Study the prevalence of some urinary tract bacterial pathogens

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Summary

A study was conducted during the period January to June 2008, a total of 856 urine samples were collected from male and female in variable ages group with symptoms suggesting urinary tract infections (UTIs) at AL-Sheffa General Hospital in Basrah district. (36.1%) of samples collected from males gave positive results for infected with UTI, while positive samples from females were (63.9%). Women age 10-49 years recorded high rate of infected with UTI in comparison to those age above 50 years. In male patients, the highest rate of UTIs was recorded in age group above 50 years. Overall, *Escherichia coli* was the most common uropathogens and was found in (52.12%) of all samples. Other bacterial uropathogens were identified and their percentages were as follow: *Klebsiella pneumoniae* (16.98%), *Enterococcus faecalis* (13.21%), *Pseudomonas aeruginosa* (7.1%), *Citrobacter* species (5.9%) and *Staphylococcus aureus* (4.72%). Antimicrobial activity of 12 antibiotics were tested against all pathogens identified. The highest resistance was shown to ampicillin (81.32%) and amikacin (62.97%) of all uropathogens isolated, while ciprofloxacin and gentamicin shown a high antimicrobial activity, (13.62%) and (14.62%) respectively.

دراسة تفشي بعض الجراثيم الممرضة للقناة البولية

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الخلاصة

تمت الدراسة خلال فترة كانون الثاني إلى حزيران 2008 . تم جمع (856)عينة ادرارمن المرضى الذكور والاناث لاعمار مختلفة الذين لديهم اعراض الاصابة بالتهابات المجاري البولية في مستشفى الشفاء العام في مدينة البصرة. (36.1%)من العينات المجموعة من المرضى الذكور اعطت نتائج موجبة للاصابة لالتهابات المجاري البولية بينما كانت نسبة العينات الموجبة للاناث (63.9%). الاناث اللواتي تتراوح اعماهم بين 10-49 سنة سجلت نسبة عالية لاصابات المجاري البولية مقارنة مع اللواتي اعمارهم فوق 50 سنة, وفي المرضى الذكور سجلت اعلى نسبة اصابة في الذكور الذين اعمارهم فوق 50 سنة. عموما, اظهرت طرق التشخيص ان جرثومة الاشريكية القولونية*Escherichia coli*هي العامل المسبب الاكبر لاصابات المجاري البولية حيث وجدت بنسبة (52.12%)من العينات الموجبة, وكانت النسب المئوية لتواجد بقية الممرضات الجرثومية كما يلي:

Klebsiella pneumoniae (16.98%), *Enterococcus faecalis* (13.21%), *Pseudomonas aeruginosa* (7.1%), *Citrobacter* species (5.9%) and *Staphylococcus aureus* (4.72%)

واستخدم 12 مضاد حيوي في فحوص الحساسية الدوائية للجراثيم المسببة لاصابات المجاري البولية,سجلت اعلى نسبة للمقاومة من قبل الممرضات الجرثومية للمضادين الحيويين ampicillinبنسبة (81.32%) وamikacinبنسبة (62.97%). بينما وجد ان ciprofloxacinوgentamicinهما المضادان الحيويان الاكثر تاثيرا على كل الانواع الجرثومية حيث كانت نسبة الممرضات الحساسة لهما (13.62%)و(14.62%) تعاقبا.

Introduction

The term urinary tract infections (UTIs) denote several distinct entities with the common feature of significant. Pyuria and bacteriuria it is the most frequent cause of illness in humans after respiratory tract infections[1]. It poses a significant health risk because it can lead to urosepsis and/or renal scarring, progressive kidney damage with associated high mortality, morbidity, and economic loss [2]. UTIs are the leading cause of gram-negative bacteremia in patients of all ages and are associated with a high rate risk especially in the elderly, the most common pathogens identified in patients with UTIs are the enteric gram-negative rods with *E.coli* being the most frequent[3] approximately 85% of cases of urethrocystitis, about 80% of cases of chronic bacterial prostatitis and up to 90% of cases of acute pyelonephritis[4]. The genera *E.coli, Klebsiella, Enterobacter, serratia, Citrobacter* and *Proteus* include obligatory and opportunistic pathogens responsible for a wide range of infections. Many species are members of the normal intestinal flora[4]. In addition *Pseudomonas aeruginosa*, an organism that is ubiquitous in the environment can infect almost any tissue or body site, including localized lesions in the urinary tract. UTIs due to *Pseudomonas aeruginosa* are more common among the elderly[5,6].

