Original Research Article

A Study on Extended Spectrum Beta Lactamse and Amp C Beta Lactamse Producing Enterobacteriaceae Isolates from Various Clinical Samples in a Tertiary Care Hospital

Radhika Katragadda[1]*, Sowmya A. Venkateswaran[1] and J. Padmakumari[2]

[1]*Department of Microbiology, Government Medical College, Omandurar Govt. Estate, Chennai-2, Tamil Nadu, India*
[2]*Institute of Microbiology, Madras Medical College, Chennai, Tamil Nadu, India*

*Corresponding author

A B S T R A C T

Over the period of years infections caused by multidrug resistance organisms has emerged as a major public health problem. Their prevalence rates vary in different parts of the world and hence local data regarding these pathogens were important. Our study was aimed to identify the presence of ESBL and AmpC producing Enterobacteriaceae isolates from various clinical samples in our hospital setup various clinical samples were processed consecutively during the study period for microbiological analysis as per standard operating procedure. Enterobacteriaceae isolates were further tested by phenotypic confirmatory methods for ESBL and AmpC production, as per CLSI guidelines. Out of 1583 samples processed, 522 samples were culture positives (32.97%). 74.52% of isolates belongs to Enterobacteriaceae family. Most common Enterobacteriaceae isolate was *E.coli* (42.42%) followed by *Klebsiella* species (41.90%) and Proteus species (11.06%). Among the total 389 Enterobacteriaceae isolates 152(39.07%) were ESBL producers and 8(2.11%) were Amp C producers. *E.coli* and *Klebsiella* species were the most common ESBL producing isolates (41.45% each), whereas the majority of AmpC producers were *K.pneumoniae* (75%). Early detection and proper management of infections caused by these MDR organisms are very important in preventing their emergence and spread. Time to time knowledge about their prevalence and their antibiotic resistance pattern can become a powerful tool in handling infections caused by them.

Introduction

Over the last two decades, infections caused by multidrug resistance (MDR) organisms have emerged as a major public health problem, especially with those that have become resistance to third generation cephalosporins and carbapenems. Their widespread global dissemination has become a significant problem worldwide. Enterobacteriaceae group of Gram negative bacteria, are the most common bacteria's isolated from majority of clinical samples. Antibiotic resistance among these group of bacteria is a rapidly emerging problem in public health sector, as they are capable to

acquire, transmit and mutate plasmids and other mobile genetic elements carrying antimicrobial resistance genes among each other and also among closely related bacteria's with ease (Binita Bhuyan *et al*, 2018).

The detection of antibiotics by our pioneers was a great boon to mankind, which protected us from infections. However bacteria's were constantly evolving and developing various strategies to become immune against the cidal effects of antimicrobial agents. One of the important mechanisms of antimicrobial resistance was the production of certain enzymes by the bacteria, which can inhibit or destroy the action of antimicrobial drugs. Among Gram negative bacteria production of beta lactamases, especially extended spectrum beta lactamases (ESBLs), Metallo beta-lactamases (MBLs) and AmpC production has emerged as a major cause of antimicrobial resistance **(Pfeifer Y *et al.,* 2010).** Infections caused by such multidrug resistance bacteria pose significant threat to treating clinician and also to patients, by means of prolonged hospital stay, high health care costs and high mortality and morbidity rates.

ESBLs are beta lactamases producing bacteria that belong to Group 2be of Bush-Jacoby-Medeiros classification. They work by hydrolyzing the beta lactam ring of beta lactam antibiotics, like cephalosporins, aztreonam etc. They are inhibited by using beta-lactamase inhibitors like sulbactam, tazobactam, clavulanic acid (Rawat *et al.*, 2010)**.** AmpC beta-lactamases are well defined enzymes, belonging to group 1 of Bush-Jacoby-Medeiros classification. These enzymes, both chromosomal and plasmid mediated show an action spectrum similar to ESBLs. However they are not inhibited by beta lacatmase inhibitors and they respond to carbapenem group of drugs (Tamang *et al.*, 2012). This study was conducted to analyse

the presence of ESBL producing and AmpC producing Enterobacteriaceae isolates from various clinical samples in our tertiary care centre.

