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Prevalence of Extended spectrum β-lactamase and AmpC β-lactamase mediated resistance in *gram negative organisms* from central laboratory, RIMS teaching hospital, Raichur, India – A Retrospective Study

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Abstract
The increasing reports on multidrug resistant Escherichia coli have become a potential threat to global health. Here, we present a Retrospective study to characterize Extended Spectrum β-lactamase and AmpC β-lactamase in Gram negative organisms isolated from different human clinical samples. ***Methods:*** *A total of 576 Gram negative bacterial isolates were collected and subjected to standard microbiological techniques, which were further subjected to antimicrobial susceptibility testing by Kirby-Bauer disk diffusion and confirmed by double disk diffusion method.* ***Results:*** *Out of 576 Gram negative bacterial isolates collected, we found Amp C [By Double disc confirmation test] 343(59.54%) was more prevalent than ESBL [By Double disc confirmation test] 230(39.93%) & KPC 3(0.52%) in our study. Escherichia coli (50.17%) [ESBL-Amp C/46.08%-53.06%] were the most frequently isolated pathogen followed by Klebsiella spp (23.61%)[ESBL-Amp C/16.08%-28.27%].* ***Conclusions:*** *Increased prevalence of AmpC more than ESBL in Gram negative organisms emphasizes the need for a continuous surveillance to detect the resistant strains, strict guidelines for the antibiotic therapy and the implementation of infection control measures to reduce the increasing burden of multidrug resistance, also early detection of ESBL & Amp C is necessary to avoid treatment failure and prevent the spread of MDR.*
Keywords: *AmpC β-Lactamase, KPC, Extended Spectrum β-Lactamase*

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I. Introduction

The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major problem that has been observed over the last decade. [1] Countries where infection prevention and control (IPC) protocols are lacking have become the main foci for developing multidrug resistance. [2.] *E. coli* serovars ranges from highly pathogenic to nonpathogenic strains, and cause several clinical manifestations, including bacteremia, sepsis, meningitis, gastroenteritis and Urinary Tract Infections (UTIs).[3] In the recent past, there are alarming reports about the emergence and spread of antimicrobial resistant *E. coli* strains from all around the world. These strains are associated with high morbidity, mortality, increased length of hospitalization and cost of health care. [4] Resistance to third-generation cephalosporin's, poses a great challenge in a developing country like India. [5] Among tropical countries, India has emerged as the focal point of antimicrobial resistance. Various strategies are used by bacteria to remain immune against the deleterious effects of antibiotics. The most important mechanism of resistance is antibiotic hydrolysis mediated by the bacterial enzyme β-lactamase. Beta-lactamase are bacterial enzymes which make the β-lactam antibiotics inactive by hydrolyzing the β-lactam ring. [6] The present study was undertaken to isolate and characterize ESBL and AmpC in *gram negative organisms* from different clinical samples at Central laboratory, RIMS teaching hospital, Raichur, India.

II. Review Of Literature

Isolates producing AmpC & β-lactamase raise special concerns as these isolates have been responsible for several nosocomial outbreaks and high rate of clinical failure among infected patients. [7] Till date, several phenotypic tests for the identification of AmpC producing isolates have been developed. However, there are presently no CLSI approved tests for identification of AmpC β-lactamase producing bacterial pathogens. AmpC β-lactamase producing *E. coli* is being increasingly reported from many parts of the world. [8,9] However, several other studies have reported much higher incidence ranging from 14-49% of AmpC producing isolates of *E. coli*.[10,11] The increasing prevalence of AmpC β-lactamase resistance among *E. coli* is becoming a serious problem worldwide. High-level AmpC production is typically associated with in-vitro resistance to third-

generation cephalosporin's and cephamycins. In connection with this, high clinical treatment failures with broad-spectrum cephalosporins have been documented. [12]

