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Original article

Escherichia coli, the Predominant Uropathogen in Women and it's Antimicrobial Susceptibility.

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Abstract:

Urinary tract infection (UTI) is one of the most common bacterial infection in humans and a major cause of morbidity. Most commonly, members of Enterobacteriaceae family, particularly the uropathogenic strains of *Escherichia coli is the* primary causative organism of UTIs in different parts of the world. The aim of this retrospective study was to determine the frequency of uropathogens in different age groups of both sex and to evaluate *Escherichia coli* as predominant bacteria for urinary tract infection in women with their antimicrobial sensitivity pattern. A total of 1616 urine samples were collected during January to December, 2012, from patients attending at Delta Medical College & Hospital, Dhaka and analyzed for different uropathogens. Urine cultures were carried out and the isolates were identified by standard procedures as needed. Antimicrobial susceptibility testing was performed by disk diffusion method according to 'The Clinical Laboratory Standard Institute' (CLSI) guidelines. In this present study, prevalence of uropathogens were higher in female patients (77.2%) than male patients (22.8%). The isolates were *Escherichia coli* (80%), *Klebsiella spp.* (7.9%), *Enterococcus spp.* (4.9%), *Staphylococcus saprophyticus* (2%), *Proteus* (1.4%), *Pseudomonas spp.* and *Staphylococcus aureus* (1.2%). The rate of *Escherichia coli* infection in females of reproductive age group; 15-50 years was higher (61%) and in that only a particular age group (21-30) was more prone to this pathogen (22.9%). Among the drugs used for testing the antimicrobial susceptibility, Imipenem, Amikacin, Nitrofurantoin and Gentamycin were found to be effective in the treatment of urinary tract infection. As drug resistance to commonly used antimicrobials is increasing, improvement in overall sanitary condition, proper knowledge on personal hygiene would play an important role in reducing the incidence and occurrence of urinary tract infection.

Key words : UTI, Uropathogen, Escherichia coli, Antibiotic susceptibility.

Introduction:

Urinary tract infection (UTI) is caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the urothelium. UTI may be asymptomatic or accompanied by fever, dysuria, urinary frequency & urgency, cloudy urine, supra-pubic or lower abdominal discomfort and pyuria. The hallmark of UTI has been presence of a single type of microorganism in numbers > 10^5 colony forming units (cfu) per ml in a clean-catch or midstream urine specimen, with lower numbers usually indicating contamination.[1]

The common uropathogens causing UTI include enteric gram-negative bacteria, predominantly *Escherichia coli*

(Esch.coli) followed by *Klebsiella*, *Proteus*, *Pseudomonas* and *Enterobacter*. [2,3] Gram-positive isolates mostly responsible for UTI are coagulase-negative *Staphylococcus saprophyticus* [4,5] and *Enterococcus*. The predisposing factors for UTI are poor perineal hygiene, sexual intercourse, pregnancy, urinary tract obstruction, urethral reflux, catheterization and neurogenic bladder.

UTI is an extremely common bacterial infection in humans and have been reported in all age groups in both sexes.[6] It has been estimated that symptomatic UTI occurs about 150 million per annum worldwide.[7]

Urinary tract infection is common in females than in males because female urethra (shorter & wider) appears to be less effective in preventing the entry of bacteria. Also anatomic, hormonal and behavioral differences between male and female are responsible for more female UTI.

UTI is challenging, not because of the large number of infections that occur each year, but also the diagnosis of UTI is not always straight forward, rather accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria in particular, *Escherichia coli* remain the most frequent cause of UTI.[8] Antimicrobial resistance among urinary tract isolates has recently been reported with an increase frequency all over the world[9-12] and makes its treatment more complicated. In Bangladesh, general practitioners, at large, recommend antibiotics without isolation and sensitivity test for pathogens. But therapeutic decision should be based on accurate and up-to-date antimicrobial susceptibility. Therefore it is necessary to identify the causative agent and spectrum of it's antimicrobial susceptibilities in order to treat UTI promptly and accurately.

The present retrospective study was undertaken to find out the frequency of different urinary tract pathogens in different age groups of both sex and to evaluate *Escherichia coli* as predominant pathogen for UTI in women with their antimicrobial susceptibility pattern, so as to guide in empirical treatment and prevent the development of antimicrobial resistance.