Materials and Methods

The study was conducted after getting Institutional Ethical Committee approval. During the three months study period various consecutive samples like urine, pus, throat swabs, wound swabs, body fluids, sputum and blood received for culture and sensitivity were processed according to standard operating guidelines. Samples were inoculated onto Nutrient agar, MacConkey agar and Blood agar plates by sterile technique. The inoculated plates were incubated at 37^0C overnight and the resultant colonies were identified using Gram's stain and conventional biochemical reactions like catalase, oxidase, oxidation –fermentation test, triple sugar iron test, citrate test and urease test. Antibiotic sensitivity testing of the isolates was carried out by modified Kirby Bauer disc diffusion technique, according to CLSI guidelines. Isolates belonging to Enterobacteriaceae family were further tested along with appropriate controls for the production of extended spectrum beta lactamase (ESBL) and AmpC enzymes as per CLSI guidelines 2017.

Detection of ESBL production

Enterobacteriaceae isolates which showed resistance to cefotaxime (30 μg) (≤27mm) were presumptively identified as ESBL producers and confirmed by phenotypic combined double disc diffusion testing method. 0.5 McFarland suspension of the isolate was inoculated onto Mueller Hinton agar. Cefotaxime (30 μg) disc and cefotaxime + clavulanic (30/10 μg) disc were placed on the surface of the inoculum at 20mm apart. The plates were incubated at 37^0C overnight.

A increase of zone of inhibition of ≥ 5mm in the combined disc (cefotaxime + clavulanic (30/10 μg) when compared to cefotaxime disc (30 μg) were confirmed as ESBL producers. The sensitivity and specificity range of this double disc diffusion testing ranges from 79% to 97% and 94% to 100% respectively (Randegger C *et al*, 2001) (Vercauteren E *et al.*, 1997).

Detection of Amp C production

Enterobacteriaceae isolates which showed resistance to cefoxitin (30μg) (≤14mm) as tested by Kirby Bauer disc diffusion technique, were presumptively identified as AmpC producers and were further subjected to combined disc assay confirmatory test. 0.5 McFarland suspension of the isolate was inoculated onto Mueller Hinton agar. Cefoxitin disc (30μg) and Cefoxitin +Cloxacillin combination disc (30μg+ 200μg) were placed onto the surface of the inoculum at 20mm apart. The plates were incubated at 37^0C overnight. An increase of zone of inhibition of ≥ 5mm in the combined disc (cefoxitin + cloxacillin (30μg+ 200μg)) when compared to cefoxitin disc (30 μg) were confirmed as Amp C producers (Rituparna Tewari *et al.*, 2018).

The results were documented and analyzed statistically.

Results and Discussion

The study was conducted over a period of three months, during which around 1583 varied consecutive clinical samples that were sent to microbiology laboratory for culture and sensitivity testing were included in the study. Out of the total 1583 samples processed, 522 samples came out as culture positives (32.97%).

Out of the total culture positives, 389 (74.52%) of isolates belongs to

Enterobacteriaceae family, 98 isolates (18.78%) were Gram positive organisms and 35 isolates (6.70%) were non fermentors.

Most common Enterobacteriaceae isolate was *E.coli* (42.42%) followed by *Klebsiella* species (41.90%) and *Proteus* species (11.06%). Majority of the Enterobacteriaceae isolates were from urine samples (57.58%) followed by pus samples (32.91%).

Among the total 389 Enterobacteriaceae isolates 152 (39.07%) were ESBL producers and 8 (2.11%) were Amp C producers.

E.coli and *Klebsiella* species were the most common ESBL producing isolates (41.45% each), whereas the majority of AmpC producers were *K. pneumoniae* (75%).

