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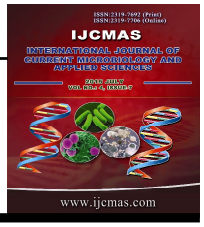

Original Research Article

Extended spectrum beta lactamases in urinary isolates of Gram Negative organisms- Prevalence and susceptibility pattern in a tertiary care hospital of North East India

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Keywords

beta lactamases, Gram negative urinary isolates, susceptibility pattern

A B S T R A C T

A total of 211 Gram negative urinary isolates (113 *Escherichia coli, 48 Klebsiella pneumonia, 14 Proteus spp, 10 Enterobacter spp, 14 Citrobacter spp, 7 Providencia spp and 5 Serratia marscecens*) were studied for extended spectrum beta lactamase (ESBL) production by double disk approximation test and NCCLS confirmatory test. ESBL production was found to be present in 74 (35.07%) isolates of which 30.08 % in *E. coli*, 37.50 % in *K. pneumonia*, 50.0 % in *Proteus* spp, 40% in *Enterobacter* spp, 21.42 % in *Citrobacter* spp and 100% in *Serratia marcescens*. 8.11 % of ESBL producers showed false susceptibility to ceftazidime and cefotaxime in routine susceptibility testing. The susceptibility of ESBL producers to Polymixin B, Imipenem, Nitrofurantoin, Doxycycline, Fluoroquinolones and Amikacin was found to be 100%, 98.65% , 35.13%, 44.59%, 47.29% and 43.24% respectively.

Introduction

Extended spectrum beta-lactamases (ESBLs) are defined as beta-lactamases capable of hydrolyzing oxyimino-cephalosporins and are inhibited by beta-lactamase inhibitors[1] The wide spread use of antibiotics in hospitals has led to emergence of multidrug resistant organisms of low virulence like *Klebsiella & Escherichia coli* causing serious opportunistic infections. Over the last 15 years numerous outbreaks of infection with organisms producing extended spectrum beta-lactamases (ESBLs) have been observed worldwide. [2] The advent of ESBL producers has posed a great threat to the use of many classes of antibiotics particularly cephalosporins. There are indications that poor outcome occurs when patients with serious infections due to ESBL producing organisms are treated with antibiotics to which the organism is resistant. [3] ESBL producing Gram negative organisms were first reported in 1983 from Germany and since then a steady increase in resistance against cephalosporins has been seen. ESBLs are encoded by transferable conjugative plasmids which also quite often code resistant determinants to other antibiotics. [4] An ESBL variant may be selected *de novo* in a given hospital or it may be introduced from another centre.

Its further spread within the hospital can be consequence of plasmid transmission. Persistence and outbreaks of ESBL producers have been convincingly correlated with extensive use of cephalosporins. [5] The plasmid mediated resistance against cephalosporins can be spread among related and unrelated gram negative bacteria. Microorganisms responsible for urinary tract infection (UTI) such as *E.coli* and *Klebsiella* spp. have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat.[6] Infections due to ESBL producing Gram negative organisms are of concern as third generation cephalosporins (3GC) are commonly used for treatment of infections due to gram negative organisms. These infections are difficult to control as they are usually associated with resistance to aminoglycosides. [7] Hence, the present study was undertaken to find out prevalence of ESBL producers in urinary isolates of Gram Negative organisms and also their susceptibility to non-beta lactam antibiotics.

The main aim of this study to prevalence of ESBL producers in urinary isolates of Gram negative organisms and also to determine their susceptibility pattern against non- beta lactam antibiotics

Materials and Methods

Between July and October 2014, a total of 3861 urine samples were processed for significant bacteruria in the department of microbiology from patients clinically suspected to have UTI. All Gram negative organisms isolated in significant numbers were included in the study. Clinico-demographic data of study patients was noted. Chi-square test was used to analyze the susceptibility pattern of non beta lactam antibiotics in ESBL producers and non-producers.

Antibiotic susceptibility testing

The above isolates were tested for antimicrobial susceptibility by Modified Kirby Bauer disc diffusion technique according to NCCLS guidelines.[8] The following antibiotic discs (drug concentration in mg) were used: Amikacin (30), Ceftazidime (30), Cefotaxime (30), Imipenem (10), Polymixin B (300), Ciprofloxacin (5), Doxycycline (10) and Nitrofurantoin (300).