Materials & Methods:

For this retrospective study, mid-stream urine samples from patients attending at Clinical Microbiology Laboratory of Delta Medical College & Hospital, Dhaka, Bangladesh were collected during January to December, 2012, using sterile, dry, wide mouthed & leak-proof plastic containers and analyzed them for different organisms responsible for UTI.

Standard methods for isolation and identification of the organisms were carried out with all urine samples. Blood agar and Mac Conkey agar were plated using a calibrated loop withdrawing 0.001 ml of urine sample. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. Significant growth was determined as $\geq 10^5$ colony-forming units (CFU)/ mL of midstream urine samples and $\geq 10^2$ CFU/mL of a catheter specimen.[13] Gram negative bacteria were identified by morphological study, oxidase test, routine biochemical tests such as motility test, indole and urease production and Triple Sugar Iron reaction.[14] Gram positive microorganisms were identified by catalase, coagulase and Novobiocin tests.[15]

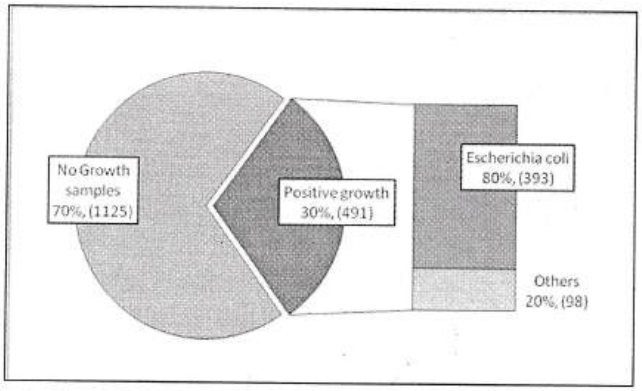
All the bacterial isolates including *Escherichia coli* of all age groups were tested for anti-microbial sensitivity by Disc diffusion of Bauer and co-workers with use of commercially prepared antibiotic disks.[16] Interpretation of results was done measuring the sizes of zones of inhibition and reported according to 'The Clinical Laboratory Standard Institute' (CLSI) guidelines.[17] Antibiotics for uropathogens were tested include; ampicillin, cotrimoxazole, ciprofloxacin, ceftriaxone, cephradine, cefixime, ceftazidime, nitrofurantoin, nalidixic acid, gentamicin, amikacin, imipenem, aztreonam & cefotaxime.

Data were categorized according to age and sex. The patients were divided into age groups as <1 years, 1-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, 61-70 years, 71-80 years, 81-90 years and 90+ years. They were further categorized into reproductive age group (15-50 years) and elderly women (>50 years).

Results:

During January to December, 2012, a total 1616 urine samples were tested from patients of all age groups in both sex. Among them, 491 (30.39%) were culture positive, of which 393 (80.05%) were Escherichia coli (Figure 1).

Figure-1: Isolation rate of bacteria in urine samples



Among culture positive samples, predominant gram negative bacteria was *Escherichia coli* (80.05%) followed by *Klebsiella* (7.9%), *Proteus* (1.4%), *Pseudomonas* (1.2%) and *Acinetobacter* (0.2%). Gram positive isolates were *Enterococcus* (4.9%), *Staphylococcus saprophyticus* (2%) and *Staphylococcus aureus* (1.2%). Only 5 (1%) urine samples showed growth of Candida species. Of 393 Escherichia coli, 315 (83.1%) isolates were found from urine samples of female patients. All other bacteria showed the same trend except Pseudomonas which occur in same proportion between male and female patient's sample (Table-1).

Table-1: Distribution of Isolates in Urine samples

Organisms	Isolates from male (112)*	%	Isolates from female (379)*	%	Total Isolates (491)*	%
Gram negative						
Escherichia coli	78	69.6%	315	83.1%	393	80.0%
Klebsiella	11	9.8%	28	7.4%	39	7.9%
Proteus	4	3.6%	3	0.8%	7	1.4%
Pseudomonas	3	2.7%	3	0.8%	6	1.2%
Acinatobacter	0	0.0%	1	0.3%	1	0.2%
Gram positive						
Enterococcus species	8	7.1%	16	4.2%	24	4.9%
Staphylococcus saprophyticus	1	0.9%	9	2.4%	10	2.0%
Staphylococcus aureus	5	4.5%	1	0.3%	6	1.2%
Fungus						
Candida	2	1.8%	3	0.8%	5	1%

Table-2 showed predominance of *Escherichia coli* isolation in female patients of 21-50 years age group (179; 57%).

