ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Biological Sciences 4 (7): 548-555, 2011
ISSN 1996-3351 / DOI: 10.3923/ajbs.2011.548.555

A Study of Antibacterial Susceptibility and Resistance Pattern of *E. coli* Causing Urinary Tract Infection in Chittagong, Bangladesh

[1]Zinnat Shahina, [2]Md. Jahedul Islam, [2]Jesmin Abedin, [1]A.H.M. Ishaque Chowdhury and [2]Md. Arifuzzaman

[1]Department of Microbiology, University of Science and Technology Chittagong, Chittagong, Bangladesh
[2]Department of Biochemistry and Biotechnology, University of Science and Technology Chittagong, Chittagong, Bangladesh

Corresponding Author: Md. Arifuzzaman, Department of Biochemistry and Biotechnology, University of Science and Technology Chittagong, Chittagong, Bangladesh Tel: +88-031-659070-71

ABSTRACT

Urinary tract infection is a very common disease that can affect anyone at any age where the infection rate is higher in woman than man. This study aimed to ascertain the current situation of Urinary Tract Infections (UTIs) and drug resistance determination caused by *Escherichia coli* in different age groups of patients. This prospective study determined the antibiotic susceptibility of 131 isolates of *Escherichia coli* from the urine culture of 665 patients. Urine samples were aseptically collected in sterile containers and then cultured on Nutrient media. Susceptibility to 9 antimicrobial agents was tested by the disc diffusion technique. Data analysis was performed by employing SPSS software, Version 10.0 and group's t-test was done as a test of significance. Among 131 *E. coli* confirmed patients 69 were females and 31% were males. It state that female are more vulnerable to UTI than male ($p<0.001$). The onset of UTIs in female was high at the age between 21-30 years (28.90%). The occurrence of the disease was high in March-October. Studies revealed that the most resistance rates for *E. coli* detected from urine culture were Amoxicillin, Doxycycline, Cephalexin, Cephradine, Cotrimoxazole, Cefixime, Ceftroxone and Ciprofloxacin except, Cefuraoxime which was significantly sensitive ($p<0.001$). This study was evaluated for the prevalence of *E. coli* in UTI especially to the women and the antibiotic resistance pattern of *E. coli*.

Key words: Uropathogens, *Escherichia coli*, urinary tract infection, antibiotic susceptibility, cefuraoxime, chittagong

INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infections in many developing countries like Bangladesh where proper sanitation is not maintained adequately. It has been reported that up to 150 million individuals are affected annually worldwide (Stamm and Norrby, 2001). UTIs affect all age group individuals and diagnosed in both hospitalized as well as outpatients. This burden causes serious impact on the socioeconomic life of individuals and also leads to a large proportion of antibacterial drug consumption (Dada-Adegbola and Muili, 2010). The main identified causes of UTIs are related to poor perineal hygiene, pregnancy, urinary tract obstruction, urethral reflux, long time catheterization, sexual intercourse, spermicidal contraception and a history of UTIs (Manges *et al*., 2008; Nahar *et al*., 2010). Women are more susceptible to UTIs due to their anatomical relation of

genitourinary tract (Levinson and Jawetz, 2000). It has been observed that, up to one-third of all women will experience UTI at some point during their lifetime (Valiquette, 2001). This finding is attributed to three features that facilitate ascending infection into bladder, namely a short urethra, the proximity of the urethra to the anus and colonization of the vagina by members of the fecal flora (Levinson and Jawetz, 2000).

Generally, the predominant uropathogens for UTIs are Gram negative bacteria and *Escherichia coli* accounting for the highest prevalence in most instances (Gupta *et al*., 1999; Moges *et al*., 2002; Sibi *et al*., 2011).

Studies revealed that uropathogens produce resistance against many common antibiotics where *E. coli* is one of them. This is because of the frequent misuse of antibiotics, inadequate dosages and easy availability of antimicrobial drugs (Tamberkar *et al*., 2006; Okeke *et al*., 2000; Lamikanra and Ndep, 1989; Okeke *et al*., 1999). Consequently, antibiotic resistance complicates UTIs treatment and this fact necessitates the appropriate use of antibiotics along with the formulation of new antibiotics (Hasan *et al*., 2007). The aims of the present study were, to investigate the frequency of UTIs in both males and females in different age groups in Chittagong district of Bangladesh, comparing the diversity of *E. coli* sensitivity to antibiotics and to evaluate the antibiotic resistance pattern which develops gradually in this particular region.

