

ORIGINAL RESEARCH

Comparison of association of ESBL producing bacteria to the duration of the diabetes: An original research

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ABSTRACT

Introduction: Diabetes is a common disease of the current population and its most common complication is recurrent urinary tract infection. Recurrent infections are treated with recurrent antibiotic therapy which leads to emergence of resistant strains of bacteria like ESBL, which can prove to be fatal.

Materials and Methods: Patients with confirmed cases of diabetes were selected based on inclusion and exclusion criteria. The urine samples of all patients were analyzed for bacterial isolates and further analyzed for ESBL producing organisms. ESBL producing strains were tested for their antibiotic sensitivity

Results: A total of 164 patients comprising of 82 males patients and 82 female patients of both genders in the age groups of 30-60yrs were selected. Out of this 58 females and 32 males had recurrent UTI. 45.73% urine samples showed gram negative isolates and among them 25.33% were found to be positive for ESBL isolates using combination disc method. The predominant micro organism was found to be E coli (33%) in urine samples of both genders. E coli was the largest producer of EBSL (57.9%) followed by K pneumoniae (21.1%). We found a strong association between increasing years of diabetes with ESBL production.

Conclusion: We found that recurrent UTI in diabetics was most common in sixth decade of life and was noted to be more in female population. E coli and K pneumoniae were the most common GNB isolates as well as ESBL producers respectively. They were resistant to 3rd generation cephalosporin but were highly sensitive to amikacin and nitrofurantoin.

Key words: Recurrent urinary tract infections, Gram negative bacteria, ESBL, Diabetics, combination disc method.

INTRODUCTION

Urinary Tract Infections (UTI) is considered to be the most common infectious disease in clinical practice in developing countries, with incidence of 250 million people are affected worldwide despite wide spread availability of antibiotics.¹ Diabetes is a metabolic disease due to defect in insulin secretion, insulin action or both. It is characterized by polyuria, polyphagia and polydipsia. Diabetes has affected more than 200 million people worldwide and can be considered a global threat. Patients with diabetes suffer from complication like cardiovascular disease, kidney disease, retinopathy, diabetic foot, diabetic neuropathy, diabetic ketoacidosis, urinary tract infections etc. Urinary tract infection is thought to be the most common infection among diabetes as the high blood sugar level favors the colonization of microorganism in the urinary tract.^{2,3} *Escherichia coli* is the primary urinary tract pathogen while *Klebsiella* spp., and *Staphylococcus* spp., are less common pathogens.⁴ The most common antibiotic used to treat UTI in diabetes by using beta lactam antibiotics. As diabetics are prone to get recurrent urinary tract infection it is treated by recurrent administration of beta lactam antibiotics, thus multi drug resistant strain has emerged known as Extended spectrum β -lactamase (ESBL). ESBL is an enzyme produced by some strains of bacilli from the Enterobacteriaceae family. The prevalence of ESBL producing strains among Gram negative bacilli (GNB) varies widely depending upon the clinical setting and the geographic region.⁵ Though the available data suggest that ESBL is prevalent in patients with diabetic above 65 yrs, but data regarding the prevalence of ESBL in diabetics of younger age group is unavailable. Thus this current study is taken up to assess the prevalence of ESBL producing organisms among diabetics of various age groups.

MATERIALS AND METHODS

DESIGN OF THE STUDY

A hospital based cross-sectional study carried out among 164 type 2 diabetics with clinically suspected Urinary Tract Infection patients visiting the OPD of Medicine, Sree Balaji Medical College and Hospital Chrompet, Chennai over a period of 1 year. The clinical sample was collected processed and sent to the Department of Microbiology for further assessment of pathogens.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria: Patients in the age group of 30 to 60 yrs of both gender with history of diabetes mellitus without any other debilitary or co-morbid condition like retinopathy, nephropathy, polyneuropathy etc. Exclusion criteria: Diabetic patients without symptoms of UTI, with complications and debilitary or co-morbid conditions. This study was conducted after obtaining permission from institutional ethical committee. The diabetic group was selected as per inclusion and exclusion criteria. Informed written consent was obtained from all patients. Detail history was obtained from the study group and complete clinical examination was carried out for each UTI patients. Sample collection and testing: Clean catch mid stream urine (CCMSU) method was explained to the patients for collecting urine sample. About 5 ml urine collected in a sterile urine container. The collected sample were then transported to the Central Microbiology Laboratory Sree Balaji Medical College and Hospital (SBMCH) within one hour. Urine obtained was tested for fasting, postprandial and glycosylated haemoglobin (HbA1C) and measured. Urine samples were collected and gram staining and culture were done. Organisms identified after biochemical reaction were taken up to study the prevalence of ESBL. The urine samples were centrifuged and the sediments obtained were analyzed under high power objective (40x) for presence of pus cells and bacteria. The microorganisms in the urine were cultured on nutrient agar, Macconkey agar and special media CLED agar. The culture plates were then incubated at 37°C overnight in an

