Original Article

Microbiology Section

The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India

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ABSTRACT

Background and Objectives: Extended-spectrum β-lactamase (ESBL) production in the members of the family *Enterobacteriaceae* can confer resistance to expanded–spectrum cephalosporins such as aztreonam and the penicillins. In the recent years, there has been an increased incidence and prevalence of ESBLs all over the world and also in various parts of India. As there was no data which was available on the prevalence of ESBL in this region and as multi-drug resistance was rampant, the current study was undertaken to know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre.

Aim: To know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre.

Materials and Methods: This study was carried out on 218 clinical isolates of Enterobacteriaceae. The screening for ESBL production was done by the disc diffusion test which was recommended by the Clinical and Laboratory Standards Institute(CLSI) and the screen positive isolates were confirmed by the the double disc synergy test (DDST) and phenotypic disc confirmatory test (PDCT).

Results: *E.coli* (57.8%) was most common isolate, followed by *K. pneumoniae* (25.6%). ESBL production was confirmed in 70(32.1%) isolates. The isolates of *K. pneumoniae* (46.4%) were the most common ESBL producers, followed by the isolates of *E coli* (31.7%) and others. ESBL production was most commonly seen in the Enterobacteriaceae which were isolated from the intensive care unit patients.

Conclusion: There is a high prevalence of ESBL production in our hospital. Specific tests to detect ESBL production should be done routinely and an empirical therapy policy should be applied to the high risk units, based on the prevalence of the ESBL producing Enterobacteriaceae.

Key Words: Extended-Spectrum β-lactamases, Enterobacteriaceae, Double disc synergy test, Phenotypic disc confirmatory test

KEY MESSAGE

- There is high prevalence of ESBL producing Enterobacteriaceae.
- Specific tests to detect ESBL production among the Enterobacteriaceae should be done on a routine basis in all the clinical laboratories.
- Based on the prevalence of ESBL producing Enterobacteriaceae, each hospital should formulate a policy of empirical therapy in the high risk units.

INTRODUCTION

Microbes are remarkably adaptable and amazingly versatile. Through the course of evolution, they have developed sophisticated mechanisms for preserving genetic information and disseminating it efficiently in the interests of their survival. They recognize no boundaries. The resistance developing in one part of the country, or indeed in the world, can be disseminated readily [1].

The problem of microbial drug resistance is a major public health concern due to its global dimension and alarming magnitude, although the epidemiology of resistance can exhibit a remarkable geographical variability and a rapid temporal evolution. The major resistance issues overall, are those which are related to the methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase producing Enterobacteriaceae, and the multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [2].

The extended- spectrum β-lactamases (ESBLs) are mutant, plasmid mediated β-lactamases which are derived from the older, broad spectrum β-lactamases and they confer resistance to all the extended spectrum cephalosporins and aztreonam, except to the cephamycins and the carbapenems [3], [4]. ESBLs have spread threateningly in many regions of the world and they presently comprise over 300 variants [5].

These enzymes are the result of the mutations of the TEM-1 and TEM-2 and the SHV-1 enzymes. All of the β-lactamase enzymes are commonly found in the *Enterobacteriaceae* family. Normally the TEM-1, TEM-2 and the SHV-1 enzymes confer a high level resistance to the early penicillins and a low level resistance to the first generation cephalosporins. The widespread use of the third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes, that has led to the emergence of the ESBLs. Because of their greatly extended

substrate range, these enzymes were called as the extended-spectrum β-lactamases [6].

The first ESBL isolates were discovered in Western Europe in the mid 1980s and subsequently in the US in the late 1980s [7]. ESBLs can be found in a variety of Entrerobacteriaceae species, but however, the majority of ESBL producing strains are *K. pneumoniae, K. oxytoca* and *E.coli*. Other organisms which are reported to harbour ESBLs include *Enterobacter* spp., *Salmonella spp*., *Morganella morganii, Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeruginosa*. However, the frequency of ESBL production in these organisms is low [6].

