Incidence & Estimation of Beta – Lactamase Enzymes (ESBL Ampc, Carbapenemase Enzymes) singly and their coexistence in clinical isolates of Gram Negative bacteria by Vitek – 2 Compact System

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Abstract

Introduction: With increase in Beta – Lactamases producing microbial infections there is need for rapid method for identification of different types of Beta Lactamases enzyme production & their coexistence in clinical isolates. Vitek – 2 compact play very crucial role in detection of different types of Beta- Lactamases & their coexistence.

Materials & Methods: 240 clinical specimen like Urine, Tracheal Secretion, Sputum, pus & Stool were isolated from clinical samples of OPD & IPD Patients of Tertiary Care Hospitals attached to Dr. S.N. Medical College, Jodhpur. Identification of Clinical isolates & detection of Betalactamase enzymes with their antibiotic susceptibility pattern was done by automated Vitek – 2 compact System.

Results: 146 (60.83%) E. coli, 67 (27.91%) Klebsiella, 22 (9.17%) Enterobacter, 2 (0.83%) Citrobacter & 3 (1.25%) Serratia spp. were included in present study. Out of 240 isolates 195 (81.25%) were enzymes producers & 45(8.75%) were non-enzyme producer. Of the 195 isolates 147(61.25) were single enzyme producer & 48(20%) isolates were multiple enzyme producers. 105(44%) isolates shows only ESBL enzyme production, 26 (11%) isolates shows AMPc enzyme & 16(7%) isolates shows carbapenemase enzymes production. Of the 48 isolates producing mixed enzymes 45.83% shows ESBL+AMPc and ESBL+CARBAPENEMASE each and 16.66% shows all enzymes (ESBL+AMPc+CARBAPENEMASE) production. Isolates producing ESBL are maximally resistant to Cefepime followed by Cefuroxime, & Ceftriaxone. Organism producing AMP care maximally Resistant to Cefuroxime followed by Trimethoprim - Sulfmethoxazole, Piperacillin-Tazobactum, Ampicillin. Organism producing CARBAPENEMASE are maximally Resistant to Ampicillin, Cefipime followed by Cefuroxime+Sulbactum, and Amoxicillin+Clavulinic acid. Organism producing mixed enzymes are maximally resistant to Cefuroxime, followed by Amoxicillin+Clavulinic acid and Ciprofloxacin.

Conclusion: The study show that Betalactamase producing microbial organism are very common in Hospital environment and responsible for majority of infections. Vitek-2 compact is found to be rapid and reliable method to identify Betalactamase and their co-existence. It is better than conventional method.

Keywords: Beta lactamases, Vitek – 2, E. coli, Klebseilla

Introduction

Members of Family Enterobacteriaceae are among the most important bacterial human pathogens accounting for the majority of bacterial isolated from clinical samples These gram Negative bacilli are Rapidly acquiring Resistance to one or more antimicrobial agents traditionally used for treatment is matter of concern.[1]

This group of bacteria is responsible for several disease like UTI, blood stream infection, hospital – health care associated pneumonias, intra abdominae infections, gastroenteritis, etc. These drug resistant Enterobacteriaceae are associated with high mortality, morbidity.[2]

The increasing number of Beta lactamase producing organism leaves very few treatment options for clinicians. It is unusual to find a single isolate which expresses multiple Beta lactamases enzymes. This study was under taken to detect the coexistence of different Beta lactamase enzymes in clinical isolates of Gram Negative Bacteria.[3]

Materials & Methods

This study was carried out during the period of 2012 to July 2015 March. Clinical samples were collected from indoor & out door patient's specimen such as wound swab, pus, drain fluid, urine, sputum, Tracheal Secretions were included in this study. Samples were collected under aseptic conditions after obtaining informed oral consent from the patients.

Primary isolation of clinical isolates= samples were inoculated on Blood Agar, MacConkey agar, Thioglycollate broth and incubated at 37°C for 24 hrs. Growth observed next day and processed in Vitek – 2 after performing preliminary test viz gram staining and oxidase disctest. Pure colonies of GNB which grown on MacConkey agar will be used for identification and Antibiotic sensitivity test.