incubator. Cultures grown on the media were counted using hand lens and sample showing colony count less than 10^5 /ml were excluded from the study. The organism grown on culture plates were thus isolated and characterized on the basis of Gram's Staining, motility and standard biochemical reactions such as slide and tube catalase test, slide and tube coagulase test, indole test, MR test, VP test, citrate utilisation test, urease test, nitrate reduction, oxidase test, triple sugar iron (TSI) test, carbohydrate fermentation tests. Cartridge of Antimicrobial Disc was stored in a tightly sealed container and refrigerated at $4-8^{\circ}\text{C}$ or kept frozen at -14°C . β - Lactam antibiotics were stored frozen. Disc containers were brought to room temperature before use. To standardize the inoculum density for a susceptibility test, a Barium Sulfate (BaSO_4) turbidity standard equivalent to a 0.5 McFarland standard was used. About 3-5 well isolated colonies were picked up and inoculated in 4-5ml of peptone water broth and incubated at 37°C for 2-6 hrs. Inoculation was done by streaking the swab over the entire sterile agar surface. Discs were distributed evenly 24mm from centre to centre. Plates were inverted and incubated at 37°C for 16-18 hrs. The diameter of the zones of complete inhibition was measured using a sliding caliper. The tiny colonies which was detected only with the magnifying lens was ignored. The sizes of the zones of inhibition were interpreted by referring to the Clinical and Laboratory Standards Institute (CLSI) standards and reported as susceptible, intermediate, or resistant to the agents that have been tested. Routine disc diffusion susceptibility testing of the strains was performed by modified Kirby Bauer Method in MHA medium. Controls: Used with each batch were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923. Isolates found to be resistant (or) with decreased susceptibility ($<5\text{mm}$) to cefotaxime ($30\mu\text{g}$) and ceftazidime ($30\mu\text{g}$) were tested for the presence of ESBLs according to the CLSI guidelines. An overnight culture suspension of the isolate that was adjusted to 0.5 McFarland's standard was inoculated on the surface of a MHA plate using a sterile cotton swab. The ceftazidime ($30\mu\text{g}$) and ceftazidime-clavulanic acid ($30\mu\text{g}/10\mu\text{g}$) were placed 20mm apart on the agar. The inoculated agar plate was incubated overnight at 37°C . The zone diameter of $\geq 5\text{mm}$ for both the antimicrobial agent cefpodoxime and its combination with clavulanic acid was interpreted as positive for ESBL production. The descriptive data, clinical examination, Fasting blood sugar (FBS), Post prandial blood sugar (PPBS), HbA1C, microscopic examination of urine, urine culture sensitivity were recorded.

STATISTICAL ANALYSIS OF DATA

The master charts of diabetics with UTI and ESBL production were prepared. Descriptive statistics were computed by arithmetic mean and standard deviation. Pearson Chi Square test was used to quantify the extent of relationship between the ESBL producers and other quantitative variables. SPSS version 15.0 was used for statistical analysis. All the statistical tests used for analysis were two tailed. $P < 0.05$ was considered as statistically significant.

RESULTS

Bacterial isolates obtained from eighty patients out of one hundred and sixty four diabetic patients with symptoms of UTI from Sree Balaji Medical College and Hospital were studied during period of about one year to find the prevalence of Extended Spectrum Beta Lactamases (ESBL) producing gram negative bacilli among them. Study included patients of both sexes between 30 and 70 years of age. Specimen included was urine. Out of one hundred and sixty four specimens collected, in 81 specimens organisms were isolated. Seventy isolates were identified as gram negative bacilli which included *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Proteus mirabilis* by biochemical reactions. Six out of them were gram positive cocci.

The results were statistically analyzed as follows.