Test for ESBL production

a) Double Disc Synergy test (DDST) [9]

The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10 mg) and cefotaxime (30 mg) were placed at a distance of 15 mm apart centre to centre and incubated. Organism that showed a clear extension of cefotaxime inhibition zone towards the disc containing clavulanate was considered as ESBL producer.

b) NCCLS Phenotypic Confirmatory Disc Diffusion test (PCDDT) [8]

While performing antibiotic testing, ceftazidime (30 mg) and ceftazidime plus clavulanic acid (30/10 mg) discs were placed on Mueller-Hinton agar and incubated.

Organism was considered as ESBL producer if there was a 5 mm increase in zone diameter around the ceftazidime/clavulanate disc as compared to that of ceftazidime disc alone.

Results and observations

Of the 3861 urine samples processed, 211 samples yielded various Gram negative

bacterial isolates. There were 113 *E.coli* , 48 *K.pneumoniae 14 Proteus spp, 10 Enterobacter spp, 14 Citrobacter spp , 7 Providencia spp and 5 Serratia marcescens* among them. ESBL production was found to be 30.08 % in E. coli, 37.50 % in *K. pneumonia*, 50.0 % in *Proteus* spp, 40% in *Enterobacter* spp, 21.42 % in *Citrobacter* spp, 42.85% in *Providencia* spp and 100% in *Serratia marcescens* by NCCLS confirmatory test [Table 1, Fig 1].

The Double Disk Synnergy test (DDST) failed to detect ESBLs in 4 (four) isolates of *E.coli*, 4 (four) isolates of *K. pneumonia*, 3 (three) isolates of *Proteus* spp and 2 (two) isolates of *Citrobacter* spp. These ESBL positive isolates were obtained from 45 female and 29 male patients with a male: female ratio of 1:1.55. When compared with non-ESBL producers, it was observed that there is no significant statistical correlation between gender and ESBL production (p= 0.07234). They were distributed in the age group of 12 years to 82 years, with as many as 31 (41.90%) patients being above 60 years of age [Table 2, Figure 2].

The highest number of ESBL producers i.e 22 (29.73%) were isolated from ICU/ITU followed by Surgery and Urology Departments (16.22% each) [Table 3, Fig 3].

The antimicrobial susceptibility results of ESBL producers are shown in [Table – 4, Figure – 4]. Susceptibility of ESBL producers to Polymixin B, Imipenem, Nitrofurantoin and Amikacin were found to be 100%, 98.65%, 35.13% and 43.24% respectively. False susceptibility to ceftazidime/cefotaxime was observed in 8.11%. Co-resistance to non-betalactam antibiotics was observed more (p=0.001528258) with ESBL producers.

ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates vary greatly worldwide and in geographic areas and are rapidly changing over time.[10] The occurrence of ESBL producers in urinary isolates of Gram Negative organisms in our study was found to be 35.07%. Much higher (58%) prevalence of ESBL producers in urinary isolates of gram negative bacilli was observed in India by Mathur *et al*.[11] ESBL production coexisted with resistance to several other antibiotics. ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics.[12] We found in our study such associated resistance with Doxycycline – 55.41%, Amikacin – 56.76%, Nitrofurantoin – 64.87% and Fluroquinolones – 52.71% (p=0.001528258). Other workers in India have reported such association only with Gentamicin.[13]

In our study, it was observed that the majority of patients infected with ESBL producers comprised of geriatric patients above 60 years of age (41.90%). This could be explained to some extent by the repeated insult by resistant microorganisms over a long period of time as well as waning immunity which predisposes these patients to infection repeatedly.

In our study it was also seen that the Double Disk Synergy test (DDST) failed to detect ESBLs in 4 (four) isolates of *E.coli*, 4 (four) isolates of *K. pneumonia* , 3 (three) isolates of *Proteus* spp and 2 (two) isolates of *Citrobacter* spp. DDST lacks sensitivity because of problem of optimal disc space and correct storage of the clavulanate containing discs.

Table.1 ESBL producing Gram -ve organisms isolated

Organism	Total no of isolates	No of ESBL producers	% of ESBL producers
Escherichia coli	113	34	30.08%
Klebsiella pneumoniae	48	18	37.50%
Proteus spp	14	7	50.0%
Citrobacter spp	14	3	21.42%
Enterobacter spp	10	4	40.0%
Providencia spp	7	3	42.85%
Serratia marcescens	5	5	100%

Table.2 Age distribution of patients harbouring ESBL Producers

Age Group (In Years)	No. of Patients	% of Patients
0-15	2	02.70%
16-30	2	02.70%
31-45	14	18.92%
46-60	25	33.78%
>60	31	41.90%
TOTAL	74	100%