MATERIALS AND METHODS

This is a retrospective study of the antibiotic sensitivity profile of 131 *E. coli* positive culture samples including 91 females and 40 males from urine culture of 665 urine samples over a period of 36 months (from January, 2008-December, 2010) at Bango Bondhu Memorial Hospital (BBMH), Chittagong, Bangladesh. Patients were identified and data were extracted using the hospital information and support system.

Culture examination: Urine samples were aseptically collected in sterile containers and then cultured on Nutrient agar media using a calibrated loop, delivering 10 μL of the sample. Then, the medium was incubated overnight at 37°C.

Isolation of colonies: All specimens with significant bacteria were further processed and cultured on Mac Conkey's agar and Nutrient agar media. After over night incubation rose pink and opaque white colonies of *E. coli* were appeared in Mac Conkey's agar and nutrient agar media, respectively.

Microscopic examination: The colonies obtained from Mac Conkey's agar plate were processed for Gram staining. Then the size, shape and color of the organism were observed. It was found rod shaped and red colored bacilli.

Sensitivity test: Susceptibility to 9 antimicrobial agents was tested by the disc diffusion technique according to the guidelines by the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards). The antibiotic discs were used, Amoxicillin (25 μg), Doxycycline (30 μg), Cephalexin (30 μg), Cephradin (30 μg), Cotrimoxazole (25 μg), Cefuraoxime (30 μg), Cefixime (10 μg), Ceftroxone (30 μg) and Ciprofloxacin (5 μg). After incubation at 37°C for 24 h the zone of inhibition in diameters were measured to the nearest millimeter and isolates were classified as susceptible, intermediate, or resistant according to CLSI-specified interpretive criteria (Clinical and Laboratory Standards Institute, 2005).

Name, surname, sex, age, date of isolation, organism isolated and susceptibility results of positive UTI isolates were recorded. The patients were categorized into 6 major age groups, <10, 11-20, 21-30, 31-40, 41-50 and >50 years.

Statistical analysis: Analysis was performed by employing Statistical Package for Social Science (SPSS Version 10.0) software and Excel office program for the statistical analysis of this study. To compare mean values between groups t-test was done as a test of significance.

RESULTS

A total of 131 UTIs caused by *E. coli* were confirmed on urine culture of 665 patients. Among them 69% patients were females (91) and 31% were males (40). It revealed that female were more vulnerable to UTI than male ($p<0.001$).

To analyze the prevalence of UTI at different age group, a gender based age group variation of UTI was analyzed. It was observed that, in each and every age group the percentage of female patients were higher than male. The percentage of male and female patients was highest in the age group between 21-30 years which was 7 and 28.90%, respectively. The lowest percentage of male and female patients was in the age group <10 years which was 2.10 and 2.80%, respectively. The present data stated that, the onset of UTIs in female were high at the age between 21-30 years (28.90%) (Fig. 1).

Furthermore, monthwise observation of 131 UTI cases showed that, during March-June the percentage of male and female patients were 14.50 and 24.43%, respectively. During July-October male and female patients were 9.20 and 27.50%, respectively. During November-February male and female patients were 6.90 and 17.56%, respectively (Fig. 2). This data suggested that, the onset of the disease was high in March-June and July-October. The number of female patients were significantly ($p = 0.05$ and $p<0.01$, respectively) high in both of these month groups than the male patients. But the onset of the disease was lower in November-February and the differences of male and female patients counts showed statistically insignificant ($p>0.10$).

The sensitivity tests to *E. coli* were carried out with 9 antibiotics such as, Amoxicillin, Doxycycline, Cephalexin, Cephradine, Cotrimoxazole, Cefixime, Ceftroxone, Cefuraoxime and Ciprofloxacin by disc diffusion technique. Among them Amoxicillin, Doxycycline, Cephalexin,