Table-2: Frequency of Escherichia coli isolates in different age group of both sex.

age group in years	E. coli Isolates in Male (N=79)*	%	E. coli Isolates in Female (N=314)*	%
< 1	1	1.3	5	1.6
1-10 yrs	6	7.6	8	2.5
11-20 yrs	0	0.0	21	6.7
21-30	7	8.9	72	22.9
31-40	2	2.5	60	19.1
41-50	16	20.3	47	15.0
51-60	17	21.5	52	16.6
61-70	17	21.5	28	8.9
71-80	11	13.9	17	5.4
81-90	2	2.5	3	1.0
91+	0	0.0	1	0.3

Mean age: Male=51± 20yrs; Female=41±18 yrs

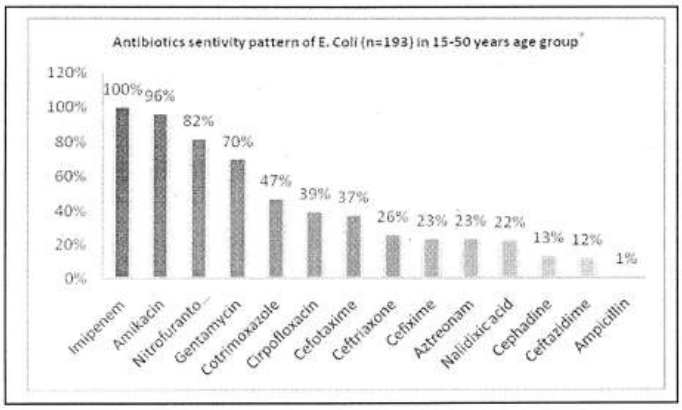
Also growth rate of *Escherichia coli* in urine sample of women was higher in reproductive age group; 15-50 years (193; 61%) than > 50 years (101; 32%). Other non-*Escherichia coli* isolates also follow same trend (Table-3).

Table-3: Uropathogen among reproductive age and elderly women

Uropathogen	Frequency among women by age group 15-50 years (N=217)*	>50 years (N=108)*
Escherichia coli	193	101
Klebsiella	21	5
Proteus	2	0
Pseudomonas	1	2

Escherichia coli of reproductive age group were found highly sensitive to Imipenem (100%), Amikacin (96%), Nitrofurantoin (82%) and Gentamicin (70%). Ciprofloxacin (39%) and Ceftriaxone (26%) were also effective to treat Urinary tract infection caused by *Escherichia coli*, though less than Cotrimoxazole (47%). Nalidixic acid has lost its efficacy (22%) in the treatment of UTI and Ampicillin is of no use now-a-days as it showed high resistance rate to *Escherichia coli*, only 1% found sensitive (Figure 2).

Figure-2: Antibiotics sensitivity pattern of *Escherichia coli* among the women of reproductive age.



Discussion:

In spite of the availability and use of the antimicrobials, urinary tract infections (UTI) caused by bacteria have been showing increasing trends in recent years.[18]

The common uropathogen was significantly gram-negative bacteria, with *Escherechia. Coli* (E. coli) being the most common. The remainders of infections are caused by *Staphylococcus saprophyticus*, *Proteus*, *Klebsiella*, and *Enterococcus* which account for less than 5%.[19-21] Our findings are consistent with these reports where *E. coli* has 80% prevalence followed by *Klebsiella*, *Enterococcus*, *Staphylococcus saprophyticus* and *Proteus*. In two other studies in Pakistan, Khan[21] reported a uropathogens prevalence of 45.6% for *E. coli*, while Farooqy revealed 42%.[22]

In this study, the prevalence of UTI in females (77.2%) is more than in males (22.8%). This is in agreement with other reports which stress that UTI is more frequent in females than males.[23-28] Amin et al.[29] reported that the urine cultures bacteriologically positive were found in 68% and 32% of the examined females and males subjects respectively. The frequency reported in this work is also similar to that reported by Okafor et al.[30] in which only 20.7% urinary tract infection was reported in males.

Women of different age groups were observed to be very much prone to UTI.[31] Different studies[32,33] have shown that women were the usual victims of urinary pathogens, predominantly of *E. coli*. This supports our finding of *E. coli* (83.1%) in female UTI.