Out of 152 ESBL isolates, 60.53% were isolated from urine samples followed by pus samples (28.28%). 75% of AmpC isolates were from urine samples and the rest of 25% of Amp C producers were from pus samples.

One of the major concerns in clinical practice, especially in developing countries is the increasing prevalence of infections caused by multidrug resistance organisms. Their prevalence and the type of infections they are associated with vary in different regions of the world owing to the different patterns of antibiotic policies they follow. This study is conducted in our tertiary care hospital to analyse the presence of ESBL and AmpC producing Enterobacteriaceae isolates from varied clinical samples.

Out of the total 1583 samples processed, 522 samples were culture positives (32.97%). Majority of culture positives were from urine samples (54.21%) followed by pus samples (31.42%), as urine and pus samples were the most common samples received for Microbiological evaluation, in any tertiary care hospital (Wondemagegn Mulu *et al.*,

2017). Out of 522 culture isolates, 389 (74.52%) belongs to Enterobacteriaceae family, 98 isolates (18.78%) were Gram positive organisms and 35 isolates (6.70%) were non fermenters. Study conducted by Sanjo Gupta *et al.,* (2017), had documented about 60% of their clinical isolates from varied clinical samples belongs to Enterobacteriaceae group of bacteria, which is less when compared to our study.

The most common Enterobacteriaceae isolate was *E.coli* (42.42%) followed by *Klebsiella* species (41.90%) and *Proteus* species (11.06%). Similar studies conducted by Narinder Kaur *et al.,* (2017) and Ashish Jitendranath *et al.,* (2018) also showed that *E.coli* was the most common isolate (41.6%, 54% respectively), followed by *Klebsiella* species (24% 32% respectively). However

study conducted by Binita Bhuyan *et al.,* (2018) showed that *Klebsiella* spp (55.5%) was the most common isolate among Enterobacteriaceae followed by *E.coli* (23.9%)**.**

Among the total 389 Enterobacteriaceae isolates 152 (39.07%) were ESBL producers. Study conducted by Binita Bhuyan *et al* (2018) and Narinder Kaur *et al.,* (2017) revealed that about 14.75% and 25% of their Enterobacteriaceae isolates were ESBL producers. Whereas study conducted by Sanjo Gupta *et al.,* (2017) had documented 68% of ESBL producers among Enterobacteriaceae isolates. Various studies conducted in India had documented that the prevalence of ESBL production among various Gram negatives differs from 19.8%-43% (Kumar MS *et al.*, 2006).

Table.1 Nature of samples and culture positives

Sample	No. of samples processed (n=1583)		Culture positives (n=522)	
	No	%	No	%
Urine	839	53	283	54.21
Pus	321	20.28	164	31.42
Wound swab	95	6	25	4.79
Sputum	81	5.12	22	4.22
Blood	132	8.33	17	3.26
Throat swab	64	4.04	9	1.72
Body fluids	51	3.23	2	0.38
Total	1583	100	522	100

Majority of culture positives were from urine samples (54.21%) followed by pus samples (31.42%)

Table.2 Enterobacteriaceae isolates among various clinical samples (n=389)

Sample/ isolates	*E.coli*	*K. pneumoniae*	*K. oxytoca*	*E. aerogenes*	*Citrobacter sp.*	*P. mirabilis*	*P. vulgaris*	*Providencia sp.*	Total
Urine	101	66	28	5	1	8	13	2	224 (57.58%)
Pus	53	37	12	5	2	14	5	-	128 (32.91%)
Wound swab	10	1	3	1	-	3	-	-	18 (4.63%)
Sputum	-	8	3	-	1	-	-	-	12 (3.09%)
Blood	1	2	1	-	-	-	-	-	4 (1.03%)
Throat swab	-	2	-	-	-	-	-	-	2 (0.51%)
Body fluids	-	-	-	1	-	-	-	-	1 (0.25%)
Total	165 (42.42%)	116 (29.82%)	47 (12.08%)	12 (3.08%)	4 (1.03%)	25 (6.43%)	18 (4.63%)	2 (0.51%)	389 (100%)