AGE AND SEX DISTRIBUTION (n = 164)

Total number of cases taken for the study was 164 which included total of 82 diabetic males and 82 diabetic females. The mean age of diabetic male was 48.26, with the range of 31 to 59 years of age. The mean age of diabetic female group was 48.23 with the range of 31 to 58 years. Patients were closely comparable in their age distribution within the groups.

BLOOD GLUCOSE LEVELS

The mean FBS is 234.92mg/dl with a SD of 84.08mg/dl ranging from 86mg/dl to 393mg/dl. The mean PPBS are 262.76mg/dl with a SD of 79.20mg/dl ranging from 111mg/dl to 421mg/dl. Over all the level of control of blood sugar among diabetics appears to be poor. All 164 diabetic patients were analyzed for HbA1C since they are established diabetics. This group showed only a moderate control. The mean HbA1C is 7.01 to the SD of 1.61, ranging from 5 to 11.

RECURRENT URINARY TRACT INFECTION

Comparisons of percentage of recurrent UTI with gender in diabetic patients in this study showed that the total number of subjects was 164 out of with 82 were female and 82 were male. Of 82 female diabetic patients, 58 patients gave history of recurrent Urinary Tract Infection and of 82 males 32 to give history of recurrent UTI with an incidence of 70.7% and 39% respectively (Graph 1).

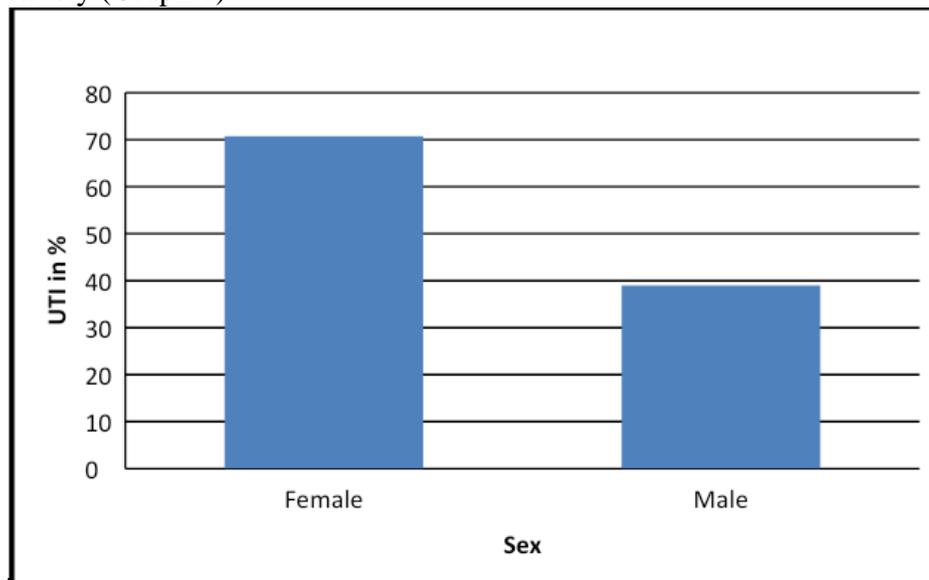


Chart 1 showing: Bar graph showing percentage of number of males and females diabetics.

ORGANISM ISOLATED FROM CULTURE POSITIVE SAMPLES n= 81

Out of 81 isolates 43(26.2%) were E.coli, 20(12.2%) K. Pneumoniae 6(3.7%) were K oxytoca, 6(3.7%) were Proteus mirabilis, 4(2.4%) were Candida, 2(1.2%) were S.aureus and 83(50.6%) showed no growth.

HbA1C WITH ESBL

This table shows the association between glycemic control and ESBL production. 12 out of 19 ESBL positives showed a good glycemic control with a range of 5 to 6.6 out of 19 ESBL isolates had a moderate glycemic control ranging from 6.1 to 8. And only 1 out of 19 ESBL isolates had a poor control of 8.1 and above thus, it is not statistically significant with a p value of 0.086. Hence this table does not establish any strong association between poor glycemic control and ESBL production.

ESBL AND RECURRENT INFECTION (n=19)

Comparison of Organism Isolated with ESBL in Percentage among diabetic Patients with recurrent UTI showing E coli to the most common organism associated with Recurrent UTI (Table 1).