The higher incidence of urinary tract infections in females might be due to close proximity of the female urethral meatus to the anus and incomplete voiding of urine which encourages infection of the urinary tract. Alternations in vaginal microflora also play a critical role in encouraging vaginal colonization with coliforms, especially *E. coli* and this can lead to urinary tract infection.[34]

In our study, the occurrence of UTI by *E. coli* among females was higher in reproductive age group (61%), of which highest prevalence was seen in the age group of 21-30 (22.9%) followed by 31-40 (19.1%). This finding correlates with the reports of earlier workers.[35] The reason could be multiparity, sexual intercourse, pregnancy and certain contraceptive methods. Women are mostly sexually active at this age. The report of this study is also similar to that of Leigh and Onuh et al.[36, 37]

In our study, *E. coli* showed very high level of susceptibility to imipenem (100%) followed by amikacin (96%), nitrofurantoin (82%) and gentamicin (70%). However, *E. coli* was poorly sensitive to cotrimoxazole (47%), ciprofloxacin (39%), and cefotaxime (37%). This pattern of sensitivity is similar to those reported by other workers.[38]

Fluoroquinolones are the most widely prescribed antibiotics for the empirical therapy of UTI followed by cotrimoxazole, cephalosporins and penicillin. But a reduced sensitivity of *E. coli* to the fluoroquinolones ciprofloxacin (39%) was observed in this study as opposed to the findings of Gupta & Prescott[39,40] who reported a higher efficacy of the drug against *E. coli.*

The antibiotic sensitivity test of this study shows that imipenem, amikacin and nitrofurantoin were the most effective antibiotic in treatment of UTI caused by *E. coli.* Similar results have been reported by other authors.[41] This low resistance of pathogens might be attributed to the fact that imipenem and amikacin are relatively newer antibiotics and have not been extensively used to warrant resistance developing against them by pathogens. Goldraichi and Manfrori reported a sensitivity of *E. coli* to nitrofurantoin of 92%, 95% and 94%, respectively over a three-year period.[42] This finding is similar to our study, in which 82% of *E. coli* were sensitive to nitrofurantoin .

Increasing antibiotic resistance of *E. coli* among urinary tract isolates (mostly against ampicillin) has been reported from many countries. The resistance rates to ampicillin were found to be 45%, 50% and 100% in Canada, Europe and Africa; respectively.[43-46] In our study, the resistance of *E. coli* among UTI isolates to ampicillin was 99%, which is consistent with other reports.[47-48] Therefore, ampicillin should be stopped in treating urinary tract infections.

Conclusion:

The present study identified eight different uropathogens, all were more frequent in female than male. Among them, *E. coli* was found to be predominant and has the highest prevalence in age group of 21-30. The organism showed high levels of resistance to ampicillin, cephradine and Cotrimoxazole thus indicating that increased consumption of a particular antibiotic can be a pathway to the development of its resistance among uropathogens. Though sensitivity of Fluoroquinolone has reduced, still it remains effective against UTI, but if frequently used or rather misused in empirical treatment, it might contribute to development of resistance. Moreover *E. coli were* found more sensitive to imipenem, amikacin but their use is limited by the fact that they have only injectable preparations but nitrofurantoin can be the drug of choice for treatment of UTI. Finally, we suggest that empirical antibiotic selection should be evidence based i.e. on the knowledge of local prevalence of bacterial organisms and their antibiotic sensitivity pattern rather than on universal guidelines.

Reference:

