International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 668-674**
http://www.ijcmas.com

Original Research Article

Antibiotic resistant pattern of urinary tract infection causing *Escherichia coli* isolated from diabetic mellitus and non-diabetic mellitus patients with special reference to Rifampicin resistance

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Keywords

Urinary tract infection (UTI) sample, Diabetic mellitus(DM), Non-diabetic mellitus (NDM) antibiotic resistance, plasmid curing.

A B S T R A C T

Urinary tract infection (UTI) is a major problem in diabetics. The risk of developing infection in diabetic patients is higher and urinary tract is the most common site for infection. Antibiotic resistance has emerged due to its frequent use. Resistance has emerged even to more potent antimicrobial agents. Treating UTI infected diabetic patients creating further complication. Hence there is a need to correlate the emergence of antibiotic resistant to diabetic and non-diabetic patients. In the present study, About 61 samples were collected from both diabetic mellitus (DM) and non-diabetic mellitus (NDM) patients who are having Urinary Tract Infections. The isolates were identified on the basis of morphology, growth and biochemical characteristics. Out of 61 samples processed 50 were positive for *E.coli* isolated from diabetic and non-diabetic patients which shows a higher incidence of 81.9% out of which 31(62%) was from DM & 19(38%) was from NDM. All the isolates offered different resistance patterns against antibiotics including Clotromazole, Chloromphenicol, Nalidixic Acid, Rifampicin, Nitrofurantoin, Amoxicillin and Ciprofloxacin. The present study concludes that a high number of *E.coli* strains isolated from both diabetic and non diabetic patients showed resistant to the antibiotic Rifampicin and Ciprofloxacin . However, the resistant to Rifampicin was more than the Ciprofloxacin.The representative isolates lost their antibiotic resistance after curing hence it was inferred that the antibiotic resistant was plasmid borne.

Introduction

Urinary Tract Infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. Nowadays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group (Kunin, 1994). The wide spread use of

antimicrobial agents has failed to eradicate microbial infections despite of their benefits. Antibiotic resistant microorganisms have been a source of ever-increasing therapeutic problem. Continued mismanaged selective pressure has contributed towards the emergence of multiple drug resistant bacteria and that has been regarded as an inevitable genetic response to antimicrobial therapy (Cohen 1992). The emergence of antibiotic resistance in the management of UTIs is a serious public health issue. Particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also high prevalence of fake and spurious drugs of questionable quality in circulation (Manikandan et al 2011). The antibiotic resistant mutants that arise spontaneously are generally resistant to only one antibiotic. The ability of bacteria to adapt to a hostile environment has been known from ancient times and development of a phenomenon of resistant against antibiotics mediated by plasmid. The R plasmids offer resistance to antibiotics and are transmissible from one cell to another by direct cell contact.

Conjugation (direct *in-vivo* gene transfer) is a convenient method of transferring drug resistant genetic determinants among intra and inter-generic bacterial populations. A surveillance study has demonstrated the emergence of highly resistant *Klebsiella spp.*, in urinary and respiratory tract infections (Bonafede & Louis, 1997). The urinary tract infection caused by *E.coli* is an excellent example of host microbe interaction and is termed to be virulent by attaching to pre-urethral flora of the urinary tract and establishes inflammation of the urinary tract named as urinary tract infection.

The present study was undertaken to assess the current antibiotic resistance pattern in the common uropathogen *Escherichia coli*. Since *Escherichia coli* is the predominant pathogen, the study was focused on it. the pathogens were isolated from Diabetic mellitus and Non-diabetic mellitus patients, in a tertiary care hospital in south India with a special emphasis on Rifampicin resistance.

Materials and Methods

Isolation and Identification

There were 61 urine samples collected from Urinary Tract Infection (UTI) patients with or without the case history of Diabetic Mellitus (DM) and Non-Diabetic Mellitus (NDM) from a private diagnostic centre at Chennai, Tamilnadu as per the standard procedure. Out of 61 urine samples 36 from DM and 25 from NDM with UTI were collected. Samples were streaked on MacConkey and EMB agar and the agar plates were incubated at 37°C for 24 hrs. The identification of the bacterium was carried out with the help of colony morphology, staining characters and biochemical properties. All the samples were subjected to Gram staining, Hanging Drop, Catalase, Oxidase. The isolates were subjected to biochemical reaction which includes Indole test, Methylred test, Voges-prauskaer test, Citrate Utilization test, Urease test, Triple Sugar Iron test etc., along with individual sugar fermentation such as glucose, sucrose, lactose and maltose.