The situation in Asia and particularly in South Asia is quite worrisome. It is more probable that, specifically in India and China, where high incidence of ESBL has been reported in early and late 1990s, CTX-M type producing bacteria have been expanded and took over other types as like in other parts of the world. In the early 1990s, reports indicate that SHV-5 and SHV-12 were more dominant in Korea and Japan [13, 14]; recent studies however indicate that CTX-M is the most dominant genotype of ESBL producers in Asia including China with exception to Japan where CTX-M-2 type has been widely disseminated. [15–17] The rate of ESBL expressing *E. coli* has been described as high as up to 68% in India [18], up to 52% in Pakistan [19], and 30% in China [20]. Our recently unpublished preliminary results based on data collected from poultry and livestock animals and their environment indicate a similar higher range of ESBL producers. More worrisomely, situation in Pakistan is quite alarming [19, 21], in part, due to missing data regarding concise surveillance and estimation of the spread of ESBL producers and due to current trends of overuse of antibiotics in hospital settings, community, livestock and poultry sectors, and agriculture.

III. Aims And Objectives

1. To know the prevalence of ESBL in gram negative isolates from different clinical samples at Central laboratory RIMS teaching hospital, Raichur.
2. To know the prevalence of Amp C in gram negative isolates from different clinical samples at Central laboratory, RIMS teaching hospital, Raichur

IV. Materials And Methods

Collection of isolates and laboratory processing

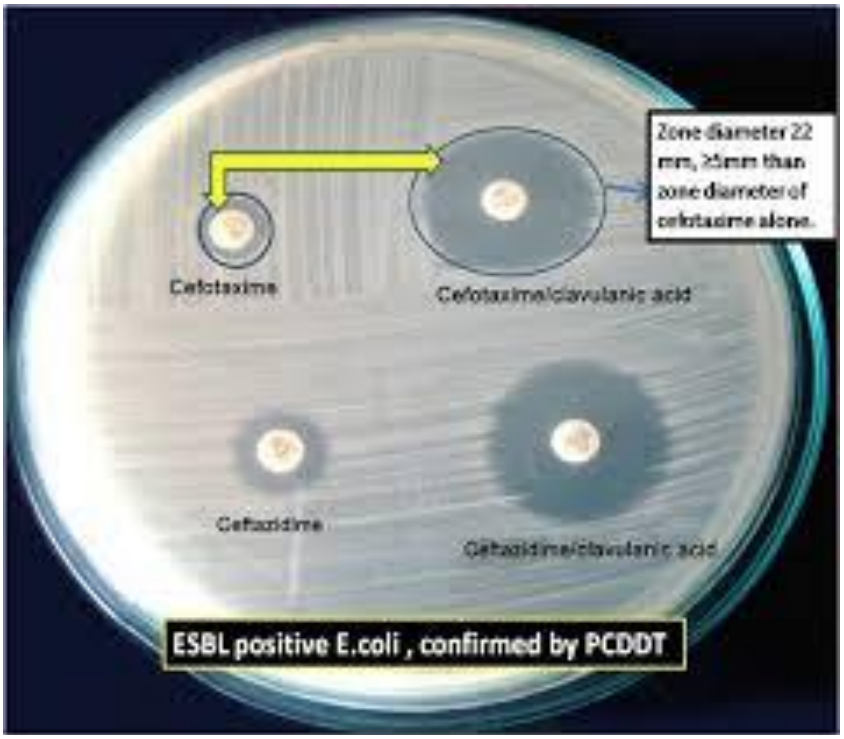
This Retrospective study was performed from January 2018 to June 2019 at Central Laboratory, RIMS teaching hospital, Raichur. A total of 576 Gram negative bacterial isolates were collected from Central Laboratory, RIMS teaching hospital, Raichur. The isolates were derived from samples of pus, urine, sputum, blood, stool, body fluids, wound and other swabs. The demographic information and the history of each patient were obtained from his/her records. The isolates were re-characterized for the identification of *gram negative organisms* following standard microbiological techniques as described by CLSI guidelines.

