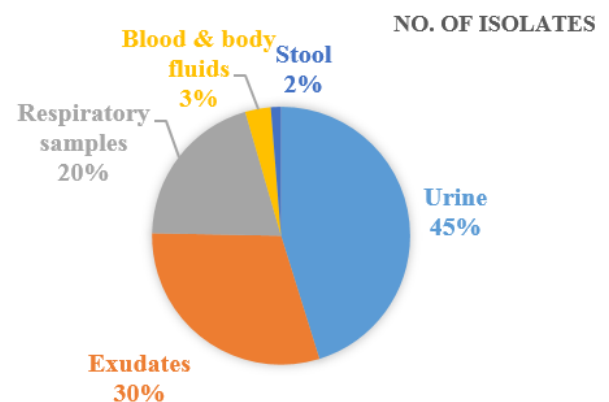


Fig. 5: Sample-wise distribution of the various isolates.

196 (62.80%) of these isolates showed resistance to either Cephotaxime or Ceftazidime and were further examined for production of Extended Spectrum beta-

lactamase enzymes by phenotypic methods.

172 isolates demonstrated the production of one or more Beta-lactamase enzymes. In total, 71 isolates were ESBL positive and 118 isolates were AmpC producers. Among the AmpC producers 91 were plasmid mediated AmpC and 27 were inducible AmpC producers.

51 isolates were resistant to either one of the carbapenems tested of which 23 isolates produced Carbapenemase enzyme. 3 isolates were Serine Beta-lactamase producers and 20 isolates were Metallo-beta-lactamase producers. Table 1 shows the distribution of various enzymes produced by the isolates.

In total, 22.76% of the isolates were ESBL producers, 37.82% were AmpC producers and 7.37% of the isolates were positive for Carbapenemase production. Figure 6 shows the organisms producing the various Beta-lactamase enzymes.

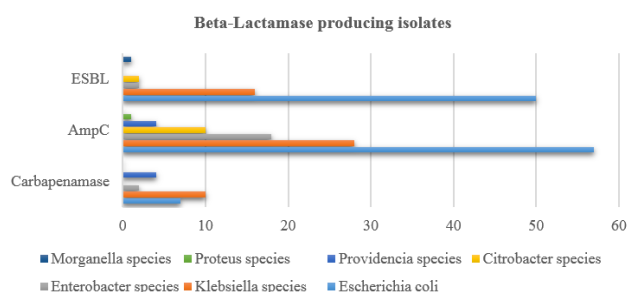


Fig. 6: Organisms producing the various Beta-lactamase enzymes

Escherichia coli was the predominant isolate to produce Extended-Spectrum Beta-Lactamase enzyme, constituting 70.4% (n=50) of the isolates followed by *Klebsiella* species accounting for 22.53% (n=16).

Likewise, among the AmpC producers *Escherichia coli* was the chief isolate forming 48.3% (n=57) of the isolates followed by *Klebsiella* species composed of 23.73% (n=28).

Among the Carbapenemase producers, *Klebsiella* species was the predominant isolate accounting for 43.48% (n=10) of the isolates followed by *Escherichia coli* accounting for 30.43% (n=7) of the isolates. Table 2 shows the production of various beta-lactamase enzymes by the isolates of Enterobacteriaceae.

The predominant isolates obtained from the various clinical samples were *Escherichia coli* and *Klebsiella pneumoniae*. Among the 161 isolates of *Escherichia coli* that were included in the study, 31.05% of the isolates produced the Extended Spectrum Beta-Lactamase enzyme, 35.40% of the isolates were AmpC producers and 4.35% of the isolates produced the Carbapenemase enzyme.

Among the isolates of *Klebsiella* species, 29.16% of the isolates were AmpC producers, 16.67% of the isolates produced ESBL enzyme and 10.42% of the isolates produced the Carbapenemase enzyme.

4. Discussion

Antimicrobial resistance is an escalating global concern. Institution of appropriate antibiotics only when indicated along with standard infection control practices is the need of the hour to curb this menace. Assessing local prevalence of antimicrobial resistant isolates and their mechanism of resistance is essential for the prescription of appropriate antibiotics. The present study was conducted to estimate the prevalence of various beta-lactamase enzymes among isolates of family Enterobacteriaceae.

In the current study, 22.76% of the isolates produced Extended Spectrum Beta-lactamase enzymes. Our findings were comparable to a study conducted by Gupta et al¹² in Chandigarh where the prevalence of ESBL was found to be 24%. However, other studies in India have shown a higher occurrence of ESBL enzymes in isolates of Enterobacteriaceae.^{13–17}

Among *Escherichia coli*, ESBL production was found in 31.05% of the isolates which is lower compared to other similar studies done in India.^{17,18}

In our study, 16.67% of the *Klebsiella* isolates were ESBL positive. In comparison, study done in Bangalore showed a higher prevalence of 46.6% among *Klebsiella pneumoniae* isolates.¹⁹