Table.3 ESBL and Amp C producing Enterobacteriaceae isolates

Isolates	ESBL producers (n=152)		AmpC producers (n=8)	
	No	**%**	**No**	**%**
E.coli	63	41.45	1	12.5
K.pneumoniae	38	25	6	75
K.oxytoca	25	16.45	1	12.5
P.mirabilis	7	4.61	-	-
P.vulgaris	18	11.84	-	-
E.aerogenes	1	0.65	-	-
Total	152	100	8	100

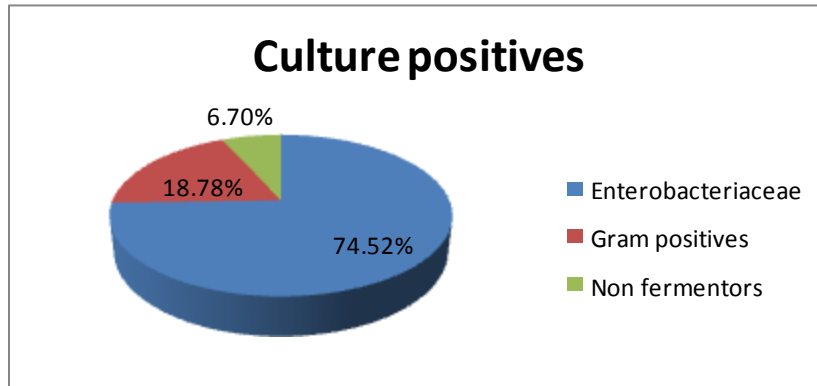**Chart.1** Culture positivity isolates (n=522)

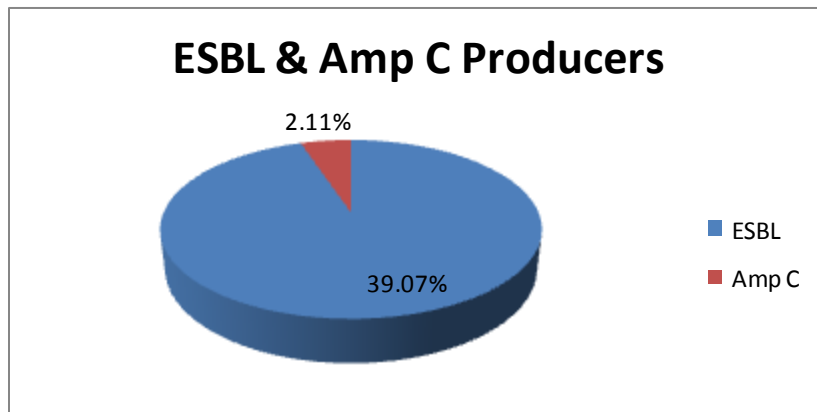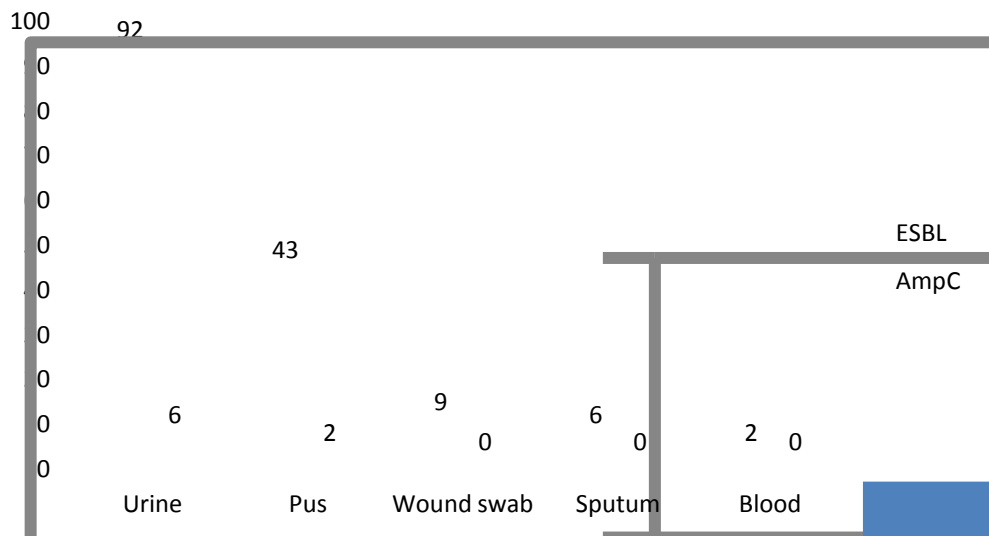**Chart.2** ESBL and AmpC producers among the Enterobacteriaceae isolates (n=389)