1. Strand, C., Bryant, J. and Sutton, K. Septicemia secondary to urinary tract infection with counts less than 105 cfu/ml. Am. J. of Clin. Pathol. 1985; 83: 619-622.
2. Orenstein, R. and Wong, E. S.1999. Urinary tract infections in adults. Am. Family Phys.; 59: 1225-1234.
3. Lutters, M. and Vogt, N. 2000. Antibiotics duration for treating uncomplicated, symptomatic lower urinary tract infections in elderly women. Cochrane Database Systematic Rev.; 2: 255-258.
4. Jacobson, S. H., Eklof, O. and Eriksson, C. G. Development of hypertension and uremia after pyelonephritis in childhood: 27 year follow up. B. M. J. 1989; 299: 703-706.
5. Steele, R. W. The epidemiology and clinical presentation of urinary tract infections in children 2years of age through adolescence. Pediatr. Ann. 1999; 28: 653-658.
6. Hooton TM, Stamm WE (1997). Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. North Am. 11: 551-581.
7. Stamm, W. E. and Norrby, S. R. 2001. Urinary tract infections: disease panorama and challenges. J.Infect. Dis.; 183 (Suppl 1): S1-S4.
8. Ojiegbe GC and Nworie WC (2000). Asymtomatic Bacteriuria among School Pupils in Enugu Urban Areas. J. Med. Sci. 9(1): 42-46.
9. Zorc JJ, Kiddoo DA, Shaw KN. Diagnosis and management of pediatric urinary tract infections. Clin Microbiol Rev 2005;18:417–22.
10. Turnidge J, Bell J, Biedenbach DJ, Jones RN. Pathogen occurrence and antimicrobial resistance trends among urinary tract infection isolates in the Asia- Western Pacific Region: report from the SENTRY Antimicrobial Surveillance Program, 1998-1999. Int J Antimicrob Agents 2002;20:10-7.
11. Zhanel GG, Karlowsky JA, Schwartz B, Jensen SB, Hoban DJ. Mecillinam activity compared to ampicillin, trimethoprim/sulfamethoxazole ciprofloxacin and nitrofurantoin against urinary tract isolates of gramnegative bacilli. Chemotherapy 1998;44:391-96.
12. Bahram F, Farhad H, Mohammad E, Marzieh A, Farrokh A, Bahram K. Detection of vancomycin resistant enterococci (vre) isolated from urinary tract infections (UTI) in Tehran, Iran. Daru 2006;14:141-45. [In Persian].
13. Forbes BA. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis, Missouri: Mosby; 1998; p:283–304.
14. Foxman, R., B.H. D'Arcy and B. Gillespie, 2000. Urinary tract infection: Self-reported incidence and associated costs. Ann., 10: 509-515.PMID: 11118930.
15. Andreu, A., J.I. Alos, M. Gobernado, M. Fdela Rosa and J.A. Garcia-Rodriguez, 2005. Etiology and antimicrobial susceptibility among uropathogens causing community-acquired lower urinary tract infections: A nationwide surveillance study. Enferm. Infect. Microbiol. Clin., 23: 4-9. PMID: 15701325.
16. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., J. Microbiol., 2: 118-123. 45: 493-496. PMID: 5325707.
17. Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
18. Newell, A., P. Riley and M. Rogers, 2000. Resistance patterns of urinary tract infections diagnosed in agenitourinary medicine clinic. Int. J. STD AIDS., 11: 499-500.
19. Orenstein, R. and E.S. Wong, 1999. Urinary tract infections In adults. Am Fam Physician. 59: 1225-1234.
20. Baerheiy, A., A. Digranes and S. Hunskar, 1999. Are resistance patterns published by microbiological laboratories valid for general practice. APMIS., 107: 676-680.
21. Khan, S.W. and A. Ahmed, 2001. Uropathogens and their susceptibility pattern: a retrospective analysis. Pak. Med. Assoc., 51: 98-100.
22. Farooqui BJ, Khurshid M, Alam M. Urinary tract infection, J Pak Med Assoc.1989; 39:129-31
23. Burbige KA, Retic AB, Colony A, Bauer SB, Lebowitz R (1984). UTI in boys. J. Urol. 132: 541-542.
24. Akinyemi KO, Alabi SA, Taiwo MA, Omonigbehin EA (1997). Antimicrobial Susceptibility Pattern and Plasmid