AmpC beta-lactamases render the bacteria resistant to various beta-lactam antibiotics including beta-lactam/beta-lactamase inhibitor combinations. Studies have shown that presence of AmpC enzymes in the isolates can lead to false sensitivity to cephalosporins leading to therapeutic failures.¹⁰ This highlights the need for identification of these enzymes in the isolates. There are no standard Clinical and Laboratory Standards Institute guidelines for the detection of AmpC enzymes.⁹ AmpC disk test for plasmid mediated AmpC and Disk antagonism test for Inducible AmpC were employed in our study.^{10,11}

Prevalence of AmpC enzyme in our isolates was found to be 37.82%. The findings of our study were comparable to similar studies conducted in Mumbai,¹³ New Delhi¹⁸ and Bangalore.²⁰ However, studies conducted in Pune and a multi-centric study involving New Delhi, Kochi, Lucknow, Wardha and Pondicherry detected lower levels of AmpC enzymes in isolates of Enterobacteriaceae.^{15,21}

35.40% of *Escherichia coli* isolates were positive for AmpC enzyme. Similar prevalence was found by Govindaswamy A et al., in New Delhi.¹⁸ *Klebsiella* species however showed a higher prevalence of AmpC enzyme in our study compared to other studies done in India.²²

Isolates in our study showed a lower prevalence of ESBL and high prevalence of AmpC enzymes. Studies have shown that co-production of AmpC and ESBL enzymes can lead to false negative results with ESBL tests.² This could be a reason for the detection of lower numbers if ESBL producers in our study.

Table 1: Distribution of beta-lactamase enzymes among Enterobacteriaceae

S. No.	Beta-lactamase Enzyme	Total Positive	% of total isolates of Enterobacteriaceae tested
1	Only ESBL	51	16.35
2	Only AmpC	78	25.00
3	Only Carbapenemase	03	0.96
4	ESBL & AmpC	20	6.41
5	AmpC & Carbapenemase	20	6.41
6	ESBL & Carbapenemase	00	00
Total		172	55.13
Total No. of ESBL producers		71	22.76
Total No. of AmpC producers		118	37.82
Total No. of Carbapenemase producers		23	7.37

Table 2: Production of various beta-lactamase enzymes by the isolates of Enterobacteriaceae

S. No.	Isolate	Total No. of isolates	Beta-lactamase enzyme production		
			ESBL positive (%)	AmpC positive (%)	Carbapenemase positive (%)
1	<i>Escherichia coli</i>	161	50 (31.05)	57 (35.40)	7 (4.35)
2	<i>Klebsiella species</i>	96	16 (16.67)	28 (29.16)	10 (10.42)
3	<i>Enterobacter species</i>	29	2 (6.90)	18 (62.06)	2 (6.90)
4	<i>Citrobacter species</i>	18	2 (11.11)	10 (55.56)	0 (0)
5	<i>Proteus species</i>	02	0 (0)	1 (50)	0 (0)
6	<i>Providencia species</i>	04	0 (0)	4 (100)	4 (100)
7	<i>Morganella morganii</i>	02	1 (50)	0 (0)	0 (0)
Total		312	71	118	23

Rate of Carbapenem resistance in our study was 16.35%. Our study shows the prevalence of Carbapenemase enzyme at 7.37% in Enterobacteriaceae. 87% of positive isolates produced Metallo-beta-lactamase. Several studies have demonstrated a predominance of metallo-beta-lactamases in India.^{18,23–25} 3 isolates of *Escherichia coli* produced Serine Beta-lactamase enzyme. Our findings were comparable to studies conducted in Pune¹⁵ and²⁶ However, studies conducted in Karad, Maharashtra,⁶ Chennai²⁷ and New Delhi²⁸ showed higher prevalence of Carbapenemase production among isolates of Enterobacteriaceae. The predominant isolate to produce Carbapenemase enzyme in our study was *Klebsiella species* similar to studies conducted by Pawar et al⁶ and.²⁸

Only 45% of the Carbapenem resistant isolates demonstrated the production of Carbapenemase enzyme. Carbapenem resistance in the absence of Carbapenemase enzyme production may be due to excessive production of Extended-spectrum beta-lactamase or AmpC enzyme along with porin loss.²⁶

The limitation of our study is that molecular methods were not employed, precluding the assessment of presence and classification of drug resistant genes in our isolates. In addition, small sample size hinders the generalization of the study results. As our study was conducted over a span of one year, we could not assess any change in the trends of distribution of beta-lactamase enzymes in our isolates from one year to another. Antibiotic policy has been formulated

and implemented in our institution in the past one year. Assessing the prevalence of these resistance mechanisms over a period of time will enable us to assess the impact of adherence to the policy.

5. Conclusion

The current study has outlined the prevalence of various beta-lactamase enzymes in isolates of Enterobacteriaceae. Awareness of institutional and regional distribution of these isolates is essential to prescribe appropriate empirical therapy. Antimicrobial resistance is a public health concern which can cause grave consequences. Inappropriate use of antimicrobials and paucity of newer antimicrobial agents will soon force us to enter the post-antibiotic era wherein hitherto curable infections will become life-threatening. A concerted effort by members of all sectors of the hospital involving judicious antimicrobial prescription, strict adherence to institutional antibiotic policy and infection control practices is essential to control the pandemic of antimicrobial resistance.

6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

7. Source of Funding

None.

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