ORIGINAL RESEARCH

Prevalence of ESBL producing bacteria in recurrent UTI of diabetics: An Original Research

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ABSTRACT

Introduction: Many Gram negative bacteria are multi resistant as they produce ESBLs which breaks down the ring in the antibiotics there by rendering them ineffective. Infections caused by ESBL can be of great consequences even with common infections like urinary tract infections. Timely detection of drug resistant bacteria is of outmost importance as it plays a role in treatment approach and fatality.

Materials and methods: 5ml of Clear midline stream urine was collected from 164 patients. The urine samples were careened for bacterial isolates and the presence of ESBL producers were confirmed by combination disc method and tested for antibiotic sensitivity and resistance.

Results: A total of 164 urine samples from 82 males and females were collected. We found that 58 females and 32 males had recurrent UTI. 43 and 38 isolates of ESBL producing bacteria were found by combination disc method in urine samples of females and males respectively. Out of 81 isolates of ESBL producers 43 were from E coli and 20 were from K pneumoniae.

Conclusion: We found that E coli is the largest producer of ESBL in urine samples of patients with diabetes. ESBL isolates were found more in female urine samples than in males. There was a strong positive association of levels of diabetes with ESBL production. The ESBL were highly sensitive to amaikacin and nitrofurantoin but resistant to 3rd generation cephalosporin.

Key words: E. coli, K pneumoniae, antibiotic resistance, combination disc method

INTRODUCTION

Extended spectrum Beta Lactamase (ESBLs) are enzymes produced by gram negative bacilli (GNB). In current times ESBLs are endemic infections found everywhere including hospitals-community settings and is widely dependent on geographical and clinical settings.[1,2] ESBL producing organisms are seen increasingly in older age population and in patients who are severely ill like malignancy or in common infections like urinary tract

infections or in patients who stay in hospital wards for long time and in ICU and also in patients who are functionally dependent. The presence of ESBL in such settings carries tremendous clinical significance as the options of antibiotics used in treating ESBL organisms is extremely limited thus resulting in increase treatment cost, increase in mortality and morbidity rate. ESBL are drug resistant pathogens which are difficult to be identified using routine laboratory methods thus delaying the diagnosis followed by delay in administration of appropriate antimicrobial therapy.[3-5] ESBLs are extended spectrum β lactamase enzymes; known so because of their ability to hydrolyze a broader spectrum of β-Lactam antibiotics than the simple parent β-lactamases from which they are derived. They are acquired plasmid-mediated β-lactamases. Many β lactam antibiotics like penicillin, ampicillin and 1st generation cephalosporins have been developed over the years which are specifically designed to be resistant to the hydrolytic action of β lactamases. But with each new class of β lactam antibiotic discovered to treat infection a new β lactamases emerges to render the organism resistant to the antibiotic thus the cycle continues.[6] The greater risk for acquiring ESBL associated infections are from recurrent UTI, previous antibiotic use, co-morbidities like Diabetes mellitus, prior catheterization of urinary tract and people above 65yrs of age.[7,8] The most common wide spread disease is diabetes of current population all around the world and the most common infection of diabetes is recurrent urinary tract infection. It is thought that high blood sugar level favors the colonization of micro organisms in the urinary tract.[9-11] Thus the aim of the present study is to identify the prevalence of various micro-organisms in the urine samples of diabetics with recurrent UTI.

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 1year. 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 60yrs of both genders with history of diabetes mellitus without any other debilitatory or co- morbid condition like retinopathy, nephropathy, polyneuropathy etc. Exclusion criteria: Diabetic patients without symptoms of UTI, with complications and debilitatory 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 was 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^{o}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, tripl8e sugar iron (TSI) test, carbohydrate fermentation tests. Cartridge of Antimicrobial Disc was stored in a tightly sealed container and refrigerated at 4-8^{o}C or kept frozen at -14^{o}C. β- Lactam antibiotics were stored frozen. Disc containers were brought to room temperature before use. To standardize the inoculums 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^{o}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^{o}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 (<5mm) to cefotaxime (30µg) and ceftazidime (30µg) 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 Mc Farland's standard was inoculated on the surface of a MHA plate using a sterile cotton swab. The ceftazidime (30µg) and ceftazidime-clavulanic acid (30µg/10µg) were placed 20mm apart on the agar. The inoculated agar plate was incubated overnight at 37^{o}C. The zone diameter of >/=5mm 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.