Identification of *Isolate*s by automated vitek-2 system: Pure colonies of GNB which grown on Mac Conkey agar are used for identification in VITEK 2. The method of identify species is[4]

Step 1: Suspension preparations for ID and AST card: Suspension Preparation for ID Card First Transfer 3ml of saline into tube. Select a isolate colony and dissolve it Mix well and check the density with densichek. Inoculum density for GNB should be 0.5- 0.63MCF. Then Place the ID card and tube into Cassette. Suspension Preparation for AST Card- First Transfer 3ml of saline into a Tube. Transfer 145ml of the ID suspension into the saline tube. Then Place the AST card and tube into the cassette.
Step 2: Filling and Loading the card into VITEK 2 System: Set all the Card and Tube with suspension in a Cassette. From PC work station print Cassette work sheet and record job ID and bar code for each card. When instrument status is OK, then press start fill button. Remove the Cassette from loading station at any time. When instruments indicate.
Step 3: Entering Specimen information Log in to window and then into VITEK 2 software with username and password. In the main view, click on the cassette icon. Find the Cassette that have been loaded in the navigation tree on the left side and enter job ID from work sheet and organism name for isolates on with AST cards only. Use define isolates button to link ID and AST cards of the same specimen. Then click the save button.
Step 4: Entering patient information in patient icon, click new patient icon. Enter patient and Specimen information and then save. Then see the result on click icon on the main view.

Observations

Table 1: Showing distribution of Clinical Isolates

S. No.	Name of Organism	No isolated	%
1	E. coli	146	60.83
2	Klebseilla	67	27.91
3	Enterobacter	22	9.17
4	Citrobacter	2	0.83
5	Serratia	3	1.25

