Original Research Article

Prevalence of extended spectrum B lactamase producing *E.coli* and *Klebsiella SPP* isolated in a tertiary care hospital, Gujarat

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Abstract

Background: Antibiotic resistance among gram negative bacilli is a rapidly expanding problem due to the organism's ability to mutate and to acquire the transmit plasmid and other genetic elements encoding resistance genes. **Objective:** This study was conducted to know the prevalence of ESBLs in *E. coli* and *Klebsiella spp*. isolates obtained from clinical samples. **Material and Methods:** A detailed history was taken and Performa was filled for each patient documenting age, sex, history of illness was obtained. Study was conducted at microbiology Department, Gujarat Adani Institute of Medial Sciences, Bhuj from May 2014 to Dec 2015. Total 2500 clinical samples like Urine, Sputum, Blood, Pus, CSF, Pleural fluid were collected in sterile containers. Samples from which *E.coli* and *Klebsiella spp* were isolated were considered for this study. Detection of ESBL was done as per CLSI guidelines. **Results:** Total 2500 clinical samples were tested for culture and sensitivity during August 2015 to July 2017. Out of this 500 samples showed growth of E. coli and Klebsiella spp. Among 500 isolates coli had 268 and from them 189 were ESBL positive. Similarly 232 were isolates from Klebsiella spp and among them 185 were positives for ESBL. **Conclusion:** In the present study, ESBL prevalence was 49.99% (*E. coli* = 50.53% and *K. pneumoniae* = 49.46%). A moderately high prevalence of ESBL producing *E. coli* and *K. pneumoniae* was observed and confirmed in the urine, sputum, pus, CSF and Blood. A strict hospital infection control policy and a prudent anti-microbials use regimens are to be adopted by the physicians.

Key words: Bhuj, *E.coli*, extended spectrum B lactamase, Gram negative bacilli

Introduction

Antibiotic resistance is one the biggest threat that the world is facing currently. Antibiotic resistance among gram negative bacilli is a rapidly expanding problem due to the organism's ability to mutate and to acquire the transmit plasmid and other genetic elements encoding resistance genes [1]. The first report of plasmid encoded B lactamases capable of hydrolysing the Extended spectrum cephalosporins was published in 1983 [2].

B lactam antibiotics are one of the most commonly used antimicrobials against bacterial infection. In recent years emergence of resistance of these antimicrobials agents due to production of B lactamases has become serious global health concern. It leads to antibiotic ineffective, increase severity of illness and cost of treatment [3]. These enzymes are numerous and they mutate continuously in response to overuse or misuse of B lactam antibiotics and have lead to the development of extended spectrum B lactamases (ESBL). Risk factors for infection with ESBL producing organism are prolong antibiotic usage, ICU stay, Recent invasive procedures, Pressure sores, anaemia, permanent urinary catheter [4]. This study was conducted to know the prevalence of ESBLs in *E. coli* and *Klebsiella spp.* isolates obtained from clinical samples.

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Material and Method

Study setting, duration and type of study- Study was conducted at microbiology Department, Gujarat Adani Institute of Medial Sciences, Bhuj from May 2014 to Dec 2015.

Sampling method and sample size calculation- Total 2500 clinical samples like Urine, Sputum, Blood, Pus, CSF, Pleural fluid were collected in sterile containers.

Samples from which *E. coli* and *Klebsiella spp* were isolated were considered for this study.

Ethical approval and informed consent- Institutional ethical committee permission was obtained. Consent was taken from all the patients.

Inclusion criteria- Patients who were willing to participate and give consent.

Exclusion criteria- Patients who had systemic disease and those who were not willing to participate.

A detailed history was taken and Performa was filled for each patient documenting age, sex, history of illness was obtained. Detection of ESBL was done as per CLSI guidelines. Screening and phenotypic confirmation tests were performed for all *E. coli*, *Klebsiella spp.* as per CLSI guidelines which is described below [5].