Tests for ESBL-production

***Screening test*:** All the *gram negative organisms'* isolates were screened for ESBLs by disc diffusion method. In the presumptive test to detect potential ESBL producers, all the isolates were screened for susceptibility to Ceftazidime (30µg) and Cefotaxime (30µg) antibiotic discs (Hi-media, Mumbai). Results were interpreted based on the CLSI guidelines as follows: zones of inhibition of ≤22mm for Ceftazidime and ≤27mm for Cefotaxime indicated ESBL production.

Confirmatory test: The ESBL producing *gram negative organisms* isolates were confirmed by CLSI phenotypic confirmatory test of combined disc assay method. One disc each of Ceftazidime (30µg) and Cefotaxime (30µg) alone and one in combination with Clavulanic acid (10µg) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The ESBL-producing strains showed ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone. *E. coli* ATCC 25922 was used as quality Control strain.

Phenotypic representation of ESBL - figure 1

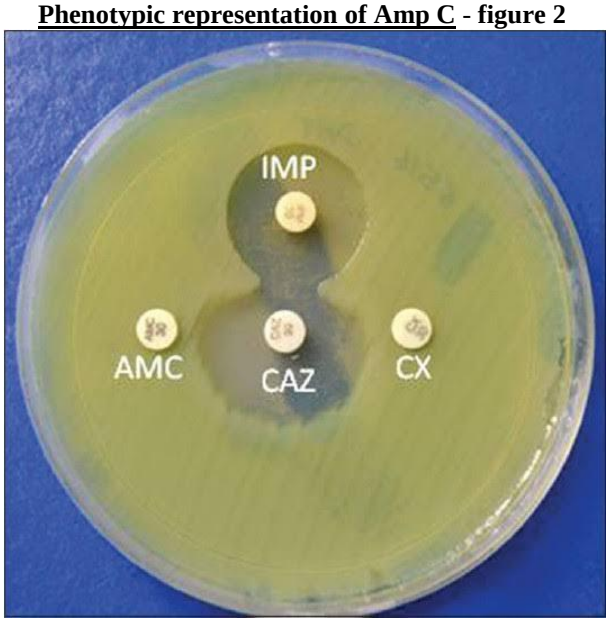


Tests for AmpC-production

Screening test: The isolates were screened for presumptive AmpC production by testing their susceptibility to Cefoxitin (30µg) and Cefotetan (30µg) antibiotic discs (Hi-media, Mumbai) using Kirby Bauer disk diffusion method. All the isolates with an inhibition zone diameter of ≤18mm for Cefoxitin and ≤16mm for Cefotetan were labeled as AmpC positive and were subjected to confirmatory test.

Confirmatory test: AmpC producers were confirmed by phenotypic confirmatory test of combined disc assay method. One disc of Cefoxitin (30µg) alone and one in combination with Cloxacillin (200µg) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The isolated strains demonstrating a zone diameter around the Cefoxitin + Cloxacillin disc ≥5mm than the zone diameter around the Cefoxitin disc alone were considered as AmpC producers. *E. coli* ATCC 25922 was used as quality control strain.

Phenotypic representation of Amp C - figure 2



INCLUSION CRITERIA: All Gram negative isolates
EXCLUSION CRITERIA: All Gram positive isolates

Statistical analysis: The data were entered and analyzed using Statistical Package for Social Sciences SPSS software version21.0. P - Value < 0.05 was considered statistically significant.