Determination of antibiotic resistance pattern (Bauer et al. 1963)

Antibiotic sensitivity test was carried out by disc diffusion technique on Muller Hinton agar plates of pH 7.2 – 7.4. The

following antibiotics such as Clotromazole, Chloromphenicol, Nalidixic Acid, Rifampicin, Nitrofurantoin, Amoxicillin and Ciprofloxacin were used to determine antibiotic resistance pattern. Isolated colonies were picked up from a fresh isolation plate, inoculated on Trypticase Soya broth medium and incubated for 2 to 6 hrs at 37°C until good visible growth. The growth is adjusted to 0.5 Mc-Farland standard equal to 10^8 cells/ ml and swab inoculated onto Mueller Hinton Agar. The antimicrobial discs were placed at equi-distance and the discs were pressed gently with forceps. After 16-18 hrs incubation of the plates at 37°C, the zone of inhibition were read with metallic rulers in mm and interpreted using standard zone of inhibition charts.

Plasmid isolation and curing experiments (Birnboim and Doly 1979; Pramod 1997):

The culture was incubated overnight in peptone water and serial dilution was performed. One drop of the culture placed on haemocytometer and cells were counted. Dilution containing 10-15 cells per ml were taken and inoculated into Luria-Bertani (LB) broth containing 4μg/ml of Ethidium bromide in a light proof flask and incubated at 37°C. The culture was then streaked on nutrient agar and the resulting colonies were taken and antibiogram was performed.

Results and Discussion

The wide spread use of antimicrobial agents has failed to eradicate microbial diseases despite of their benefits. The resistance of bacteria causing urinary tract infection (UTI) to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to more potent antimicrobial agents. Treating UTI infected diabetic patients creating further complication. Hence there is a need to correlate the emergence of antibiotic resistant to diabetic and non-diabetic patients.

Isolation and identification

It was found that, the total of 61 samples processed 50 were positive for *E.coli* isolated from diabetic and non-diabetic patients which shows a higher incidence of 81.9% out of which 31(62%) was from DM & 19(38%) was from NDM (Table 3.1). Hence the report suggests that the majority of *E.coli* was isolated from UTI of diabetic patient. Out of 61 urine samples processed 50 were found to be positive for UTI. The organisms were identified by gram staining, hanging drop method, cultural characteristics on growth media, and biochemical characteristics.

The present study relates to the isolation and identification of Rifampicin resistant *E.coli* from UTI patients, who are either diabetic or non-diabetics. The isolation and identification was based on Morphological, cultural and biochemical characteristics. All the isolates were screened for their antibiotic resistance profiles to a battery of seven antibiotics representing different groups. Stamm et al (2005) in their studies on management of Urinary Tract Infections collected from individuals with the age group below 30 years and above 60 years. In The present study the urine samples were collected samples from diabetic patients (31) and Non diabetic patients (19) who are infected with UTI. The samples were from different age group ranging from 7-60 years and above, out of which 23 were females and 38 males (Graph 3.1& 3.2). A total of 100 urine samples were collected

from patients with urinary tract infection out of which 50% of the isolates were identified as *E.coli*. The ratio of incidence of *E.coli* infection in diabetic mellitus was 62% and 83% in non diabetic patients (Shah et al 2005).In our study a total of 61 urine samples were collected from UTI of diabetic and non diabetic patients out of which 51 samples (81%) were found to be positive for *E.coli.*

Antibiotic resistant pattern

The graph 3.3 & 3.4 showed antibiotic resistant pattern of *E.coli* isolates, isolated from both diabetic and nondiabetic patients. The antibiotic resistant pattern was studied from hospitalized diabetic patients (Shah et al 2005). It was found that the *E.coli* showed an increasing resistance to Rifampicin and Ciprofloxacin. In the present study the anti-biogram of the isolates were carried out using antibiotics like Ciprofloxacin, Rifampicin, Nitrofurantoin etc and increased resistance to Rifampicin and Ciprofloxacin was observed. Stamm et al (2005) reported that *E.coli* maintains antibiotic resistance R-factor and based on the Rifampicin resistance, isolates were subjected to plasmid analysis and found to be positive. In the present study 14 Rifampicin resistance isolates were subjected to plasmid isolation technique and a similar banding pattern of molecular weight (2.45kb) was observed.

Plasmid isolation and curing

The Plasmid DNA were subjected to plasmid analysis profile and run along with the super ladder marker of molecular weight of about 500bp – 54000bp. Based on the molecular weight all the 14 isolates showed band of identity revealing that they were all of same molecular weight of 24.5kb**.** The plasmid curing was performed for 14 isolates of *E.coli* using Ethidium bromide as a curing agent. After curing the Rifampicin resistant property of the isolates was lost and isolates became sensitive towards Rifampicin. This was indicated by the formation of a large zone on Muller Hinton Agar plate.