Screening test: Mueller Hinton agar plates were inoculated with standardized inoculum (0.5 McFarland standard) of the isolate to form a lawn culture. Disks of Ceftazidime (30µg) and Cefotaxime (30µg) were placed on inoculated Mueller Hinton agar plates. Plates were inverted and incubated at 35 ± 2^0C in ambient air for 16 to 18 hours. Zone of inhibition was measured. Ceftazidime ≤ 22mm and/or Cefotaxime ≤ 27mm were taken as indicator of suspicion for ESBL production. Such isolates are subjected to testing for phenotypic confirmation.

Phenotypic confirmation by combination disk test: Mueller Hinton agar plates were inoculated with standardized inoculum (0.5 Mc Farland standard) of the isolate to form a lawn culture. Disks of Cefotaxime (30 µg), Ceftazidime (30 µg), Cefotaxime plus clavulanic acid (30 µg/10µg), Ceftazidime plus clavulanic acid (30 µg/10 µg) are placed on inoculated Mueller Hinton agar plate. Plates were incubated at 35 ± 20C in ambient air for 16 to 18 hours. Zone of inhibition was measured.

An increase of ≥ 5 mm in zone diameter with any of the antibiotic tested in combination with clavulanic acid versus tested alone was taken as indication of ESBL production by the isolates. Increase in zone of inhibition of ≤ 5 mm was considered as negative for ESBL production.

ESBL isolates which were resistant to Cefoxitin were not considered for the study. This is to exclude associated Amp C type of B lactamases [6, 7].

Results

Total 2500 clinical samples were tested for culture and sensitivity during August 2015 to July 2017. Out of this 500 samples showed growth of *E. coli* and *Klebsiella spp*.

Table-1: Distribution as per gender.

Sex	ESBL	Non ESBL	Total
Female	260	86	346
Male	114	40	154
Total	**374**	**126**	**500**

Out of all ESBL positive isolates 374, (260) 69.51 % were females and (114) 30.49 % were Males.

Table-2: Distribution of isolates.

Sample	Total	*E. coli*	ESBL positive *E. Coli*	*Klebsiella spp*	ESBL positive *Klebsiella spp.*	Total
Urine	399	81	60	69	52	150
Sputum	539	99	56	61	53	160
Pus	246	35	20	45	43	80
CSF	42	3	1	7	6	10
Blood	338	66	50	34	31	100

Table 2 Describes Distribution of isolates. Strains isolated from different clinical specimens 399 from urine, 539 from sputum, 246 from pus, 42 from CSF and 338 from Blood. ESBL positive *E. coli* was highest from Urine followed by Sputum and Blood. Similarly, ESBL positive *Klebsiella spp* were from sputum followed by urine, pus and blood.

Table-3: Prevalence of ESBL.

Organism	Total	ESBL
E. coli	268	189 (50.53%)
Klebsiella spp	232	185 (49.46%)
Total	500	374

Table 3 Describes Prevalence of ESBL. Among 500 isolates *E. coli* had 268 and from them 189 were ESBL positive. Similarly 232 were isolates from *Klebsiella spp* and among them 185 were positives for ESBL.

Discussion

Many researchers have examined the prevalence of ESBL producing *K. pneumonia*, for example, in India; high prevalence of ESBL-producing *K. pneumoniae* strains has been reported by various groups. In the present study the prevalence of ESBL was 69.77% in *E. coli* and 79.74% in *Klebsiella spp*. Similar results are reported by Mathur et al [8] Some other authors like Sahni et al [9] has reported 32% of ESBL. Percentage of ESBL-producing organisms ranged from 4% to 83% in India [10, 11]. Rodrigues et al [12] reported a lower prevalence for ESBL producers in Maharashtra.

They reported that four isolates (8.5%) were positive ESBL-producers among 47 *K. pneumoniae* isolates. This ratio probably reflects the emerging phase of ESBL production, which by now should have increased at the same location. This is understandable since the prevalence of ESBL producers in any hospital depends upon various factors such antibiotic policy, the carriage rate among the hospital personal, and the type of disinfectant used especially in the ICU [13].