V. Results

Table 1: Prevalence of ESBL, AmpC and KPC in E. coli and other Gram negative isolates from various clinical samples

Organisms	No	%	ESBL	%	Amp C	%	Both ESBL & Amp C	%	KPC	%
Escherichia coli	289	50.17	106	46.08	182	53.06	3	60	1	33.33
Klebsiella	136	23.61	37	16.08	97	28.27	2	40	2	66.66
Pseudomonas	86	14.93	66	28.69	20	5.83	0	0	0	0
Acinetobacter	46	7.98	8	3.47	38	11.07	0	0	0	0
Proteus	12	2.08	8	3.47	4	1.16	0	0	0	0
Shigella	1	0.17	1	0.43	0	0	0	0	0	0
Citrobacter	2	0.34	2	0.86	0	0	0	0	0	0
Others	4	0.69	2	0.86	2	0.58	0	0	0	0
Total	576	-	230(39.93)	-	343(59.54)	-	5	-	3(0.52)	-

Table 2: Prevalence of ESBL and Amp C among various clinical samples

Sample	No	%	ESBL	%	AmpC	%	Both ESBL & Amp C	%	KPC	%
Pus	273	47.39	115	50	157	45.77	3	60	1	33.33
Blood	60	10.41	17	7.39	41	11.95	0	0	2	66.66
Urine	134	23.26	52	22.60	82	23.90	0	0	0	0
Stool	21	3.64	5	2.17	16	4.66	0	0	0	0
Sputum	15	2.60	11	4.78	4	1.16	0	0	0	0
Wound	21	3.64	11	4.78	10	2.91	0	0	0	0
Body fluids	18	3.12	5	2.17	13	3.79	2	40	0	0
others	34	5.90	14	6.08	20	5.83	0	0	0	0
Total	576	-	230 (39.93)	-	343 (59.54)	-	5	-	3 (0.52)	-

Table 3: Sex comparison in Outpatient and Inpatient cases

Sex	Outpatient -397(68.92%)	Inpatient-179(31.07%)
Male-304(52.77%)	205(67.65%)	99(32.56%)
Female-272(47.22%)	192(70.58%)	80(29.41%)

Table 4: Prevalence of ESBL, AmpC and KPC among Outpatient and Inpatient cases

OP/IP (Sex Ratio)	No	ESBL	%	AmpC	%	KPC	%
Outpatient Male	205	88	49.43	115	52.99	2	0.50
Outpatient Female	192	90	48.12	102	47	0	0
Outpatient Total	**397**	**178**	**50.56**	**217**	**54.65**	**2**	**0.50**
Inpatient Male	99	28	53.84	70	55.55	1	0
Inpatient Female	80	24	46.15	56	44.8	0	0
Inpatient Total	**179**	**52**	**29.05**	**126**	**70.39**	**1**	**0.50**
Total	576	230	-	343	-	3	-

Table 5: Total number of ESBL and Amp C Screened and Confirmed Cases

Tests Performed	**Amp C** (n=576)	**ESBL** (n=576)
Screening test	Cefoxitin/Cefotetan	Cefotaxime/Ceftazidime
	435 (75.52%)	292 (50.69%)
Confirmatory test	Cefoxitin/Cefotetan + Cloxacillin	Cefotaxime/Ceftazidime + Clavulanic Acid
	343 (59.54%)	230 (39.93%)

Among the 576 gram negative isolates, the prevalence of Amp C 343 (59.54%) was more prevalent than ESBL 230 (39.93%) and KpC 3 (0.52%). The present study identified 289 (50.17%) E. coli isolates and Klebsiella 136(23.61%), Pseudomonas 86(14.93%), Acinetobacter 46(7.98%), Proteus 12(2.08%), Citrobacter 2(0.34%), Shigella 1(0.17%) others 4(0.69%) out of 576 Gram negative bacteria collected from central lab, RIMS, Raichur. Among these major Amp C/ESBL producer was Escherichia coli 182(53.06%)/106(46.08%), followed by Klebsiella spp 97(28.27%)/37(16.08%) in this study [Table-1].