Table.3 Department wise Breakup of Patients

Department	No of patients from whom ESBL producers isolated	% of patients
Burn Ward	11	14.86%
Casualty Department	4	05.40%
Dermatology	1	01.35%
ICU/ITU	22	29.73%
Medicine	6	08.11%
Obstetrics & Gynaecology	3	04.05%
Paediatrics	1	01.35%
Orthopedics	2	02.70%
Surgery	12	16.22%
Urology	12	16.22%
TOTAL	74	100%

Table.4 Antimicrobial Susceptibility Results of ESBL Producers

ANTIBIOTIC	SENSITIVE	% SENSITIVE	RESISTANT	% RESISTANT
Ceftazidime/cefotaxime	6	08.11%	68	91.89%
Amikacin	32	43.24%	42	56.76%
Doxycycline	33	44.59%	41	55.41%
Nitrofurantoin	26	35.14%	48	64.86%
Fluoroquinolones	35	47.29%	39	52.71%
Imipenem	73	98.65%	1	01.35%
Polymixin B	74	100%	0	0%
Piperacillin tazobactam	22	29.73%	52	70.27%

Fig.1 ESBL Producing Gram -ve organisms isolated

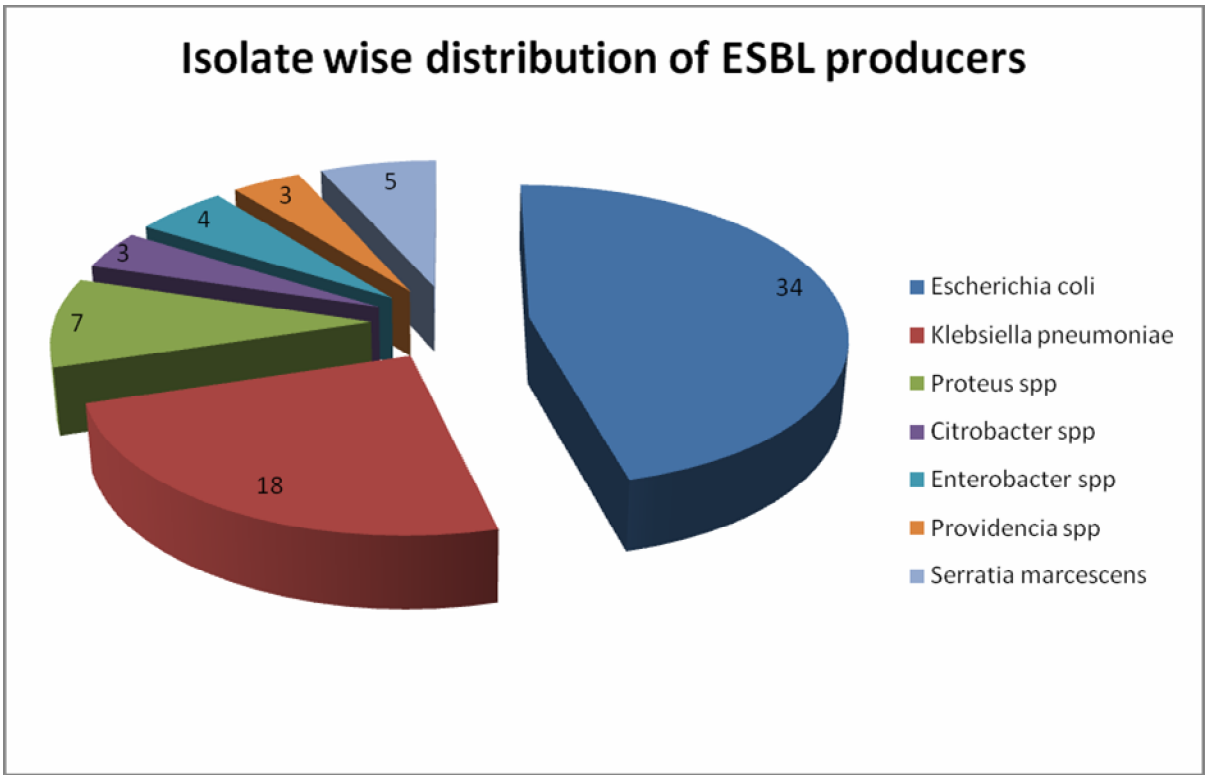


Fig.2 Age distribution of patients harbouring ESBL producers

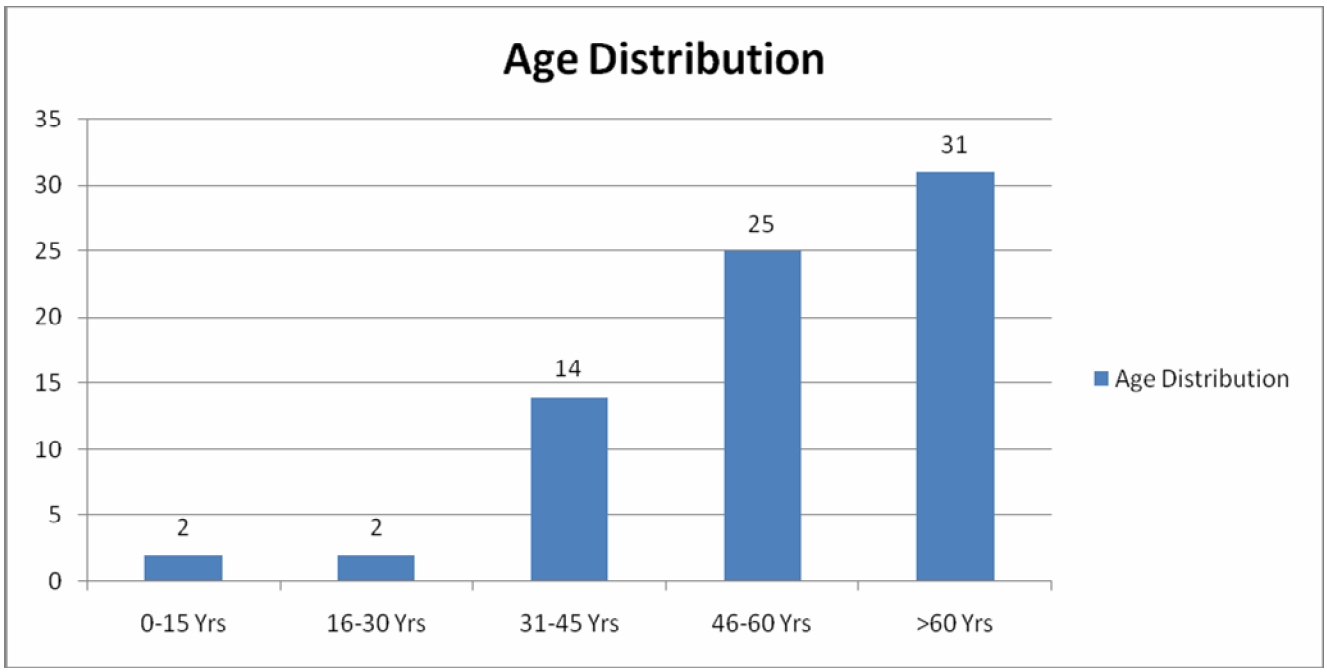


Fig.3 Department wise Breakup of Patients

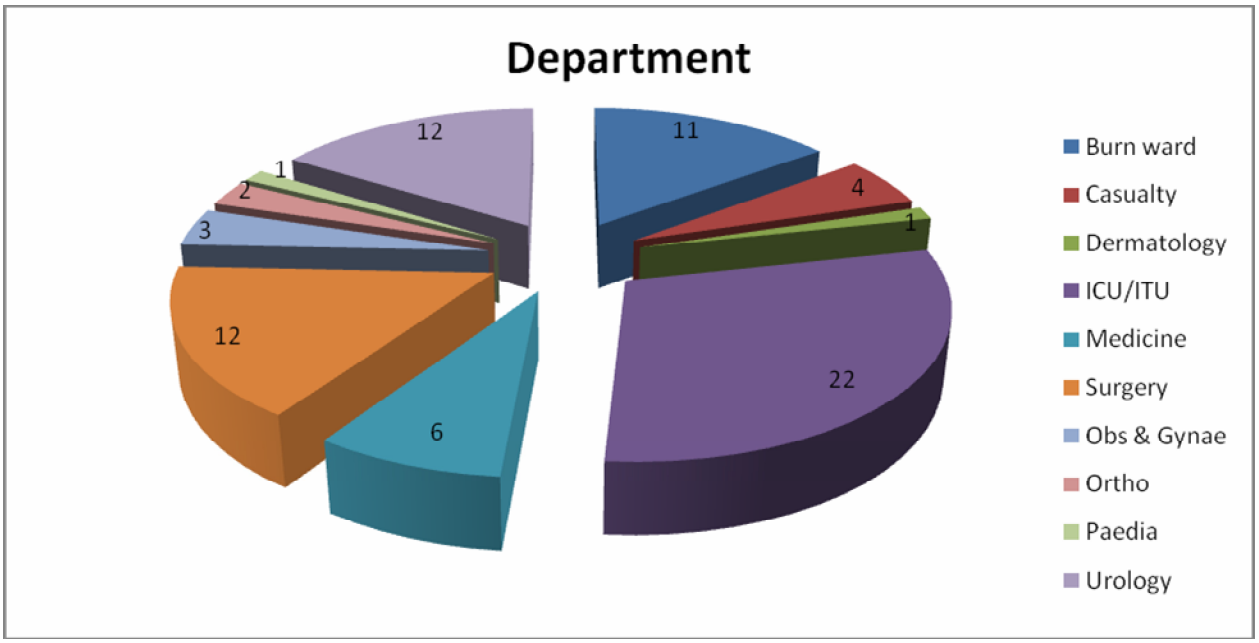
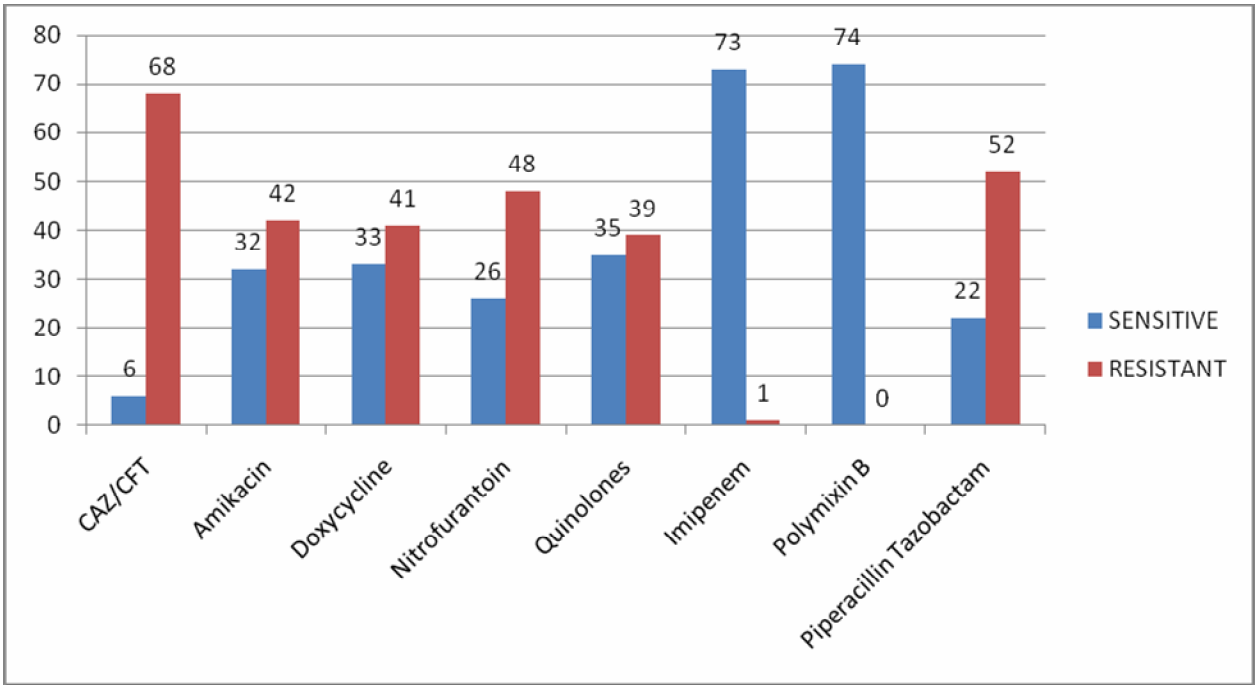


Fig.4 Antimicrobial Susceptibility Results of ESBL Producers



Assuming that a laboratory is currently testing sensitivity for ceftazidime and cefotaxime with disc diffusion and since for the NCCLS Phenotypic Confirmatory Disc Diffusion test (PCDDT) only two discs are required to be added to the sensitivity plate, we can confidently say that PCDDT is a technically simple and inexpensive method that would screen all gram negative bacteria in the diagnostic laboratory for ESBL production.

Admission in ICU/ITU, Urology, Surgery and Burn wards were found to be the major risk factors for ESBL production in our study. Patients infected with these ESBL producing strains cannot be treated with b-lactam antibiotics and monobactams. Since coresistance to non-b lactam antibiotics like Fluoroquinolones, Doxycycline, Nitrofurantoin and Amikacin was observed, Polymixin B and Imipenem are found to be alternatives for treating such patients although at a higher cost. Hence, it is the need of the hour as well as the moral responsibility of both the clinicians and microbiologists alike to check the menace at the earliest by promoting judicious use of antibiotics.

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