Organism isolated	ESBL %	Recurrent infection%
Candida	0	1.1
E.coli	57.9	33.3
K.oxytoca	5.3	4.4
K.pneumoniae	21.1	18.9
Proteus mirabilis	15.8	4.4
S. aureus	0	1.1

Table 1 Comparison of organisms isolated with ESBL in recurrent UTI

RESISTANT STRAIN AMONG GNB

The total number of gram negative isolate obtained were 75 (45.73%) out of which 24 (32%) were resistance to 3rd generation cephalosporins (Chart 2)

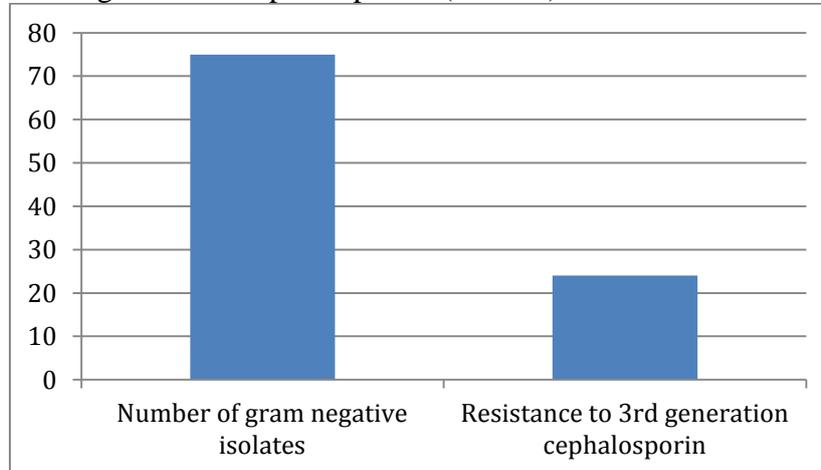


Chart 2: Bar graph showing resistant strains of GNB to 3rd generation cephalosporins

ESBL POSITIVE ISOLATES AMONG GRAM NEGATIVE BACILLI

Out of 75 (45.73%) gram negative bacilli 19 (25.33%) were found to be ESBL producers by combination disc method. (Chart 3)

COMPARISON OF DURATION OF DIABETES AND NUMBER OF ESBL PRODUCERS

15 out of 19 ESBL isolates had history of diabetes for more than five years. Of which only one in the group 15-20 years due to minimal number of patients in that group due to avoidance of subjects with complications. Hence this table establishes a strong association between increasing years of diabetes with ESBL production.(Table 2)

Duration of Diabetes	ESBL	Percentage	P Value
1 to 5 years	4	21.1%	0.000
6 to 10 years	6	31.6%	
11 to 15 years	8	42.1%	
16 to 20 years	1	5.3%	

Table 2 showing association of duration of diabetes in years with ESBL production.

COMPARISON OF DIABETIC PATIENTS AGE AND ESBL ISOLATES AMONG ESBL PRODUCERS

Among the 9 isolates of ESBL producers 3 were isolated in the age range of 31-40yrs and 41-50yrs each. Whereas a majority of ESBL producers 13 were isolated in the age range of 51-60yrs. This shows that there is a positive correlation between the increasing age of diabetic patients with ESBL producing isolates. (Table 3)

Age range of diabetic patients	ESBL producers (N=19)
31 to 40	03
41 to 50	03
51 to 60	13

Table 3 shows prevalence of ESBL producers in the age range of diabetic patients

DURATION OF UTI SYMPTOMS (DAYS) AND ESBL

This table shows the association of number of days of UTI symptoms with ESBL production. Out of 19 ESBL isolates, 13 diabetic patients had history of UTI symptoms of more than 4 days and only 3 had symptoms 3 days and below. This table gives a strong association of increasing days of UTI symptoms with ESBL production. However the p value is 0.165 and hence not statistically significant. (Table 4)

Duration of UTI symptoms (days)	ESBL	Percentage	P value
1 to 3 days	6	31.6%	0.165
4 to 6 days	10	52.6%	
7 to 9 days	2	10.5%	
10 to 12 days	1	5.3%	

Table 4 showing correlation of duration of UTI and ESBL

PERCENTAGE OF ANTIBIOTIC SUSCEPTIBILITY FOR ESBL PRODUCED ORGANISMS N=19

Retrospectively, the ESBLs Isolated had been highly susceptible to Amikacin(98.94%), moderately susceptible to Nitrofurantoin(68.4%) and Ofloxacin (57.8%), very least susceptible to ciprofloxacin (21.05%).