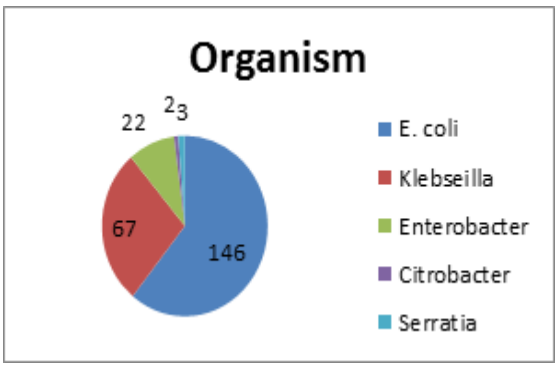


Fig.1: Pie – chart showing distribution of different isolates

Table 2: Percentage of Enzyme producers & Non Enzyme producers

S. No.	Enzyme Producer	%	Non enzyme Producer	%
1	195	81.25	45	18.75

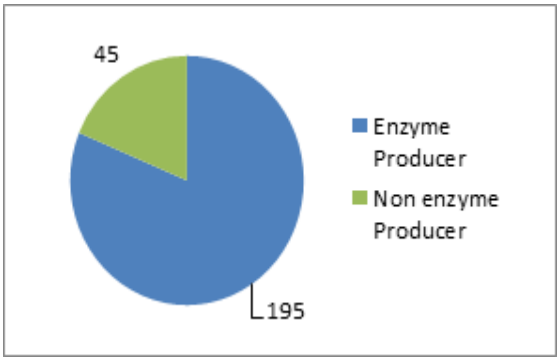


Fig. 2: Pie – chart showing enzyme producers by study isolates

Table 3: Percentage of Single Enzyme Producers & Multiple Enzyme Producers

S. No.	Single Enzyme Producer	%	Multiple Enzyme Producer	%
1	147	61.25	48	20

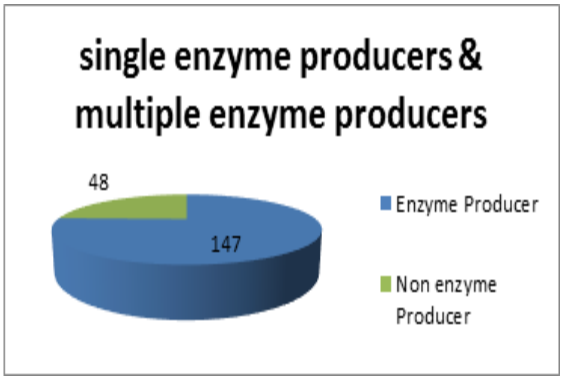


Fig. 3: Pie – chart showing single & multiple enzyme producers

Table 4: Percentage of Organism Showing Single Enzymes

S. No.	Name of Enzyme	No.	%
1	ESBL	105	44
2	Ampc	26	11
3	Carbapenemase	16	7

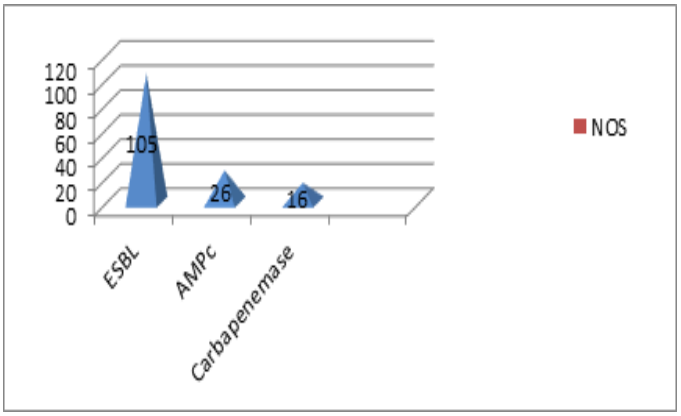


Fig. 4: Pie – chart showing different beta lactamase % among isolates

Table 5: Distribution of total ESBL (in combination with other enzymes), AMPc (in combination with other enzymes) & carbapenemase (in combination with other enzymes) in different Gram Negative Bacteria

S. No.	Organism	ESBL	%	AMPc	%	Carbapenemase	%
1	E. coli	102	69.38	34	23.12	10	6.80
2	Klebsiella	56	38.09	15	10.20	27	18.36
3	Enterobacter	4	2.72	2	1.36	4	2.72
4	Citrobacter	3	2.04	2	1.36	2	1.36
5	Serratia	3	2.04	3	2.04	2	1.36
6	Total	168	70	56	23.33	45	18.75

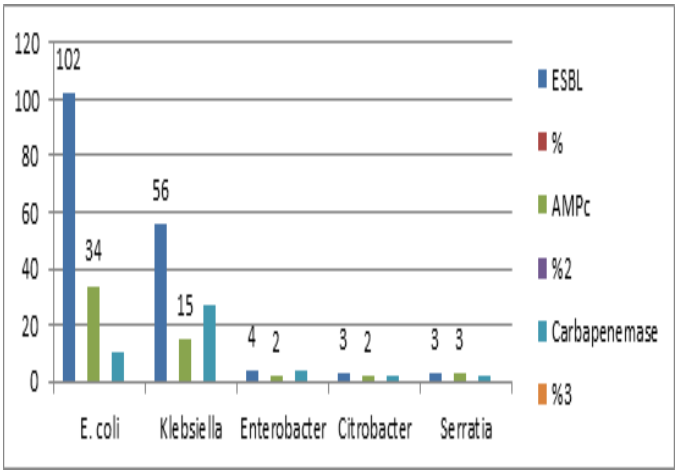


Fig. 5: Graphical representation of different beta lactamases % among different spp

Table 6: Showing enzymes produced by different organism and the percentage of each enzyme/enzyme combination produced by these organism

S. No	Organism	Enzyme Produced						
		ESBL	ESBL + AMPc	ESBL+ Carbapenemase	ESBL+AMPc+ Carbapenemase	AMPc	AMPc+ Carbapenemase	Carbapenemase
1	E. coli	75	9	5	3	22	0	2
2	Klebsiella	27	10	14	4	1	0	9
3	Enterobacter	2	1	1	0	1	0	3
4	Citrobacter	1	1	1	0	1	0	2
5	Serratia	0	1	1	1	1	0	0
6	Total	105	22	22	8	25	0	16
7	Percentage	71.42	45.83	45.83	16.66	17.00	0	10.88

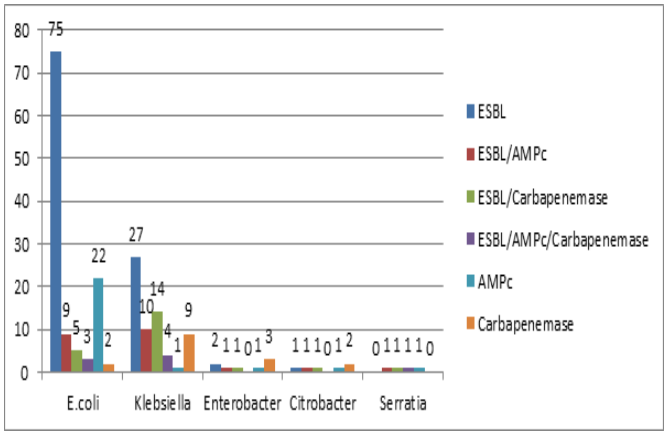


Fig. 6: Graphical representation of different single & multiple beta lactamases % among different spp

Table 7: Sensitivity Pattern of different Isolates producing single & mixed Enzymes