The resistant organisms are now a world wide problem. They are found in variety of Enterobacteriaceae species. Over the last 15 years, numerous outbreaks of infections which were caused by ESBL producing organisms have been observed world wide [8].

The overall prevalence of ESBL-positive Enterobacteriaceae varies greatly among different geographical areas. According to published reports from Europe, ESBLs appeared to be increasing among Enterobacteriaceae in the periods 1997 through 1999 to 2001 and 2002. In Enterobacteriaceae, classical ESBLs evolved from the TEM and the SHV families. In the recent years, several new ESBLs of the non-TEM and the non-SHV types emerged, such as the enzymes of the CTX-M, PER, VEB, and the GES lineages [9].

In India, the ESBL producing strains of Enterobacteriaceae have emerged as a challenge in the hospitalized as well as the community based patients. These have been studied at New Delhi [10], Varnasi [11], Chennai [12], Coimbatore [13], Pondicherry [14], Mumbai [15], Aligarh [16] and also in various parts of the country. In Karnataka, they have been studied at Gulbarga [17], Bangalore [18], Hubli [19] and Davangere [20].

The advent of the ESBL producers has posed a great threat to the use of many classes of antibiotics, particularly the cephalosporins. The detection of ESBL expression has proved to be difficult for many laboratories because the resistant ESBL producing organisms appear to be susceptible in the *in vitro* routine testing and result in treatment failure [21], [22].

Hence, ESBL detection should be routinely undertaken by using specific detection methods for the proper management of infections. As there was no data which was available on the prevalence of ESBL production in this region and as multi-drug resistance was rampant, the current study was undertaken to know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology at the Shri B M Patil Medical College, Bijapur, from June 2009 to May 2010.

Sample size: All the clinical samples that came to the Microbiology laboratory during the study period constituted the material for the study.

A total of 218 random, non repetitive, clinical isolates of Enterobacteriaceae, which were recovered in the microbiology laboratory over a period of one year, were identified, based on the colony morphology and the biochemical reactions from a variety of clinical specimens like urine, stool, sputum, blood, exudates, pus and other body fluids.

Inclusion criterion: The samples which yielded Enterobacteriaceae were included in the study.

Exclusion criterion: The samples which did not yield Enterobacteriaceae were excluded from the study.

Antimicrobial susceptibility tests were performed by using the Kirby Bauer disc diffusion method as per the CLSI guidelines [23]. The antimicrobials which were tested were ampicillin (10μg), amikacin (30μg), cefuroxime (30μg), ciprofloxacin (5μg), gentamicin (10μg), co-trimoxazole (25μg), nalidixic acid (30μg), gatifloxacin(5 μg), nitrofurantoin(300μg), ceftazidime and imipenem (10μg). All the discs were obtained from Hi-Media, Mumbai, India.

Screening for ESBL producers by the disk diffusion methods: The screening for ESBL producers was done by the disc diffusion test as was recommended by the CLSI [23], [24]. Ceftazidime (30 μg) was used as indicator drug. Those with a zone diameter of ≤22mm were suspected of possible ESBL production and these were confirmed by the double disc synergy test and the phenotypic disc confirmatory test.

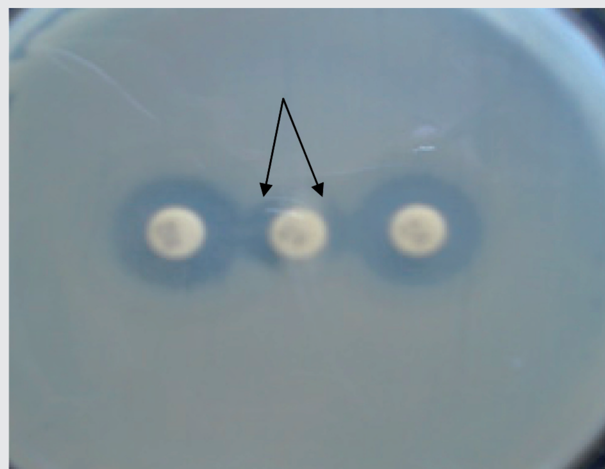
The Detection of ESBLs by the Confirmatory Tests