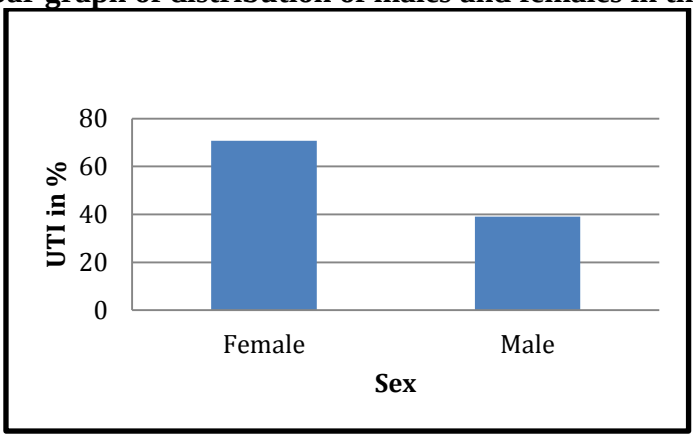
RESULTS

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 gram negative bacilli producing Extended Spectrum Beta Lactamases (ESBL) among them. Study included patients of both genders between 30 and 70 years of age. Sample specimen collected from patients was urine.

AGE AND SEX DISTRIBUTION (n = 164)

In this study the total number of subjects was 164, out of which 82 were female and 82 were male. Of 82 female diabetic patients, 58 patients gave history of recurrent UTI and of 82 males 32 to give history of recurrent UTI with an incidence of 70.7% and 39% respectively.

Graph 1 showing bar graph of distribution of males and females in the study with UTI



BLOOD GLUCOSE LEVELS

The overall blood levels of control of blood sugar among diabetics appear to be poor with PPBS raging from 111mg/dl to 421mg/dl and the mean being 262.76%. The HbA1c ranged from 5 to 11 with mean being 7.01.

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 which 82 were female and 82 were male. Of 82 female diabetic patients, 58 (70.7%) patients gave history of recurrent Urinary Tract Infection and of 82 males 32 (39%) gave history of recurrent UTI. (Table 1)

Table 1 showing number of ESBL positive isolates from the Gram negative bacilli isolates by combination disc method.

Number of females with recurrent UTI	Number of males with recurrent UTI
58(70.7%)	32(39%)

ISOLATION OF ORGANISM

Out of 82 urine samples of females, 43 were found to be culture positive and 39 were culture negative. Out of 82 urine samples of males 38 were found to be culture positive and 44 were culture negative.

RESISTANT STRAIN AMONG GNB

Out of 81 bacterial isolates 75 isolates (45.73%) of bacteria were Gram Negative Organisms among which 24 isolates were resistant to 3rd generation cephalosporin. (Table 2)

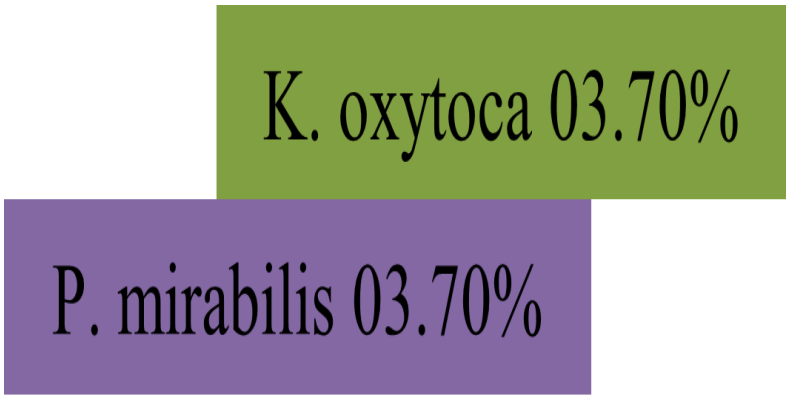
Table 2 showing the incidence of gram negative isolates and their resistance to 3rd generation cephalosporin.

