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Determination of Antimicrobial Resistance Pattern and Production of Extended-Spectrum B-Lactamases amongst *Escherichia coli* and *Klebsiella pneumoniae* from Clinical Isolates

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ABSTRACT

Background: The prevalence of antibiotic resistance among extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* has been increased markedly in recent years. The present study was done to know the prevalence of ESBL production among isolates of *E. coli* and *K. pneumoniae* and to study the susceptibility pattern of isolates against different antibiotics.

Methods: Extended-spectrum β-lactamase producing *E. coli* and *K. pneumoniae* were isolated from various samples obtained from outdoor and indoor patients of the Prathima Institute of medical sciences, Andhra Pradesh, India. They were tested for ESBL production by double disc synergy test and resistance to various antibiotics like fluoroquinolones, cephalosporins, aminoglycosides and ß-lactamase inhibitor combinations and susceptibility to carbapenems were determined by Kirby-Bauer disc diffusion method.

Results: A total of 94 ESBL producing isolates were obtained. Of them 60 were *E. coli* and 34 *K. pneumoniae*. They were obtained from urine, sputum, pus, wound swabs blood & tracheal aspirates. Urine (38.29%) was the main source of ESBL-producing isolates from all patients, followed by sputum (34.04%). About 37.23% of these isolates were collected from medical wards and 27.65% were collected from outdoor. All isolates were susceptible to imipenem. The resistance to cephalosporins (1-4 generations) was almost 100%. Resistance to Aztreonam, Ampicillin and Co-amoxyclav was also 100%. A high degree of resistance was observed to other antibiotics.

Conclusion: The highest prevalence of resistance to ESBL in *E. coli* and *K. pneumonia* is associated with a multitude of infections in hospitalized patients with a significant longer duration of hospital stay, increased morbidity and greater hospital charges. Advanced drug resistance surveillance and molecular characteristics of ESBL isolates is necessary to guide the proper and judicious antibiotic use.

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Introduction

Extended spectrum beta-lactamases (ESBL) are plasmid mediated with TEM-1, TEM-2 and SHV-1 derived enzymes conferring broad resistance to penicillin, cephalosporin and many other antibiotics (1). They are mainly found in *Escherichia coli*, *Klebsiella species* and *Proteus* species but can also occur in other members of Enterobacteriaceae family (2, 3). The first ESBL-producing organism was isolated in Germany in 1983. Then, such organisms were reported in the USA following outbreaks of infections caused by these pathogens (4-6). Since the ESBL genes are usually found in large plasmids, they also contain other antimicrobial resistance genes. Therefore most ESBL producing organisms are also resistant to aminogylcosides, fluororquinolones, tetracyclines, chloramphenicol and sulfonamides. Carbapenems are the mainstay of therapy for infections caused by ESBL producing organisms (7).

Several factors have been reported to increase the risk of colonization and infection with ESBL producing bacteria especially Enterobacteriaceae including prolonged antibiotic usage, increased hospital stay, continued presence of invasive device and coexisting morbid conditions. It is necessary to identify the risk factors for infection with ESBL producing organisms so that effective strategies can be formulated to decrease the spread of these strains (8-10).

We conducted the current study to identify the prevalence of ESBL in *E. coli* and *K. pneumoniae* and various risk factors responsible for infection with ESBL-producing *E. coli or K. pneumoniae*. The objective of this study was to find out the prevalence of ESBL in *E. coli* and *K. pneumoniae*, to study the susceptibility pattern of various isolates of *E. coli* and *K. pneumoniae* against different antibiotics, to identify various risk factors responsible for infection with ESBL-producing *E. coli* and *K. pneumonia* to know whether clinical outcomes differed between patients with infections caused by resistant organisms and those with infections caused by susceptible organisms and to formulate antibiotic policy in our hospital for proper and judicious antibiotic use.

Materials and Methods

This investigation was conducted in the Prathima Institute of Medical Sciences, a 750 bed academic tertiary care hospital located in Karimnagar, Andhra pradesh. During the study period various specimens (urine, sputum, respiratory secretions, blood, body fluids and swabs) were processed for significant bacteremia in the microbiology laboratory of Prathima medical college Hospital from clinically suspected patients. This study was done on 94 Gram-negative bacilli that were confirmed as ESBL producing isolates.

Medical and demographic data of the patients were collected using patients' files. Data recorded were as follows: demographic data (age, sex) presence of urinary catheter / abdominal tubes / respiratory tubes / others, admission ward and so on.

The presence of the following comorbid conditions was documented: hepatic dysfunction, malignancy, diabetes mellitus, renal insufficiency (indicated by a creatinine level of >

2.0 mg/dL), HIV infection, neutropenia, corticosteroid use, prior organ transplantation, use of an immunosuppressive agent in the 30 days prior to admission to the hospital, and surgical procedure or trauma in the 30 days before admission. Samples were collected aseptically in sterilized bottles or disposable sterile tubes and submitted to the clinical microbiology laboratory.

The specimens received were inoculated on blood and MacConkey agar plates. Then all plates were incubated at 37°C for 24 hours. Significant isolates were identified to species level using conventional bacteriological methods (11).