1. The double disc synergy test (DDST) [25]

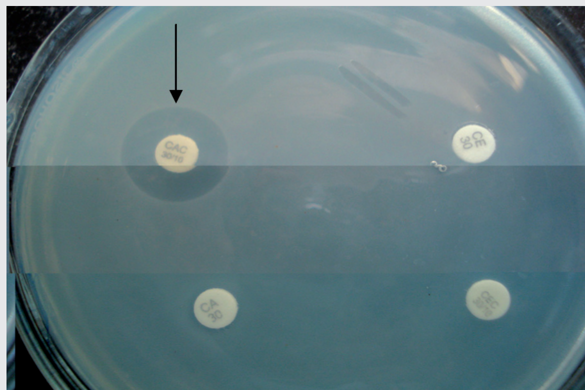
The test inoculum (0.5 McFarland's turbidity) was spread onto Mueller-Hinton agar(MHA) by using a sterile cotton swab. A disc of augmentin (20 μg amoxycillin + 10 μg clavulanate) was placed on the surface of the MHA; then, discs of cefotaxime (30 μg) and ceftazidime (30 μg) were kept 16 to 20 mm apart from the augmentin disc (centre to centre). The plate was incubated at 37 °C overnight. The enhancement of the zone of inhibition of the cephalosporin disc towards the clavulanic acid disc was inferred as synergy and the strain was considered as an ESBL producer. [Table/Fig-1]

2. The phenotypic disc confirmatory test (PDCT) [24]

This test was performed as a disc diffusion test, as recommended by the CLSI. The test inoculum (0.5 McFarland's turbidity) was spread onto the MHA by using a sterile cotton swab; then, a) a ceftazidime(CA) disc containing 30 μg of the antibiotic and a ceftazidime- clavulanic acid (CAC) disc containing 20+10 μg of the antibiotics were placed at a distance of 30 mm from each other b) a cefotaxime(CE) disc containing 30 μg of the antibiotic and a cefotaxime-clavulanic acid (CEC) disc containing 20+10 μg of the antibiotics were placed at a distance of 30 mm from each other. [Table/Fig-2]



[Table/Fig-1]: Organism showing (arrow) enhanced zone of inhibition between ceftazidime/cefotaxime and clavulanic acid disc indicating positive ESBL.



[Table/Fig-2]: A ≥ 5 mm increase in zone of inhibition (arrow) for ceftazidime clavulanic acid (CAC) versus its zone diameter when tested alone by ceftazidime , confirmed an ESBL-producing organism. ESBL was not detected by cefotaxime clavulanic acid(CEC) and cefotaxime as there is no increase in in zone of inhibition for CEC.

The plates were incubated overnight at 37°C and the results were read. A ≥ 5 mm increase in the zone diameter for CAC, versus its zone diameter when it was tested alone by CA and/or a ≥ 5 mm increase in the zone diameter for CEC, versus its zone diameter when it was tested alone by CE, confirmed an ESBL-producing organism. All the discs were obtained from Hi- Media, Mumbai, India.

Quality control when performing the screening and the phenotypic confirmatory tests [24].

Simultaneous tests with a non-ESBL-producing organism (*Escherichia coli* ATCC 25922) and an ESBL-producing organism (*Klebsiella pneumoniae* ATCC 700603) were performed.

RESULTS

The present study was conducted in the Department of Microbiology at the Shri B M Patil Medical College, Bijapur, from June 2009 to May 2010, to know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre.

The antibiotic sensitivity pattern of the isolates revealed that 92.6% of the isolates were sensitive to imipenem, 67.6% were sensitive to amikacin, 28.5% were sensitive to gatifloxacin and 23.2% were sensitive to gentamicin. High resistance was seen for cefuroxime (99.1%), ampicillin (94.5%), ceftazidime (91.8%), co-trimoxazole (91.7%), ciprofloxacin (84.4%), nitrofurantoin (83%) and nalidixic acid (81%).