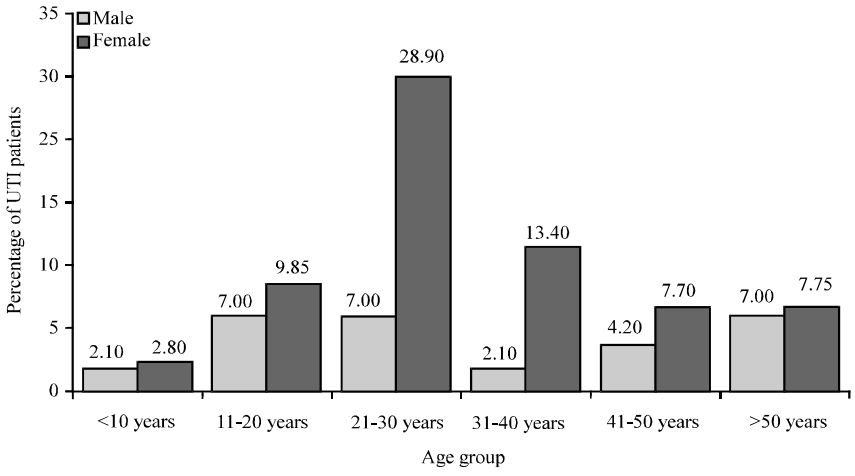

Fig. 1: Percentage of male and female UTI patients by *E. coli* in different age groups in this study

Cephradine, Cotrimoxazole, Cefixime, Ceftroxone and Ciprofloxacin showed less sensitivity to *E. coli*. But Cefuraoxime showed significant (mean p-value <0.001) level of sensitivity to *E. coli* (Fig. 3).

Furthermore, it was observed that, among study populations Amoxicillin, Doxycycline, Cephalexin, Cephradine, Cotrimoxazole, Cefixime, Ceftroxone and Ciprofloxacin showed resistance by *E. coli*. The resistance pattern of the resistant antibiotics was comparably high of Amoxicillin, Cephradine and Cotrimoxazole which were 40, 23 and 21%, respectively. The resistance pattern of other antibiotics such as, Doxycycline, Cephalexin, Cefixime, Ceftroxone and Ciprofloxacin were comparably low which were 6, 2, 4, 2 and 2%, respectively. Among all tested antibiotics Cefuraoxime showed no resistance by *E. coli* at all. This data suggested that, Amoxicillin, Cephradine and Cotrimoxazole were significantly (mean p-value <0.001) resistant by *E. coli* (Fig. 4).