The skin and soft tissue infections [Pus sample] 273 (47.39%) were the most common infections, followed by urinary tract infections [Urine sample] 134 (23.26%) and blood stream infections [Blood sample] 60 (10.41%), Stool 21 (3.64%), Sputum 15(2.60 %), Wound 21(3.64%), Body fluids18 (3.12%) and others 34 (5.25%) out of 576 samples. In pus samples, Amp C 157 (45.77%) was more prevalent than ESBL 115 (50 %) in this study [Table-2].

Positive culture was more Prevalent among Males 304 (52.77%) than Females 272 (47.22%) and Outpatients 397 (68.92%) were more than the Inpatients179 (31.07%) in this study [Table-3].

Among Outpatient male Amp C 115 (52.99%) was slightly more than ESBL 88(49.43%) & in Outpatient female Amp C 102 (47%) was slightly less than ESBL 90(48.12%). While in Inpatient male Amp C 70 (55.55%) was slightly more than ESBL 28(53.84%) & in Inpatient female Amp C 56 (44.8%) was slightly less than ESBL 24(46.15%) in this study [Table 4].

Initial screening test by (Cefoxitin/Cefotetan) disk diffusion method identified possible Amp C producing Gram negative organisms was 435 (75.52%) and Amp C positive confirmatory test by (Cefoxitin/Cefotetan + Cloxacillin) double disk diffusion method showed 343 (59.54%), and ESBL initial screening test by (Cefotaxime/Ceftazidime) disk diffusion method identified 292 (50.69%) and ESBL positive confirmatory test by (Cefotaxime/Ceftazidime + Clavulanic Acid) double disk diffusion method showed 230 (39.93%) in this study [Table-5]. Others have also reported 50-70% prevalence of ESBL producing E. coli. [22, 23]

The present study showed much higher prevalence rates of AmpC producing E. coli, than the ones ranging from 2% to 10% reported from various parts of the world. [12,24], However, several other studies have reported much higher incidence ranging from 14-49% of AmpC producing isolates of E. coli. [10, 11]

VI. Discussion & Conclusion

Isolates producing AmpC β-lactamase raise special concerns as these isolates have been responsible for several nosocomial outbreaks and high rate of clinical failure among infected patients. [7] Till date, several phenotypic tests for the identification of AmpC producing isolates have been developed. However, there are presently no CLSI approved tests for identification of AmpC β-lactamase producing bacterial pathogens. AmpC β-lactamase producing E. coli is being increasingly reported from many parts of the world. [8, 9]

The increasing prevalence of AmpC β-lactamase resistance among E. coli is becoming a serious problem worldwide. High level AmpC production is typically associated with in-vitro resistance to third-generation cephalosporins and cephamycins. In connection with this, high clinical treatment failures with broad-spectrum cephalosporins have been documented. [12]

In our study prevalence of AmpC is more than ESBL among gram negative organisms. The most common organism was Escherichia coli around 289 (50.17%) and Amp C prevalence was 182 (53.06%) among Uropathogens. Carbapenems are considered to be one of the antibiotics of last resort for treatment of infections caused by multi drug resistant bacteria such as E. coli. [11] Alarms have been raised over and over on the dangers of spreading of Carbapenem resistant bacteria in hospitals.

Communities; where they can be acquired if IPC programs are not in place. Antibiotic overuse is an important contributor for the emergence and spread of resistance; association between Carbapenem consumption and resistance has been previously documented.

ESBL production varies from hospital to hospital because of variation in selection of type of antibiotics. The selective pressures which are generated by the indiscriminate use of the beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamase. [25]

The early detection of beta lactamase producing E. coli would be important for the reduction of morbidity and mortality and also to avoid the dissemination of such strains within the community. The present study observed increased prevalence of ESBL and AmpC producing E. coli. This study underlines a real threat from the emergence of pan drug-resistant bacteria in near future. The spread of ESBL/AmpC/KPC producing Gram negative organisms has been noticeably rapid worldwide including India, indicating that continuous monitoring systems and effective infection control measures area absolutely required.

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