Out of 218 enterobacteriaceae isolates, 200 were suspected to be ESBL producers, based on the screening method which was suggested by the CLSI. Out of the 200 suspected isolates, 70 (32.1) were confirmed as ESBL producers. DDST detected only 61 ESBL producers and all the 70 were detected by the PDCT [Table/Fig-3].

Organism	No. of isolates	Percentage
E. coli.	126	57.8
K. pneumoniae.	56	25.6
Citrobacter spp.	14	6.5
Proteus spp.	14	6.5
Salmonella spp.	4	1.8
Enterobacter spp.	4	1.8
Total	218	100

[Table/Fig-3:] Isolates of family Enterobacteriaceae from clinical samples

[Table/Fig 3] shows different members of family *Enterobacteriaceae* isolated from clinical samples. *E.coli* was the most common (57.8%) isolate followed by *K. pneumoniae* (25.6%) and others.

K. pneumoniae was the most common ESBL producing Enterobacteriaceae, followed by *E. coli* and others, as shown in [Table/Fig-4], by both the PDCT and the DDST.

Organism	ESBL Producers	
	PDCT*	DDST†
E.coli.	40 (31.7%)	36 (28.6)
K. pneumoniae.	26 (46.4)	24 (42.9)
Citrobacter spp.	02 (14.3)	00
Proteus spp.	02 (14.3)	01 (07.1)
Salmonella spp.	00	00
Enterobacter spp.	00	00
Total	70 (32.1)	61 (27.9)

[Table/Fig-4]: ESBL producers among different isolates of family *Enterobacteriaceae*
*Phenotypic disc confirmatory test; † Double disc synergy test.

Specimen wise distribution of ESBL producers is shown in [Table/Fig-5]. Maximum ESBL producers were seen in urine samples.

Specimen	No of isolates	ESBL producers	Percentage
Urine	117	46	39.1
Blood	39	12	30.7
Exudates/pus	30	7	23.3
Sputum	23	5	21.7
Others	9	0	0
Total	218	70	32.1

[Table/Fig-5]: Specimen wise distribution of ESBL producers

[Table/Fig-6] shows that maximum ESBL producing isolates were from ICCU, surgical ICU and medical ICU.

Wards	No. of isolates	ESBL producers	Percentage
Medicine	55	06	10.9
Surgery	34	03	08.9
OBG	33	09	27.2
Orthopedic	21	05	23.9
Pediatric	13	06	46.2
ENT	7	02	28.6
Medical ICU	20	13	65.0
Surgical ICU	30	22	73.33
ICCU	5	04	80.00
Total	218	70	32.1

[Table/Fig-6]: Department wise distribution of ESBL producers

The age and sex wise distribution of the ESBL producers which is shown [Table/Fig-7], revealed that the maximum number of ESBL producers were seen in the 41-60 years age group and that the prevalence was more among the females than among the males.

DISCUSSION

The spread of ESBL-producing bacteria has been strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required.

Parameter	Value	No. of isolates (n=218)	ESBL produces (n=70)	Percentage
Sex	Male	87	33	37.9
	Female	141	37	26.2
Age group	1–20	32	08	25
	21–40	54	16	29.6
	41–60	72	28	38.9
	>61	60	18	30

[Table/Fig-7]: Age and sex wise distribution of ESBL producers

Therapeutic options for the infections which are caused by the ESBL producers have also become increasingly limited [5].

Antibiotic resistance has been noted as a serious problem, even at our medical college hospital. The third generation cephalosporins have been used in a majority of patients and resistance even to these antibiotics has been reported. As there was no data which was available on the prevalence of ESBL production in this region, the current study was undertaken to know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre.

Out of the 218 Enterobacteriaceae isolates, a majority were *E. coli* (57.8%), followed by *Klebsiella pneumoniae* (25,6%), *Citrobacter* spp (6.5%), *Proteus* spp (6.5%), *Salmonella* spp (1.8%) and *Enterobacter* spp(1.8%). This finding was on par with those of many studies from this region. Mathur et al. [26] from New Delhi, have also reported *E. coli* and *Klebsiella pneumoniae* as the most common Enterobacteriaceae which were prevalent in their clinical samples and this was well comparable to the reports from our study. Babypadmini et al. [13] from Chennai too reported the prevalence of 49% *E. coli* and 8% *Klebsiella* spp.