Name of Anti-microbial drug	ESBL		AMPc		Carbapenemase		Mixed Enzymes	
	No	%	No	%	No	%	No	%
Ampicillin	10	9.52	6	23.07	2	12.5	5	10.41
Amoxicillin/Clavulanic Acid	14	13.33	8	30.77	3	18.75	3	6.25
Piperacillin/Tazobactam	22	20.95	4	15.38	5	31.25	8	16.67
Cefuroxime	8	7.61	2	7.69	4	25	2	4.17
Cefuroxime Axetil	8	7.61	2	7.69	4	25	9	18.75
Ceftriaxone	12	11.42	10	38.46	6	37.5	9	18.75
Cefoperazone/Sulbactam	14	13.33	12	46.15	3	18.75	11	22.91
Cefepime	6	5.71	14	53.84	2	12.5	5	10.41
Imipenem	96	91.42	22	84.61	16	100	46	95.83
Meropenem	102	97.14	26	100	16	100	42	87.5
Amikacin	92	87.61	22	84.61	12	75	48	100
Gentamicin	66	62.85	12	46.15	8	50	12	25
Nalidixic Acid	20	19.04	10	38.46	6	37.5	12	25
Ciprofloxacin	24	22.85	8	30.77	4	25	4	8.33
Nitrofurantoin	10	9.52	12	46.15	8	50	6	12.5
Colistin	105	100	26	100	16	100	48	100
Trimethoprim/Sulfamethoxazole	12	11.42	3	11.53	3	18.75	8	16.67

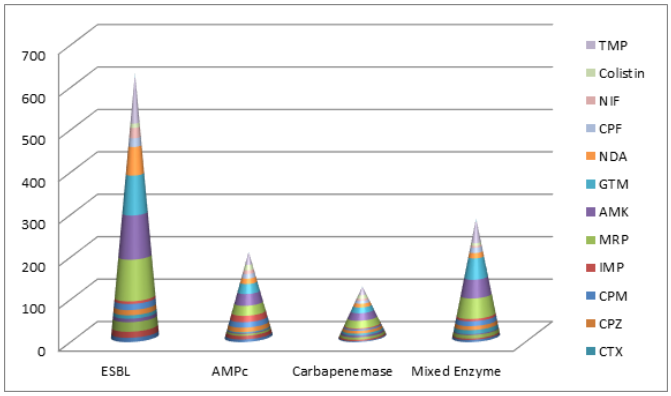
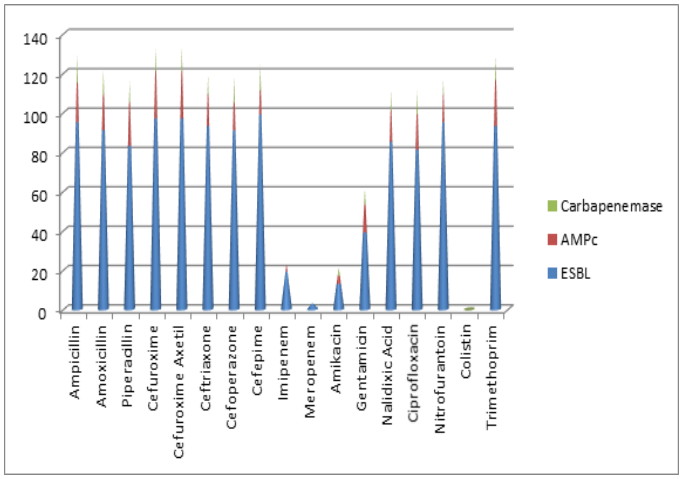


Fig. 7: Showing antibiogram of different enzymes producing isolates

Table 8: Resistant Pattern of different Isolates producing single & mixed Enzymes

Name of Anti-microbial drug	ESBL		AMPc		Carbapenemase		Mixed Enzymes	
	No	%	No	%	No	%	No	%
Ampicillin	95	90.48	20	76.93	14	87.5	43	89.95
Amoxicillin/Clavulanic Acid	91	86.67	18	69.23	13	81.25	45	93.75
Piperacillin/Tazobactam	83	79.05	22	84.62	11	68.25	40	83.33
Cefuroxime	97	92.39	24	92.31	12	75	42	95.83
Cefuroxime Axetil	97	92.39	24	92.31	12	75	39	81.25
Ceftriaxone	93	88.58	16	61.54	10	62.5	39	81.25
Cefoperazone/Sulbactam	91	86.67	14	53.85	13	81.25	37	77.09
Cefepime	99	94.29	12	46.16	14	87.5	43	89.59
Imipenem	19	8.58	4	15.36	0	0	2	4.17
Meropenem	3	2.86	0	0	0	0	6	12.5
Amikacin	13	2.86	4	15.36	4	25	0	0
Gentamicin	39	37.15	14	53.85	8	50	36	75
Nalidixic Acid	85	80.96	16	61.54	10	62.5	36	75
Ciprofloxacin	81	77.15	18	69.23	12	75	44	91.67
Nitrofurantoin	95	90.48	14	53.85	8	50	42	87.5
Colistin	0	0	0	0	0	0	0	0
Trimethoprim/Sulfamethoxazole	93	88.58	23	88.47	13	81.25	48	83.33