The Klebsiella isolates which showed different pattern of resistance to antibiotics like Ampicillin, Streptomycin and Chloromphenicol, were subjected to plasmid curing using acridine orange as a curing agent (Sheik et al 2003). In our study 14 Rifampicin resistant isolates were subjected to plasmid curing using Ethidium bromide as a curing agent and after curing the resistant property of isolate was lost indicates that it was plasmid-borne.

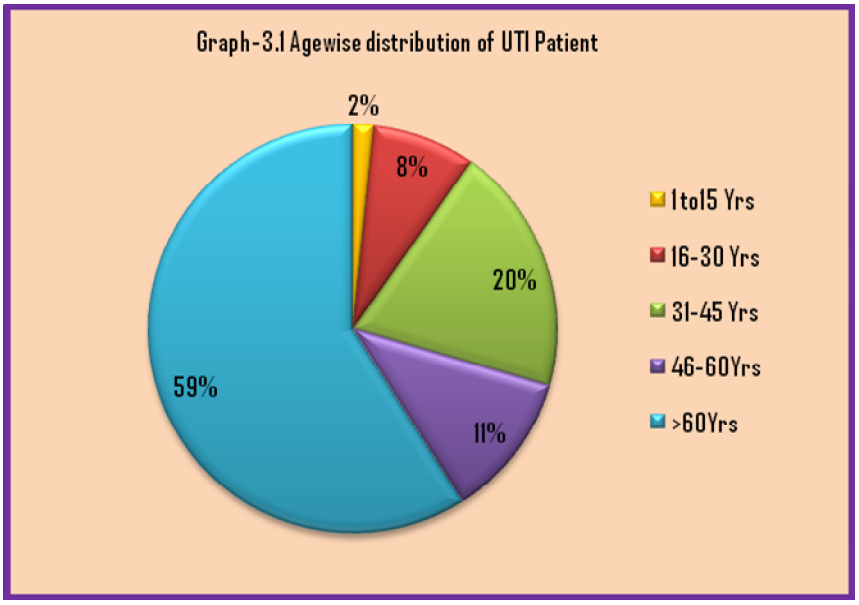
The urine samples were collected from 36 diabetic and 25 non diabetic patients. Samples were streaked on nutrient, MacConkey and Eosin methylene blue agar. *E.coli* isolates were identified as per the conventional morphological and biochemical methods. Antibiotic sensitivity test was performed and the plasmid isolation was carried out for 14isolates of *E.coli.* In the present study concludes that a high number of *E.coli* strains isolated from both diabetic and non diabetic patients showed resistant to the antibiotic Rifampicin and Ciprofloxacin.

However, the resistant to Rifampicin was more than the Ciprofloxacin. The molecular weight of the plasmid was 24.5kb when compared with standards marker. Plasmid curing was done using Ethidium bromide as curing agent. After curing the resistant property of the isolates was lost and they become sensitive to the drug.

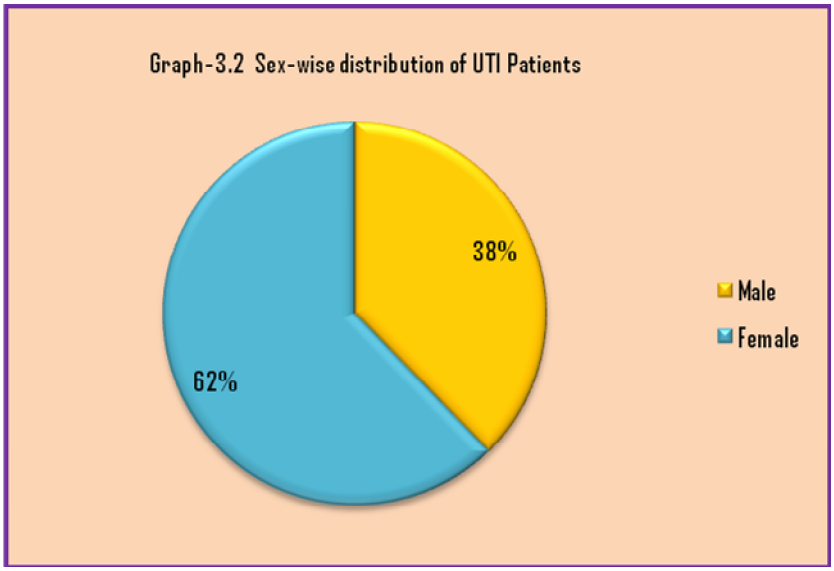
Table.3.1 Clinical specimen (urine)