As of now, no countrywide study has been conducted for the detection of the prevalence of ESBL production in India. Individual studies which were done in different parts of the country showed a varying prevalence, based on various risk factors and local reasons.

The prevalence of ESBL producing organisms in this study was found to be 32.1%, which was slightly higher than that which was reported by other studies which were done in the same region-Gulbarga [17] and Bangalore [18] and it was lower than that which was reported by studies which were done in Hubli [19] and Davangere [20].

Previous studies from India have reported the prevalence of ESBL producers to be 6.6 to 91% [Table/Fig-8]. The wide variation in the prevalence is probably due to the variation in the risk factors and in the extent of antibiotic use. The prevalence of ESBL production is high in the referral centers and the intensive care units where the patients are referred from the peripheral centers and where the antibiotic use is profuse. Studies which were undertaken in Hubli by Krishna et al. [19] and in New Delhi by Wattal et al. [27] revealed a markedly higher incidence of ESBL production, which can be attributed to the subjects from the intensive care units, where the prevalence and the risk factors which are responsible for the emergence of the ESBL producers is high. Other reasons for the high prevalence of the ESBL producers were indwelling catheters, endotracheal or nasogastric tubes, gastrostomies or tracheostomies, severity of the illness, the excessive use of cephalosporins and a high rate of patient transfer from the peripheral centers [28], [29].

The present study reveals *K. pneumoniae* and *E. coli* as the major ESBL producers. Babypadmini et al. [13] have shown 40% and 41% ESBL positivity among *K. pneumoniae* and *E. coli* respectively and Vinod Kumar et al. [17] from Gulbarga reported 16.8% and 48.6% of *E.coli* and *K. pneumoniae* respectively as the ESBL producers. In *Citrobacter* and *Proteus*, ESBL production was 14.3 %, which was consistent with the findings of the study which was carried out by Gangone et al. [30].

ESBL producing *K. pneumoniae* evolved due to a mutation in the class A TEM and SHV β-lactamases. TEM 1, SHV 2 and SHV 5 are the common types of beta lactamases which are produced by these strains. Cross-resistance to other unrelated antibiotics may occur and this resistance is transferable in association with plasmids [14].

Salmonella and *Enterobacter* [31], [32] species are also known to produce ESBLs, but in our study, none of the *Salmonella* spp. and the *Enterobacter* spp. showed ESBL production. This could be due to the few (4 each) isolates which were obtained in these genera.

Of the 218 isolates, 200 were suspected to be ESBL producers based on the screening test. When these 200 isolates were subjected to the confirmatory test, 70 (32.1%) isolates were identified as ESBL producers by using the DDST and the PDCT. Of the two tests which were used in the study, PDCT was a more sensitive procedure for the detection of ESBL production than the DDST. 61 (87%) of the 70 ESBL producing strains were detected by the DDST by using two drugs, cefotaxime and ceftazidime. The PDCT test was compared with the DDST and it was found to be an inexpensive alternative to the DDST for the detection of ESBL. The DDST lacks sensitivity because of the problem of optimal disc space and the correct storage of the clavulanate containing discs. Assuming that a laboratory is currently testing the sensitivity for ceftazidime and cefotaxime with the disc diffusion test and for the phenotypic confirmatory disc diffusion test only two discs are required to be added to the sensitivity plate and would screen all gram negative bacteria in the diagnostic laboratory for ESBL production. This method is technically simple and inexpensive [16].

A study which was conducted by Khan et al. [10] found that the DDST was less sensitive than the PDCT, since it could detect ESBLs in 25 of the 39 isolates that were confirmed as ESBL positive by the latter technique. Shukla et al. [16] also reported similar findings.