Number of gram negative isolates	Resistance to 3rd generation cephalosporin
75(45.73%)	24(32%)

NUMBER OF ORGANISM ISOLATED

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.(Graph 2)

Graph 2 Pie graph showing distribution of organism isolated from urine samples of male and female diabetics.



RESISTANT STRAIN AMONG GNB

Among the 75 GNB isolates obtained in our study 19 were found to be ESBL producers by combination disc method and 24 (32%) of them were resistant to 3rd generation cephalosporin.

NUMBER OF PUS CELLS AND ESBL

The number of Pus cells/HPF was associated positively with ESBL production. 10-20 pus cells/HPF were seen with 8 ESBL producers whereas >25 pus cells /HPF were seen with 11 ESBL producers. Hence establishing a strong association between number of pus cells with ESBL production. (Table 2)

Table 3 showing association of number of pus cells/HPF with ESBL production

No. of Pus Cells/ Hpf	ESBL
less than 10	0
10 to 14	4
15 to 20	4
More than 25	11

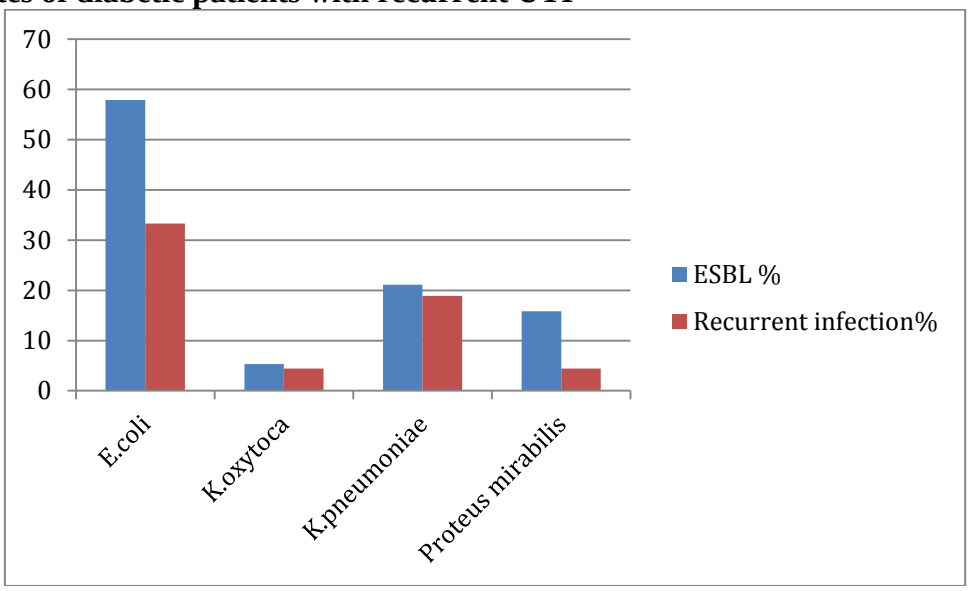
ESBL ISOLATES AND DURATION OF RECURRENT UTI

Out of 19 ESBL producers, 17 diabetics gave positive history of recurrent UTI. In the age group of 30 to 50, totally five had recurrent UTI, while above the age of 50 till 60 in the study included, 12 had recurrent UTI, which gives a strong association between the diabetics belonging to age more than 50 having recurrent UTI with ESBL production.

ESBL AND RECURRENT INFECTION (n=19)

Among the 19 ESBL producers obtained in this study, 11 were *E.coli*, of which 10 diabetic patients gave history of recurrent UTI and only female patient belonging to the age of 48 did not give history of recurrent UTI. 4 were *K.pneumoniae*, of which 3 diabetic patients gave positive history of recurrent UTI, while only one male diabetic aged 59 did not give history of recurrent UTI. 3 were *P. mirabilis* and only one was *K. oxytoca*, of which all gave positive history of recurrent UTI. (Graph 3)

Graph 3 showing Bar diagram above showing the incidence of ESBL producers among the isolates of diabetic patients with recurrent UTI



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%)