In the present study female were more prone for ESBL isolates. Similar results are shown by Gupta S et al [4]. Mendelson G et al [14] and Bazzaz B et al [15] have reported more male proneness to ESBL.

Strains isolated from different clinical specimens 399 from urine, 539 from sputum, 246 from pus, 42 from CSF and 338 from Blood. ESBL positive *E. coli* was highest from Urine followed by Sputum and Blood. Similarly, ESBL positive *Klebsiella spp* were from sputum followed by urine, pus and blood. Recently, similar reports have been presented by Gupta et al [4].

In the recent years, a significant increase in ESBL-producing *Klebsiella spp* was also reported from the USA (42-44%), Canada (4.9%) [16], Spain (20.8%) [17], Taiwan (28.4%) [18], Turkey (78.6%) [19], Algeria (20%) [20] and China (51%) [21].

Focusing on the epidemiology in Europe, there are considerable geographical differences in the occurrence of ESBLs. A recent survey of 1610 *E. coli* and 785 *K. pneumoniae* isolates from 31 centres in 10 European countries found that, the prevalence of ESBL of these organisms ranged from as low as 1.5% in Germany to as high as 39-47% in Russia, Poland and Turkey [22].

This study demonstrated that the PCDDT was more sensitive for detecting ESBL than DDST, since it, detected 46.15% of ESBL, whereas PCDDT detected 50.76% of ESBL producers.

Presence of ESBLs can be masked by the expression of Amp C β-lactamase, which can be generated by chromosomal plasmid genes. Of the 384 clinical isolates of *K. pneumoniae*, 101 randomly selected isolates were screened for ESBL production by DDST and PCDDT. Of these 79 out of 101 isolates were found to be ESBL positive and 22 were ESBL negative, whereas, Duttaroy et al. from Gujarat India in 2005 reported 58% prevalence for ESBL producing *K. pneumoniae*, isolated from different clinical specimens using DDST [23].

Babypadmini et al. reported 40% prevalence for ESBL producing *K.pneumoniae*, in urinary isolates in Coimbatore, India in 2004 [24].

ESBL prevalence was 49.99% (*E. coli* = 50.53% and *K. pneumoniae* = 49.46%). It was high as compared to a study done in India [25], which reported nearly 40% of urinary isolates of *E.coli* and *K. pneumoniae* were ESBL positive. The highest isolation rate of ESBLs producing *K. pneumoniae* had been reported from the Latin America (54.4%), the western Pacific (24.6%) and Europe (22.6%). The frequency of ESBL producing E.coli in these areas was reported to be 8.5%, 7.8% and 5.3%, respectively.

Multi-centre studies involving major health-care facilities in other parts of the country are required to have a clearer picture of ESBL producing uropathogens. Further, molecular epidemiological studies of resistance genes among the uropathogens would provide us much needed details on bacterial clones circulating in this region.

Conclusion

In the present study ESBL prevalence was 49.99% (*E. coli* = 50.53% and *K. pneumoniae* = 49.46%). A moderately high prevalence of ESBL producing *E.coli* and *K. pneumoniae* was observed and confirmed in the urine, sputum, pus, CSF and Blood. A strict hospital infection control policies and a prudent anti-microbials use regimens are to be adopted by the physicians. It is essential and mandatory to have a regular and routine monitoring of ESBL producing clinical isolates in clinical laboratories.

What this study adds to existing knowledge?

Multi-center studies involving major health-care facilities in other parts of the country are required to have a clearer picture of ESBL producing uropathogens.

It is essential and mandatory to have a regular and routine monitoring of ESBL producing clinical isolates in clinical laboratories.

Contribution from authors

- **Dr. Hitesh Assudani** formulated the aims & objectives with study design and helped in data collection from medical record department.

- **Dr. Jigar Gusani** contributed to the preparation of the manuscript and Data analysis.

Findings: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

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