DISCUSSION

Various impairments in the immune system in addition to poor metabolic control of diabetes, and incomplete bladder emptying due to autonomic neuropathy may all contribute in the pathogenesis of urinary tract infections (UTI) in diabetic patients.⁶ In addition, the high rates of antibiotic prescription, including broad-spectrum antibiotics, for UTI in these patients may further induce the development of antibiotic-resistant urinary pathogens commonly the antibiotic resistant Gram Negative bacilli (GNB) which produce ESBL's.^{7,8} The prevalence of ESBL producing strains among Gram negative bacilli (GNB) varies widely depending upon the clinical setting and the geographic region.⁹ Infections due to ESBL producing organism increase the treatment cost and also increase the morbidity and mortality rate.^{10,11} They have the ability to inactivate beta lactam antibiotics containing oxyimino group such as oxyimino cephalosporin (Eg. ceftazidime, ceftriaxone, cefotaxime). They show resistance to oxyimino-monobactam as well.¹² Totally 164 urine specimen were collected, of which 81 organisms were isolated out of which 62 were non-ESBL producers. 75 out of them were gram negative bacilli like E coli, K pneumonia, pseudomonas etc of which E coli was the major the major isolate. This finding of ours correlates with the previous work of Habeeb Khadri (2009), Umadevi S (2011) and Hemachandran K (2011). The finding of this K pneumonia is a ESBL producing species next to *E. coli* corelates with the findings of Supriya et al (2004).¹³⁻¹⁵ In

our current study it was noted that majority of diabetic patients with recurrent UTI were females compared to male diabetic patients with recurrent UTI. these samples obtained were culture positive, which correlates with the studies of Schmitt JK et al (1986), Boyko EJ et al (2002) and Geerings (2008) which showed that prevalence of bacteriuria is greater in diabetic women and not in diabetic men.¹⁶⁻¹⁸ In our current study we noted that ESBL isolates were found to be more in patient suffering from diabetes for more than 5yrs. Based on this finding we suggest a strong association between ESBL production with increase in years of diabetes. However we cannot establish this relationship due to limited sample size, excluding subjects with complications and lack of literature. Of 19 ESBL isolates 13 belonged to the age range of 50-60yrs and 6 belonged to the age range of 30 to 50yrs. This suggested that the ESBL production increased correspondingly with advancing age. Though this correlation was not significant statistically it does show clinical significance and correlates with the study of Eligius F et al.¹⁹ In our current study the HbA1c ranged from 5 to 9 and above. We compare HbA1c with ESBL producers and found that as the HbA1c range increases there seems to be decrease in the number of ESBL producers thus showed negative correlation between them. This finding of ours is supported by study of Boyko EJ et al who concluded that there is no established linear trend between HbA1c and UTI risk.¹⁷ However Kelestimur et al found a positive significant relation between HbA1C and bacteriuria and pyuria.²⁰ 75 urine samples showed gram negative bacilli and we decided to detect for ESBL by combined disc diffusion method. We compared the duration of onset of diabetes with the ESBL isolates and found that there was a strong association between increasing years of diabetes with ESBL and were found to be statistically significant. We also compared the age of the diabetic patients with the ESBL isolates and found that there was a positive correlation between increasing age of diabetic patients with ESBL isolates. This finding of ours is in contrast with the previous works of Al-Garni SM (2018) who found no significant association between age and ESBL infections.²¹⁻²³ Among the 75 gram negative bacilli isolated 24 were resistant to 3rd generation cephalosporin (32%) and 19 out of them were positive by combination disc diffusion method for ESBL (25.33%) and was found to be highly significant. this finding of ours is in agreement with the findings of Subha A et al (2001) who also found that majority of strains were resistant to 3rd generation cephalosporin. We also express the same opinion as theirs that such strains pose a threat in the management of patients.²⁴⁻²⁶ 19 ESBL producing organisms were further subjected to antibiotic susceptibility test and found that majority of organisms were susceptible for amikacin (98%) followed by nitrofurantoin (68%), ofloxacin (57%) and least susceptible to ciprofloxacin (21%). This finding of ours is consistent with studies of Mohammad MA (2016) and Gharavi MJ (2021).²⁷⁻³⁰

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