Author	Place	Year	Prévalence(%)
Subha et al. [28]	Chennai	2002	6.6
Vinodkumar et al. [17]	Gulbarga	2004	13.5
Krishnan et al. [18]	Bengaluru	1998	17
Menon et al. [12]	Chennai	2006	20
Shukla et al. [16]	Aligarh	2003	30.2
Present study	Bijapur	2010	32.1
Bhattacharjee et al. [11]	Varanasi	2008	32
Rodrigues et al. [15]	Mumbai	2004	53.3
Sridhar Rao et al. [20]	Davangere	2008	61
Mathur et al. [26]	New Delhi	2002	68
Krishna et al. [19]	Hubli	2007	71.9
Jain et al. [29]	Lucknow	2003	86.6
Wattal et al. [27]	New Delhi	2005	91.7

[Table/Fig-8]: Comparative studies in different regions of India

Among the 218 isolates of the Enterobacteriaceae family which were analyzed, their sensitivity was found to be 92.6% with imipenem and 67.6% with amikacin. Their resistance was 99.1% against cefuroxime, followed by ampicillin (94.5%), ceftazidime (91.8%) co-trimoxazole(91.7%) and ciprofloxacin (84.4%). Sahm et al. [33] reported 97.8% resistance to ampicillin and 92.8% resistance to cotrimoxazole, which was similar to the resistant pattern which was observed in the present study. The resistance to gentamicin (91%) co-trimoxazole(82.6%) and ciprofloxacin (82.6%) which was reported by Babypadmini et al. [13] was also similar to that which was found in our study.

As indicated in many previous studies, the 92 % imipenem sensitivity in the present study advocates the usage of carbapenem antibiotics as a therapeutic alternative in the wake of the increasing resistance rates which were observed with the the conventional β-lactam and non β-lactam antibiotics. However, we need to keep in mind that the carbapenems are antimicrobials that are usually kept in reserve [15] In the case of non-life-threatening infections and in non outbreak situations, it is not necessary to administer carbapenems. This approach intends to preserve the therapeutic value of these precious drugs. The heavy use of carbapenems, in fact, may favour the selection of *Stenotrophomonas maltophilia* (a species which is naturally resistant to these drugs) [9].

In the present study, the highest number of ESBL producers were obtained in the isolates from the ICCU, the surgical ICU and the medical ICU, followed by other wards and this was comparable with a study which was carried out at AIIMS, New Delhi, India [26]. This could be due to the prolonged hospital stay, inappropriate therapy, total antibiotic use, indwelling catheters, endotracheal or nasogastric tubes, gastrostomies or tracheostomies and the severity of the illness.

The present study revealed a slight female preponderance for ESBL production among the study subjects. This was similar to the findings of an earlier study which were reported by Kiratisin et al. [5] which revealed a female preponderance. The age wise distribution of the ESBL producers showed the highest prevalence among the 41-60 years age group (38.9%). This was not statistically significant ($p>0.005$). This was closely followed by the >60 years age group (30%). This may be because of the increased hospitalization of the patients with ages around 60 years in the medical and surgical units. Kiratisin et al. [5] have also reported similar findings.

The knowledge of the resistance pattern of the bacterial strains in this geographical area will help in guiding an appropriate and judicious antibiotic use. There is a possibility that a restricted use can lead to the withdrawal of the selective pressure and that the resistant bacteria will no longer have a survival advantage in such settings.

There is a high prevalence of ESBL producers among Enterobacteriaceae and the routine susceptibility tests which are done, fail to detect the ESBL positive strains. With the spread of ESBL producing strains in hospitals all over the world, it was necessary to know the prevalence of ESBL positive strains in our hospital. The reporting of ESBL producing Enterobacteriaceae from the clinical samples will be useful for the clinicians to select the appropriate antibiotics for the treatment of these strains and to take proper precautions to prevent the spread of these resistant organisms. The failure to detect these enzymes results in an uncontrolled spread of these organisms and finally, therapeutic failures. This study underscores the need for the routine detection of ESBL producers by specific tests.

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