Nosocomial acquisition of infection was defined as the infection that occurred more than 48 hours after admission to the hospital; and infection that occurred less than 48 hours after admission to the hospital in case of patients who had transferred from an outside hospital or nursing home.

Antimicrobial susceptibility testing for ESBL screening

Isolates were screened initially using Kirby-Bauer method and MIC assays using microdilution method. *K. pneumoniae* and *E. coli* isolates for which the MIC of ceftazidime was > 2 µg/mL were suspected of producing an ESBL. All ESBL producing isolates were confirmed using the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS 2004) approved double disk diffusion method (12).

A positive result required an increased zone (5 mm), using combination disk technique with antibiotic disks containing ceftazidime (30 mg), cefpodoxime (30 mg) and cefotaxime (30 mg) either alone or in combination with clavulanic acid (10 mg). Bacterial isolates of *K. pneumoniae* and *E. coli* were subjected to antibiotic susceptibility testing by disc diffusion technique according to CLSI guidelines with suitable quality controls including *E. coli* ATCC 25922 and *Klebsiella pneumoniae* 700603 (Himedia, Mumbai).

The following antibiotic disks were used: gentamicin, amikacin, tobramycin, imipenem, meropenem, and four generation of cephalosporins (cefazoline, cephalothin, cefuroxime, cefixitin, ceftazidime, cefotaxime and cefepime), aztreonam, ampicillin, amoxicillin / clavulanate, ampicillin / sulbactam, piperacillin-tazobactam, trimethoprim / sulfamethox azole, nitrofurantoin, ciprofloxacin, norfloxacin and nalidixic acid.

According to the suggestion of CLSI, the results were interpreted. The quality control check for susceptibility testing was performed once in a week.

Results

Ninety four (69.62%) out of 135 gram negative isolates of *E. coli* and *K. pneumoniae* were ESBL producers and 41 (30.37%) were non ESBL producers. Among the ESBL producers 60 were *E. coli* and 34 were *K. pneumoniae*.

The source of ESBL producing strains was urine (n = 36), sputum/respiratory secretions (n = 32), blood (n = 12), swabs (n = 10) and body fluids (n = 4) *(Table 1).*These strains were co-resistant to several antibiotics including ciprofloxacin and ofloxacin (86.8%), amikacin (22%) and pipercillin- tazobactam (10.4%). All ESBL producers were susceptible to imipenem *(Table 2).*

Among the patients, 58 (61.7%) were male and 36 (38.29%) were female.

Table 1. The source of ESBL producing strains

Sample	Number	Percentage
Urine	36	38.29
Sputum/respiratory secretions	32	34.04
Blood	12	12.76
Swabs	10	10.63
Body fluids	04	4.25
TOTAL	94	100

Table 2. Antimicrobial susceptibility pattern of ESBL producing *E. coli* and K. pneumoniae

Antibiotics	Sensitive	Resistant
Ampicillin	0	100
Ampicillin/sulbactum	0	100
Amoxicillin/clavulanate	0	100
Gentamicin	62.4	37.6
Amikacin	78	22
Cefazoline	9.3	90.7
Cefuroxime	7.2	92.8
Cefotaxime	11.4	88.6
Cefepime	23.2	76.8
Ciprofloxacin	13.2	86.8
Norfloxacin	7.6	92.4
Nalidixic acid	4.8	95.2
Nitrofurantoin	6.1	93.9
Trimethoprim/sulfamethaxole	0	100
Imipenem	100	0
Aztreonam	0	100
Piperacillin/tazobactum	89.6	10.4

Most of these isolates (42.2%) were from the medical wards, followed by outpatient clinics (30.3%) followed by Intensive Care Unit (ICU) (18.6%) and surgical wards (8.1%). The least number of ESBL producing pathogens were isolated from the pediatric wards (5.2%) and obstetrics and gynecology wards (3.7%). Mean age of patients with ESBL producing isolates was 43.69 and male patients outnumbered the female patients. Mean duration of hospital stay was 13.6 days among patients with ESBL producing organisms and 4.2 among patients with non-ESBL producing organisms.

Table 3. ESBL producing bacteria from different wards and Outpatient department

Sample collection area	ESBL Positive bacteria	Percentage
Medical wards	35	37.23
OPD	26	27.65
Intensive care units	16	17.02
Surgical ward	8	8.51
Pediatric ward	5	5.31
Obstetrics & Gynecology ward	4	4.25

Increased duration of stay in the ICU, exposure to multiple antibiotics, presence of invasive device and underlying disease were the significant risk factors. Previous exposure to beta lactam antibiotics was recorded in 36 patients with ESBL producing organisms. This is an important contributing factor. Four patients in the study population died, all of

them had been infected with ESBL producing organisms and were in the ICUs.