profiles of Pathogenic Bacteria isolated from Subjects with Urinary Tract Infections in Lagos, Nigeria. Niger. Qtr. J. Hosp. Med. 1: 7-11.
25. Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Cambridge United Press, U.K. part 27: 105.
26. Ibeawuchi R, Mbata TI (2002). Rational and Irrational Use of Antibiotics Afr. Health. 24 (2): 16-18.
27. Asinobi AO, Fatunde OJ, Brown BJ, Osinusi K, Fasina NA (2003). Urinary Tract Infection in Febrile Children with Sickle Cell Anaemia in Ibadan, Nigeria. Ann. Trop. Paediatr. 23(2): 129-134.
28. Mbata TI (2007). Prevalence and Antibiogram of U T Is Among Prisons Inmates in Nigeria. Int. J. Microbiol. 3 (2).
29. Amin, M., M. Manijeh and P. Zohreh, 2009. Study of bacteria isolated from urinary tract infections and their susceptibility to antibiotics. Jundishapur J. Microbiol., 2: 118-123.
30. Okafor HV, Okoro BA, Ibe BC (1993). Prevalence of asymptomatic bacteriuria among nursery school children. Nig. J. Paediatr. 20: 84-88.
31. Kass E. H. 1957. Bacteriuria and the diagnosis of infections of the urinary tract. Arch int. Med. 100: 709.
32. Stamm W. E. and Turck M. 1991. Urinary tract infections and pyelonephritis. In Harrison'sb principles of internal medicine.
33. Lipsky BA (1990). Urinary tract infection in men: Epidemiology, pathophysiology, diagnosis and treatment. Ann. Inter. Med. 110: 138-150.
34. Hooton TM, Stamm WE (1997). Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. North Am. 11: 551-581.
35. Macejko, A.M. and A.J. Schaeffer, 2007. Asymptomatic bacteriuria and symptomatic urinary tract infections during pregnancy. Urol. Clin. North Am. 34: 35-42. PMID: 17145359.
36. Leigh, D: Urinary Tract Infections. In: Parker, M T and Darden, B I (ends) Topple and Wilson's Principles of bacteriology,Virology and Immunity.1989; Vol.3, *the edition. B C Decker, Philadelphia. Pp197 – 211.
37. Onuh, S O, Umeora, O U J, Igberase, Go, Azikem M E and Okpere, E E. Microbiological Isolates and sensitivity pattern of urinary tract infection in pregnancy in Benin City Nigeria,Ebonyi Medical Journal. 2006; 5(2); 48 –52.
38. MF Bashir, JI Qazi, N Ahmad, and S Riaz. Diversity of Urinary Tract Pathogens and Drug Resistant Isolates of Escherichia Coli in different age and gender Groups of Pakistanis. Tropical Journal of Pharmaceutical Research, September2008; 7 (3): 1025-1031
39. Gupta K, Hooton TM, Webbe CL, Stamm WE (1999). The prevalence of antimicrobial resistance among uropathogens causing actor uncomplicated cystitis in young women. Int. J. Antimicrob Agents 11, (3-6): 305-308.
40. Prescott L, Harley J, Klein JO (2002). Microbiolgy 5th Ed. MacGraw Hill Publishers. pp. 808-823.
41. KC. Arul Prakasam, KG. Dileesh Kumar, M. Vijayan. A Cross Sectional Study on Distribution of Urinary Tract Infection and Their Antibiotic Utilisation Pattern in Kerala. International Journal of Research in Pharmaceutical and Biomedical Sciences, Jul – Sep 2012; 3 (3): 1125-30.
42. Goldraichi NP, Manfroi A (2003). Febrile urinary infection. Escherichia coli susceptibly to oral antimicrobials Paediatr. Nephrol 17 (3): 173- 176.
43. Adjei O, Opoku C. Urinary tract infections in African infants. Int J Antimicrob Agents 2004; 24:32–4.
44. Allen U, MacDonald N, Fuite L, Chan F, Stephens D. Risk factors for resistance to first-line antimicrobials among urinary tract isolates of Escherichia coli in children. CMAJ 1999; 160:1436–40.
45. Prais D, Straussberg R, Avitzur Y, Nussinovitch M, Harel L, Amir J. Bacterial susceptibility to oral antibiotics in community acquired urinary tract infection. Arch Dis Child 2003; 88:215–18.
46. Haller M, Brandis M, Berner R. Antibiotic resistance of urinary tract pathogens and rationale for empirical intravenous therapy. Pediatr Nephrol 2004; 19: 982–86.
47. Haghi-Ashteiani M, Sadeghifard N, Abedini M, Soroush S, Taheri-Kalani M. Etiology and antibacterial resistance of bacterial urinary tract infections in children's medical center, Tehran, Iran. Acta Medica Iranica 2007; 45:153-57.
48. Mostafa S, Abdulla K, Sedigheh R, Navid A. microbial sensitivity pattern in urinary tract infections in children: a single center experience of 1117 urine culture. Jpn J Infect Dis 2006; 59: 380-82.