S.No	Specimen	DM	NDM	Total	No.of positive	Percentage
1.	Urine	36	25	61	50	81.9

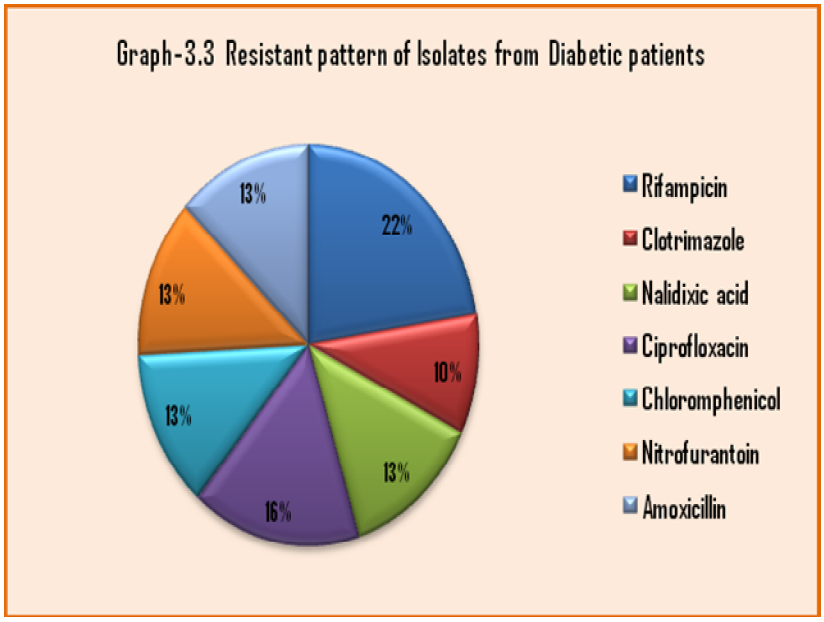
Graph.3.1 Interpret the age wise distribution of UTI patients involved in this study. The majority of the samples were obtained from UTI patients above 60 yrs



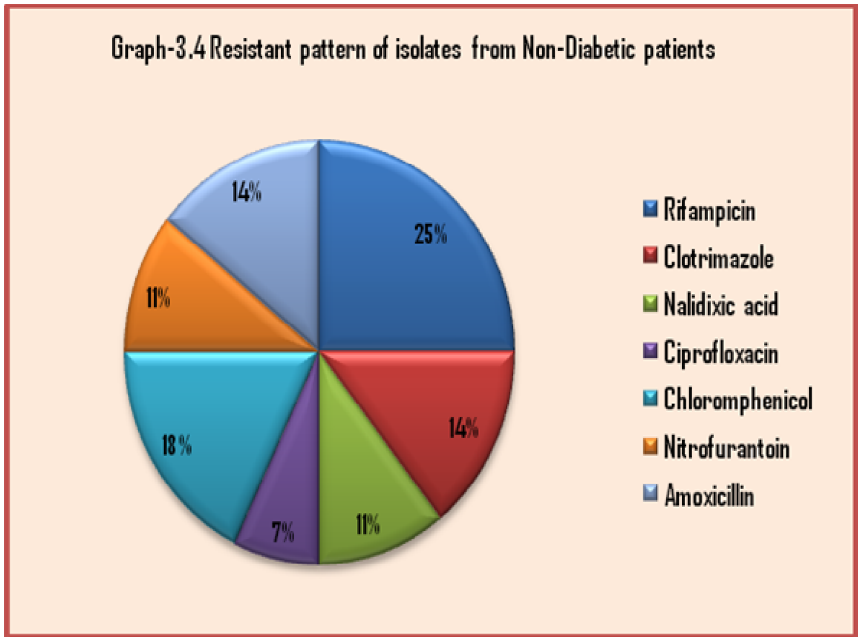
Graph.3.2 Showed sex wise distribution of Urinary Tract infection patients used in this study. Urinary Tract infection is more prevalent in female than male



Graph.3.3 Out of 7 antibiotics tested against the *E.coli* which cause UTI in diabetic mellitus all found to show various incidence of resistant pattern. The higher % of resistance is offered by Rifampicin (22.5%) followed by Ciprofloxacin and Nitrofurantoin (16.1% & 12.9% respectively). But only 3 isolates showed resistance to Clotrimazole(9.6%).



Graph.3.4 Out of 7 antibiotics tested the *E.coli* which cause UTI in non-diabetic all found to show various incidence of resistant pattern. The higher% of resistance is offered by rifiampicin36.8% followed by chloromphenicol and clotrimazole (26.3% & 21% respectively) but only 3 isolates showed resistance to ciprofloxacin(13.3%).



Antibiotic resistance has emerged due to its frequent use. This resistance was seen more in the in-patients, elderly males and females. Also the resistance to other antibiotics was also high. Increasing antibiotic resistance trends indicate that it is imperative to rationalize the use of antimicrobials in the community and also use these conservatively. The present study concludes that a high number of *E.coli* strains isolated from both diabetic and non diabetic patients showed resistant to the antibiotic Rifampicin and Ciprofloxacin . However, the resistant to Rifampicin was more than the Ciprofloxacin. Further the resistant was plasmid coded as it was determined by plasmid curing experiment.

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