Table 4. Medical & demographic data of patients with ESBL positive isolates

Features	ESBL Positive (94)	Percentage	ESBL negative (41)	Percentage
Mean age	43.69	-	38.64	
Sex–male	58	61.7	28	68.29
Sex–female	36	38.29	13	31.70
ICU Stay	32	34.04	8	19.51
Non ICU stay	62	65.95	33	80.48
Ventilated	17	18.08	02	4.87
Non ventilated	77	81.91	39	95.12
Mean duration of hospital stay	13.6	-	4.2	
Underlying illnesses Neutropenia	26	27.65	38	92.68
Diabetes	38	40.42	35	85.36
Renal insufficiency	07	17.07	01	2.43
Hiv infection	09	9.57	03	7.31
Others	14	14.89	18	43.90
No illness	43	45.74	36	87.80
Corticosteroid use	21	22.34	09	21.95
Prior organ transplantation	02	2.12	00	00
Immunosuppressive agent use	02	2.12	00	00
Surgical procedure or trauma	26	27.65	17	41.46
Previous B-lactum use	36	38.29	11	26.82
Outcome-Recovered	90	95.74	41	100
Expired	04	4.25	00	00

Discussion

ESBL producing Gram-negative bacilli especially *E. coli* and *K. pneumonia* have been emerged as serious pathogens both in hospital and community acquired infections worldwide. These Gram-negative bacilli, show resistance to penicillin, cephalosporins, β-lactams and also cross resistant to many other antibiotics.

In this study, the prevalence of ESBL production among 135 isolates of *E. coli* and *K. pneumonia* was 69.62 %, which is similar to the study done by Alipourfard I, Yeasmin Nili N (13). Moreover, among the ESBL producers *E. coli* (63.82%) was more than *Klebsiella* which is same as many other studies (14). In our study, urine (38.29%) was the main source of ESBL producing isolates from all patients, which is similar to study done by Alipourfard I, *et al* (13).

Few studies have found that the conditions of patients with urinary tract infections because of ESBL-producing organisms improve despite the patients having received treatment with agents to which the organisms are resistant (15).

The various strains of *E. coli* and *K. pneumonia* were co-resistant to several antibiotics including ciprofloxacin and ofloxacin (86.8%), cefazoline (90.7%), cefuroxime (92.8%), cefotaxime (88.6%) and cefepime (76.8%). All ESBL producers were susceptible to imipenem. Our findings are similar to many studies done by Lee SY, *et al* and M Shanthi *et al*, (9, 16). All These studies imply that to treat the infections caused by ESBL producing bacteria, carbapenems should be used. The prevalence of ESBL producing bacteria vary greatly all over the world and are rapidly changing over time (17). The risk factors for infection and colonization may indeed be different in various geographical areas (18).

The Patients who are infected with ESBL producing organisms had a history of longer

hospital stay compared to those patients without these infections. The mortality rate was also higher.

Mean duration of hospital stay among patients with ESBL producing organisms was 13.6 days and it was 4.8 days among patients with non-ESBL producing organisms.

Empirical antibiotic therapy promotes colonization in hospitalized patients with resistant strains by eradicating susceptible flora.

In this study, all the four patients who succumbed to the infection were infected by ESBL Positive bacteria. If the prevalence of ESBL was less in this Hospital, the mortality rate would have been nearly nil.

In One study, of patients with bloodstream infection because of ESBL-producing *K. pneumoniae* a 75% crude mortality rate was found among patients who received ineffective initial empirical therapy, compared with 28% among patients with initial therapy against the organism (19).

furthermore, other patients who were infected with ESBL positive bacteria may have recovered but they contributed to lengthy hospital stay and unnecessary antibiotic usage leading ultimately to economic burden in the hospitalized patients.

Few Studies showed that patients with infection such as septicaemia caused by ESBL producing organisms had a significant higher fatality rate than those with non-ESBL isolates (1).

As the percentage of ESBL producing isolates from outpatient clinics is increasing, the use of oral cephalosporins should be judiciously done. The local physicians should consider ESBL producing isolates in infections not responding to many first line antibiotics.

Presently, few options are available for treatment of ESBL organisms. Hence, prevention should be the top priority. Many studies have shown that the use of third-generation cephalosporins is a modifiable risk factor in prevention of these organisms. Therefore, restricting the use of third-generation cephalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL producing isolates.

It is imperative that risk factors for infections caused by ESBL-producing organisms be clearly identified so that effective strategies should be formulated to limit outbreak of these infections.

Conclusion

The prevalence of antibiotic resistance among extended-spectrum β-lactamase (ESBL)-producing *E. coli and K. pneumoniae* has increased markedly in recent years and is associated with a multitude of infections in hospitalized patients. Detection of ESBL producers, their treatment strategies and infection control policies are important in controlling this growing epidemic. Knowledge of Organisms producing ESBL, the risk factors for acquisition and differentiation between ESBL and other resistance mechanisms will help physicians to choose the best treatment modalities. Presently, carbapenems are the most sensitive and reliable treatment options for infections caused by ESBL producing isolates.

However, injudicious use of carbapenems may lead to resistant gram-negative organisms. Therefore, restricting the use of third-generation cephalosporins, along with implementation of infection control measures,

are the most effective means of controlling and decreasing the spread of ESBL producing isolates.

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None declared.

Conflict of Interest

None declared conflicts of interest.

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