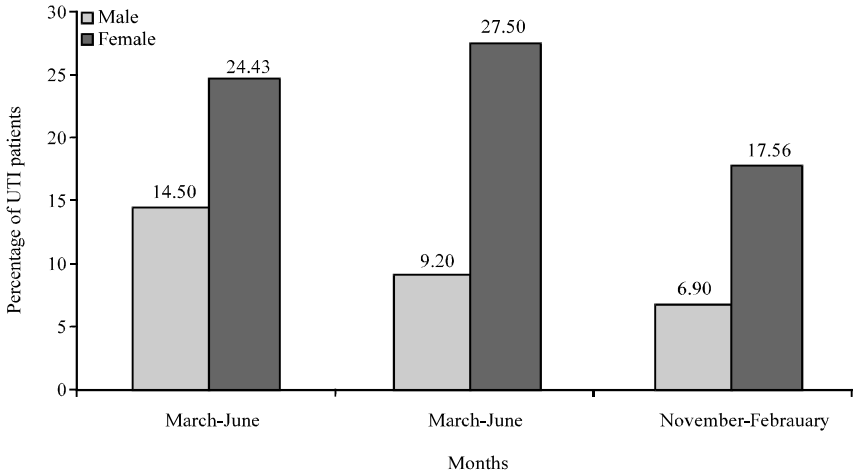

Fig. 2: Monthwise observation of UTI patients by *E. coli* in this study

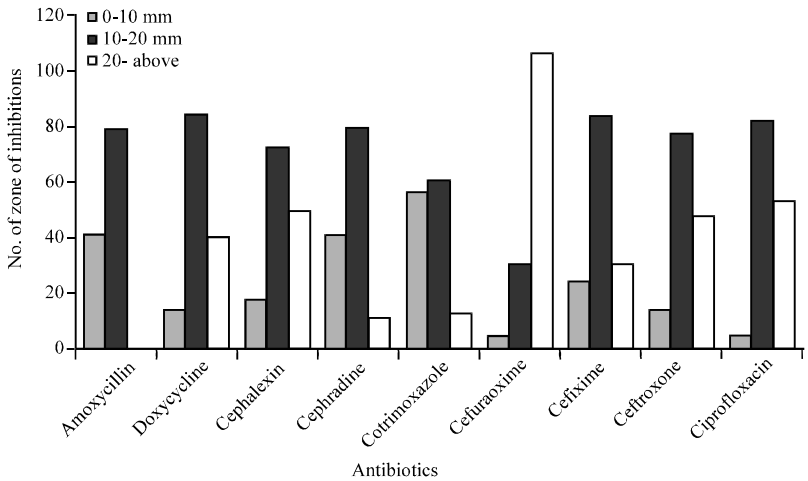

Fig. 3: Antibiotic sensitivity to *E. coli* isolated from the UTI patients in this study. The sensitivity of all antibiotics showed by bar columns. 0-10, 10-20 and 20-above mm represents the zone of inhibition by the antibiotics on the culture plates

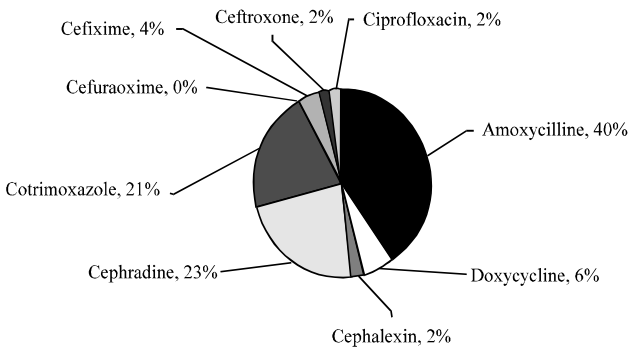

Fig. 4: Percentage of antibiotic resistance in UTI patients caused by *E. coli* in this study

DISCUSSION

In this study, the comprehensive data analysis of UTI patients in all age groups in Bango Bondhu Memorial Hospital (BBMH) in Chittagong district of Bangladesh suggested the significantly increasing resistance of antibiotics in this particular region. From the screened cases 131 were confirmed with UTIs where *E. coli* was mostly the etiologic agent (19.7%). It was individually 47.6, 53.24 and 49.4% cases which were found earlier by Gul *et al.* (2004), Khleifat *et al.* (2006) and Shareef and Yagoub (2006).

Among 131 UTI patients the percentage of female (69%) were more compared to male patients. In the study done by Rafique *et al.* (2002). The female UTI proportion was 132 (66%) out of 200 enrolled patients. The prevalence of female UTIs was higher in the age group between 21-30 years (28.9%). The lowest percentage of female patients was in the age group <10 years (2.80%). In the same age groups the male patients were 7.0 and 2.10%, respectively (Fig. 1). Among the female patients adult women were observed to be very much prone to UTIs. The similar finding was also found earlier by Nahar *et al.* (2010).

The prevalence of the UTIs were high in between March-June and July-October. The number of female patients were significantly high ($p = 0.05$ and $p<0.01$, respectively) in both of these month groups than male patients (Fig. 2). It might be due to higher atmospheric temperature in Bangladesh during March-October than other months and this increased temperature causes more sweating, therefore, urine production becomes less and concentrated which enhances the opportunity to multiply the bacteria. Conversely the prevalence of UTIs were comparably low in November-February where the difference of male and female patient counts showed statistically insignificant ($p>0.10$). It might be due to winter season in Bangladesh during November-February and winter season causes more dilute urine production which washes away any multiplying bacteria and inhibits UTIs which ultimately causes low patient counts. This statement was supported by Nahar *et al.* (2010).

The problem of bacterial antibiotic resistance emerged as soon as the first antibiotics become available for clinical use (Raco and Barez, 1998). This study is consistent with previous observation where amoxicillin resistance in UTI patients were high (Kader *et al.*, 2004; Astal, 2005; Aboderin *et al.*, 2009) following Cephradine and Cotrimoxazole (Fig. 3).

Furthermore Antibiotic resistance among the study populations were 40% for Amoxicillin, 23 for Cephradine, 21 for Cotrimoxazole, 6 for Doxycycline, 4 for Cefixime, 2 for Cephalexin, 2 for

Ceftroxone and 2% for Ciprofloxacin (Fig. 4). Amoxicillin, Cotrimoxazole and Cephradine were found 83.7, 61 and 22% resistant in previous studies (Mohammadi *et al*., 2010; Manikandan *et al*., 2011; Bhowmick and Rashid, 2004, respectively). This findings indicated that, Amoxicillin, Cephradine and Cotrimoxazole showed much more resistance by *E. coli* where their mean p-value of resistance was $p<0.001$.