Discussion

*Beta-lactamse*producing organisms have been increasingly detected worldwide. Increasing numbers and types of ESBLs have been reported. The total number of ESBLs that are characterized exceeds 200[5]. In present study total ESBL producing isolates were 68(28.9%) out of 240. The major ESBL producer was E. coli 102(42.2%) followed by *Klebsiella* 56(23.3%) and *Enertobacter 4 (1.79*%). Prevalence of ESBLs varies in different regions in India ranging from 60.7% to 73.4% in all gram negative isolates/ *Enterobactericeae*, and 22% to 79% in *E. coli* and 22% to 86.6% in *Klebsiella* spp[6-10]. In present study prevalence of ESBL in gram negative isolates is less (28.9%) as compared to a study by **Amita Jain et al**.[6] from Lucknow who found a prevalence of 73.4% in *Enterobacteriaceae*and another study by **Chaterjee et al**.[10] from Chandigarh who found a 60.7% prevalence of ESBLs in all gram-negative isolates. So also prevalence of ESBLs in *E.coli*and *Klebsiella* is much less as compared to other studies[6,8-10].

In present study, of the 240 isolates 56(23.3%) were Amp C producers .Of this highest producer of Amp C was *E.coli* 34(14.16%), followed by *Klebsiella* 15(6.25%).(Table 5) The prevalenceof Amp C in different regions in Indiaranges from 10.95% to 50.9%[10,11,12,13]. The prevalence of Amp C producers in present study is somewhatcomparable to a study by **Basak** etal.[13] from Wardha who found a prevalence of 19.3% in their study & also with study by **patwardhan** N et al[14] who found a prevalence of 26.6%.

Total carbapenemases producers were 45 out of 240 (18.9%). The prevalence of other carbapenemases in different regions in India from 8% to 37%[14-17]. In our study carbapenemases production was maximum in Klebsiella 27 (11.2%) followed by E. coli 10(4.1%) (Table 5). In a study by **Gupta V** et al.[18] Chandigarh the prevalence of carbapenemases production in Klebsiella

was 37.33% and in E.coli 8%. In present study the prevalence of Carbapenemase in Klebsiella spp was 11.2% which was much less compared to other studies[14-17], whereas **P Dutta** et al.[19] found a prevalence of 5.75% in CRE which was found to be less then present study.

Prevalence of mixed enzyme production in present study was 48(20%) which (28.5%) was similar to study done by **Patwardhan N et** al[14].

ESBL producing isolates in present study shows 79% resistance to piperacillin+Tazobactum & 2.86% resistance to carbapenem. While study done by **Dutta S[21]** et al shows 74% resistance to piperacillin+Tazobactum & 25% resistance to carbapenem.

Mazority of enzyme producing isolates were sensitive to colistin, meropenem, imipenem & amikacin in our study where done as study done by **S Mulla** et al[20] shows sensitivity to meropenem was (69.8%) & to ceftazidime was (74.1%) in CRE isolates.

Conclusion

81.2% of the isolates of gram negative bacteria in our institute produce Beta-lactamase enzymes. Single enzyme producers comprised of 61.25% of the isolates whereas coexistence of more than one enzyme was found in 20% of the isolates, which means that co-existence of different Beta-lactamse enzymes in a single isolate is not uncommon. Prevalence of ESBLs in Gram negative bacterial isolates in our Institute is much less compared to the prevalence in other places in India. Prevalence of Amp C producers is comparable to some studies in India whereas producers of carbapenemases seem to be much less at our Institute. E. coli was a major producer of ESBL and Amp C, whereas Klebsiella species was a major producer of carbapenemase.

The study show that Betalactamase producing microbial organism are very common in Hospital environment and responsible for majority of infections. Vitek-2 compact is found to be rapid and reliable method to identify Betalactamase and their co-existence. It is better than conventional method.

So present study high lights alarming increase in resistance and antibiotic use and the emergence of MDR isolates amongst E. coli & Klebsiella spp. and emphasizes on prompt remedial actions to salvage the situation. Reducing consumption by judicious use is the first intervention in direction of antibiotic surveillance. There is an urgent need for early detection of these isolates for better treatment out comes.

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