Table 4 showing antibiotic sensitivity of ESBL producing bacteria

Antibiotics	Percentage	Number of organism
Amikacin	98.94%	15
Nitrofurantoin	68.4%	13
Ceftazidime	0%	0
Nalidixic Acid	0%	0
Cefixime	0%	0
Cefuroxime	0%	0
Gentamycin	0%	9
Cefdinir	0%	0
Ciprofloxacin	21.05%	4
Norfloxacin	0%	0
Ofloxacin	57.8%	11
Aztreonam	0%	0
Cefotaxime	0%	0
Ceftriaxone	0%	0

DISCUSSION

Critically ill patients are easily prone to infection based on the clinical setting and geographic distribution. Among all, infections caused by drug resistance pathogens are of very important concern as they carry a higher mortality and morbidity.[12-14] β lactam antibiotics were used commonly for treating bacterial infections. Production of enzymes known as β lactamases is the mechanism of the organism to gain resistance to these β lactam antibiotics. Antibiotics have a β lactam ring and β lactamase enzymes break this ring by hydrolysis. Hydrolysis of penicillin produces penicillioic acid and this can be detected biochemically by acidometric method, iodometric method and chromogenic method.[15-17] Extended spectrum β lactamase

ESBLs are enzymes produced by Gram Negative Bacteria (GNB). Currently there are more than 200 ESBL producers and their presence can be confirmed by confirmatory test like combination disc method according to CLSI guidelines. Combination disc method is a phenotypic confirmatory test where cephalosporin is used in combination with clavulanic acid on agar plates and zone of inhibition of ≥ 5mm is considered positive for ESBL. Over the last 20 years many new be β lactam antibiotics were developed to resist hydrolytic action of beta lactamase producing organisms. However with each new class of antibiotics used to treat patients, a new β lactamase emerges quickly. [18-20]Datta et al in 1960 1st described TEM-1 β-1 lactamase which is well known for its antibiotic resistance against penicillin and early cephalosporins.[21] TEM-1 β lactamase enzyme was originally found in a single strain of E coli from blood culture of patient named Temoniera hence called TEM.[22]Since its discovery around 170 variants of TEM-1 has been isolated in various clinical settings and hospitals.[24] SHV1 is another important β lactamase enzyme1st isolated in K. pneumoniae and E coli in 1974.[25-27]The known risk factors for acquiring ESBL infections are recurrent UTI, previous antibiotic usage, diabetes mellitus, prior catheterization of urinary tract, females, age above 65 years.[28,29] In this present study we assessed the prevalence of various micro-organisms in the 164 urine samples of diabetics with recurrent UTI of both genders. We found that the incidence of recurrent UTI increased with age and female diabetics were more affected by recurrent UTI than males. This finding of ours correlates to the findings of Geerlings 2001 and Bokyo EJ 2002.[30-33] We found that patient with diabetes were more associated with ESBL related recurrent UTI and are similar to the findings of Rahim 2018.[34] We found that the samples of diabetic females showed more culture of bacterial isolates than the diabetic male samples. Among the isolated bacteria, gram negative bacteria like E coli, K. pneumoniae, K oxytoca, P mirabilis were found in increasing numbers and candida and S. aureus were found in small numbers in samples of both genders. This finding of ours is similar to the findings of Habeed K 2009, Lyamuya 2011.[35,36] Supriya 2004 found that K. pneumoniae is the ESBL producing species after E coli and correlates with findings of our study.[37] But contrast to this, findings of Nicolle 2001 found that the bacteria isolated from diabetic are the same to non diabetic patients with complicated UTI.[38]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.[22] 19 ESBLs isolated in our present study were highly susceptible to amikacin, moderately susceptible to nitrofurantoin and ofloxacin and least susceptible to ciprofloxacin. This finding of ours is consistent with studies of Kibret 2011, Kumar d 2014.[39,40] There is emergence of antibiotic resistant bacteria worldwide and posses a major threat to the outcome of even common diseases. Infections caused by enterobacteriaceae like urinary tract infections may produce β lactamase enzyme which develop multi drug resistance to penicillin and cephalosporins. Major outbreak of these drug resistant organisms may result in limited therapeutic options increasing the rate of mortality. Hence early and effective antibiotic strategy should be prepared to diagnose ESBL infections thereby prevent and treat such infections effectively reducing mortality rate.

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