Among the antibiotics tested, Cefuraoxime was found significantly sensitive than the other tested antibiotics in this study. So, it could be considered as a suitable alternative first-line oral antibiotic to treat UTI patients. The p-value of Cefuraoxime's sensitivity was $p<0.001$.

E. coli remained the predominant organism causing UTIs in the majority of the screened patients (Tamberkar *et al*., 2006; Inabo and Obanibi, 2006; Akhtar Khan *et al*., 2002). Antibiotic resistance by pathogenic microorganisms was recognized as an increasing global problem and had become an important factor to be considered in the treatment of infections (Tenover and Hugles, 1996; Tamberkar *et al*., 2006). The trend of increasing resistance of urinary pathogens had been published in the recent years (Gruneberg, 1994; Barrett *et al*., 1999; Gupta *et al*., 1999). As the antibiotic resistance is an important factor, urine culture and Antibiotic sensitivity tests should be routinely performed in all UTI patients. Depending on the antibiotic susceptibility, therapy should be designed of an antibacterial agent with the narrowest spectrum, least cost and few adverse effects.

CONCLUSION

Present study suggested that *E. coli* were responsible for UTIs in 131 cases out of 665 patients and female were the main sufferer especially those of 21-30 years of age. The organism developed resistance against many common antibiotics which might be due to inappropriate use, frequent misuse, inadequate dosages, easy availability of antimicrobials and duration of treatment. In most of the cases people are not aware of this problem and remain without proper laboratory investigations and treatment. So, large-scale prospective studies are urgently required from other centers in the Chittagong district to look at the similar data in order to investigate this matter further and to identify predisposing factors for urinary pathogens with antibiotic resistance. In this regards local policies for the choice of first-line oral antibiotic treatment for UTI patients should be reviewed every 3 years or so according to local resistance rates.

ACKNOWLEDGMENT

Special thanks to Mohammed Rafiq, Laboratory assistant, department of Microbiology, USTC.

REFERENCES

Aboderin, O.A., A.R. Abdu, B.W. Odetoyin and A. Lamikanra, 2009. Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections. J. Natl. Med. Assoc., 101: 1268-1273.

Akhtar Khan, N., S. Noor, S. Abdus and Q. Ali Abbas, 2002. Incidence and antibiogram patterns of *Escherichia coli* isolated from various clinical samples from patients at N.I.H. Islamabad. Pak. J. Biol. Sci., 5: 111-113.

Astal, Z.E., 2005. Increasing Ciprofloxacin resistance among prevalent urinary tract bacterial isolates in the Gaza Strip. Singapore Med. J., 46: 457-460.

Barrett, S.P., M.A. Savage, M.P. Rebec, A. Guyot, N. Andrews and S.B. Shrimpton, 1999. Antibiotic sensitivity of bacteria associated with community-acquired urinary tract infection in Britain. J. Antimicrob. Chemother., 44: 359-365.

Bhowmick, B.K. and H. Rashid, 2004. Prevalence and antibiotic susceptibility of *E. coli* isolated from urinary tract infection (UTI) in Bangladesh. Pak. J. Biol. Sci., 7: 717-720.

Clinical and Laboratory Standards Institute, 2005. Performance Standards For Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA.

Dada-Adegbola, H.O. and K.A. Muili, 2010. Antibiotic susceptibility pattern of urinary tract pathogens in Ibadan, Nigeria. Afr. J. Med. Med. Sci., 39: 173-179.

Gruneberg, R.N., 1994. Changes in urinary pathogens and their antibiotic sensitivities, 1971-1992. J. Antimicrob. Chemother., 33: 1-8.

Gul, N., T.Y. Mujahid and S. Ahmad, 2004. Isolation, identification and antibiotic resistance profile of indigenous bacterial isolates from urinary tract infection patients. Pak. J. Biol. Sci., 7: 2051-2054.

Gupta, K., D. Scholes and W.E. Stamm, 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. J. Am. Med. Assoc., 281: 736-738.