Chart.3 ESBL and AmpC producers among various clinical isolates (n=152)

E.coli and *Klebsiella* species were the most common ESBL producing isolates (41.45% each) in our study, followed by *Proteus* species (16.45%). Similar results were obtained in the study conducted by Mita D. Wadekar *et al.,* (2013) and Binita Bhuyan *et al.,* (2018) who had revealed that 50% and 21% respectively of their *E.coli* isolates were ESBL producers when compared to *Klebsiella* spp.(37.5%, 16% respectively). Binita Bhuyan *et al* (2018) had also documented that 14.3% of their *Proteus* isolates were ESBL producers, which correlates well with our study.

In our current study about 8 (2.11%) isolates were Amp C producers. Study conducted by Baha Abdalhamid *et al.,* (2017) had documented that 1% of their Enterobacteriaceae isolates were Amp C beta lactamase producers whereas study conducted by Pankaj Baral *et al.,* (2013) had revealed that about 27.8% of their Enterobacteriaceae isolates were AmpC producers whereas study by Ashish Jitendranath *et al.,* (2018) had documented 11.2% of Enterobacteriaceae isolates as AmpC producers. This wide variation among the ESBL and AmpC producers among Enterobacteriaceae isolates were due to varying prevalence of site and type of infections among various hospitals.

Klebsiella species was the predominant AmpC β lactamase producing agents (87.5%) followed by *E.coli* (12.5%). Similar results were shown in Ashish Jitendranath *et al.,* (2018) study were 53.6% of AmpC producers belongs to *Klebsiella* spp. Followed by *E.coli* (21.4%) and *Enterobacte*r (14.3%). Also Shubhdeep Kaur *et al.,* (2016) in his study has shown that about 14.4% of *Klebsiella* species and 7.8% of *E.coli* isolates were Amp C producers.

Out of 152 ESBL isolates in our study, 60.53% were isolated from urine samples followed by pus samples (28.28%). 75% of AmpC isolates were from urine samples and the rest of 25% of Amp C producers were from pus samples. Similar results have been obtained in the study conducted by Kumar MS *et al.,* (2006) and Kritu panta *et al.,* (2013), where nearly 54% and 89.2% respectively of their ESBL and Multi drug resistant (MDR) isolates respectively were from urine samples**.** This high prevalence of ESBL and Ampc isolates among urine samples may be due to indiscriminate and over the counter use of antibiotics.

In conclusion, infectious diseases caused by various β-lactamases producing bacteria are emerging as a major threat to the public health. As ESBLs and AmpC producers are resistant to most of the second and third line antibiotics, it becomes increasingly mandatory to identify them, so that appropriate infection control measures can be ensured to prevent their emergence and spread, both in the hospital setup and also in the community. The general population and healthcare professionals should be educated about appropriate use of antibiotics which will help limit further spread of these multi-drug resistant bacteria. Further periodic updates in the resistance pattern of these MDRs from time to time among different setups and areas, may pave way for formulating effective empirical therapy and in also addressing various problems associated with infections caused by MDRs.

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