Hasan, A.S., D. Nair, J. Kaur, G. Baweja, M. Deb and P. Aggarwal, 2007. Resistance patterns of urinary isolates in a tertiary Indian hospital. J. Ayub Med. Coll. Abbottabad, 19: 39-41.

Inabo, H.I. and H.B.T. Obanibi, 2006. Antimicrobial susceptibility of some urinary tract clinical isolates to commonly used antibiotics. Afr. J. Biotechnol., 5: 487-489.

Kader, A.A., A. Kumar and S.M. Dass, 2004. Antimicrobial resistance patterns of gram-negative bacteria isolated from urine cultures at a general hospital. Saudi J. Kidney Dis. Transpl., 15: 135-139.

Khleifat, K.M., M.M. Abboud, S.S. Omar and J.H. Al-Kurishy, 2006. Urinary tract infection in South Jordanian population. J. Med. Sci., 6: 5-11.

Lamikanra, A. and R.B. Ndep, 1989. Trimethoprim resistance in urinary tract pathogens in two Nigerian hospitals. J. Antimicrob. Chemother., 23: 151-154.

Levinson, W. and E. Jawetz, 2000. Medical Microbiology and Immunology: Examination and Board Review. 6th Edn., McGraw-Hill, New York.

Manges, A.R., H. Tabor, P. Tellis, C. Vincent and P.P. Tellier, 2008. Endemic and epidemic lineages of *Escherichia coli* that cause urinary tract infections. Emerg. Infect. Dis., 14: 1575-1583.

Manikandan, S., S. Ganesapandian, M. Singh and A.K. Kumaraguru, 2011. Emerging of multidrug resistance human pathogens from urinary tract infections. Curr. Res. Bacteriol., 4: 9-15.

Moges, A.F., A. Genetu and G. Mengistu, 2002. Antibiotic sensitivities of common bacterial pathogens in urinary tract infections at Gondar Hospital, Ethiopia. East Afr. Med. J., 79: 140-142.

Mohammadi, M., E. Ghasemi, H. Mokhayeri, Y. Pournia and H. Boroun, 2010. Antimicrobial resistance patterns of *E. coli* detected from hospitalized urine culture samples. Asian J. Biol. Sci., 3: 195-201.

Nahar, S.J., H. Khanum and K. Shimasaki, 2010. Occurrence of Escherichia coli infection among the women of Dhaka City. ARPN J. Agric. Biol. Sci., 5: 68-73.

Okeke, I.N., A. Lamikanra and R. Edelman, 1999. Socioeconomic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg. Infect. Dis., 5: 18-27.

Okeke, I.N., S.T. Fayinka and A. Lamikanra, 2000. Antibiotic resistance in *Escherichia coli* from Nigerian students, 1986 1998. Emerg. Infect. Dis., 6: 393-396.

Raco, M.V.O. and M.Y.C. Barez, 1998. Profile of community-acquired urinary tract infections in Davao City. Phil. J. Microbiol. Infect. Dis., 27: 62-66.

Rafique, S., M. Arifa, Q. Mazhar and Q. Ali Abbas, 2002. Prevalence patterns of community-based and nosocomial urinary tract infection caused by *Escherichia coli*. Pak. J. Biol. Sci., 5: 494-496.

Shareef, S.A. and S.O. Yagoub, 2006. Incidence and isolation of bacteria associated with nosocomial Urinary Tract Infection (UTI) in sudanese women. Res. J. Microbiol., 1: 534-539.

Sibi, G., A.P. Devi, K. Fouzia and B.R. Patil, 2011. Prevalence, microbiologic profile of urinary tract infection and its treatment with trimethoprim in diabetic patients Res. J. Microbiol., 6: 543-551.

Stamm, W.E. and S.R. Norrby, 2001. Urinary tract infections: Disease panorama and challenges. J. Infect. Dis., 183: 1-4.

Tamberkar, D.H., D.V. Dhanorkar, S.R. Gulhane, V.K. Khandelwal and M.N. Dudhane, 2006. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. Afr. J. Biotechnol., 5: 1562-1565.

Tenover, F.C. and J.M. Hugles, 1996. The challenges of emerging infectious diseases. Development and spread of multiply-resistant bacteria pathogens. J. Am. Med. Assoc., 275: 300-304.

Valiquette, L., 2001. Urinary tract infections in women. Can. J. Urol., 1: 6-12.