In addition to the gram-negative, various gram-positive organisms are commonly associated with UTIs. *Enterococcus faecalis* is a gram positive coccus previously included within the genus *Streptococcus* like *E.coli* (and most species of Enterobacteriaceae) *Enterococcus faecalis* is a member of the normal gastrointestinal flora of humans and may also be found among the normal vaginal flora, although *Entrerococcus faecalis*

is associated with various other diseases, UTIs are the most frequent diseases caused by this organism[7].

The virulence factors of the uropathogens discussed above include: adhesions, hemolsin, capsular polysaccharide, neutrophil activation etc[8,9,10].

In addition antibiotic resistance among uropathogens also constitute a major deciding factor in the establishment and eradication of the disease. Before seventies multiple drug resistance was practically nonexistent and was restricted to mutation of chromosomal genetic elements(plasmids) which simultaneously carry gene for resistance to number of antibiotics has made the position miserable, specially in gram negative bacteria. Since the plasmid mediated drug resistance can transferred to related drug sensitive bacteria, the multiple drug resistance is increasing at an alarming rate, especially environment[11].

The purpose of this study is to determine the most common agent causing urinary tract infections in a large hospital that may reflect what currently present in our community and to determine the relation ship between UTIs age and sex also to determine the antimicrobial activity of different antibiotics.

Materials and methods

***Sample collection**: between January to June 2008, 856 mid stream urine samples were analyzed from the clinically suspected patients of UTIs, attending the in patients and outpatients in AL-Sheffa General Hospital.

Mid stream clean catch specimen of urine was collected in a sterile, screw capped, wide mouth bottle. Before collection of a sample, male subjects were asked to clean the genital part with soap and water while female patients were told to do the genital toilet using soap and water and the vulva has washed and the labia was carefully separated prior to voiding the urine, in the sterile culture bottle.

***Culture**: Urine samples were processed within one hour of collection. In case of, if delay of, more than one hr was an avoidable the samples were refrigerated at 4°C. the sample were inoculated on nutrient agar, blood agar and macConkey agar (Difco). After overnight incubation at 37°C culture plates yielding

bacterial counts >10^5 CFU/ml were considered as significant while counts less than that were taken as not significant.

* **Identification**: Uropathogens were identified to the genus or species level by colonial morphology, gram stain, catalase test, coagulase test, indole production, methyl red test, voges-proskaure citrate utilization test, oxidase test, gelatine hydrolysis test, motility, fermentation test, lipase test, nitrate reduction and DNase test[12,13,14].

All above tests were performed according to[12].

***Antibiotics sensitivity testing**: Antimicrobial susceptibility testing of isolates was performed via the Kirby-Bauer disc diffusion technique on Müeller-Hinton agar to determined the antibacterial activity of 12 antibiotics [15] . These are: Rifampicin (5µg), nalidixic acid (30 µg), tetracycline (30 µg), ceftizoxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), amoxicillin (10 µg), ampicillin (10 µg) and trimethprim-sulfamethaxazole (25 µg).

Results

A total of (856) patients were studied of whom, (493) were female and (363) were male. (424 patients/856,49.53%) were proved to be infected with urinary tract infection. 153 patients were males (36.1%) and 271 were females(63.9%), as shown in **Table 1**.

Table 1: Stratification of patients with UTI according to sex

Sex	No. of patient	No. of patient with UTI	Relative frequency (%)
Male	363	153	36.1
Female	493	271	63.9
Total	856	424	49.53

Table 2 Shows patients with UTI under study with different ages ranged 10-to 60 and above years old. Noticed that women age 10-49 years recorded high rate of infected with UTI in comparable with those age above 50 years, in male high rate of UTI recorded in above 50 years.

Table 2: Demographic data of patients with UTIs by sex and age

Age group (years)	Females			Males			Total	No.(%)
	With out UTI	With UTI	No.(%)	With out UTI	With UTI	No.(%)		
10-19	113	47	17.34	43	10	6.54	57	13.44
20-29	81	56	20.66	34	11	7.19	67	15.80
30-39	105	61	22.51	32	18	11.76	79	18.63
40-49	94	59	21.77	58	21	13.73	80	18.87
50-59	46	18	6.64	73	28	18.30	46	10.85
>60	54	30	11.1	123	65	42.48	95	22.41
Total	493	271		363	153		424	

Table 3 Showed the numbers and percentages of bacterial uropathogens isolated from male and female patients. Overall, *E. coli* was the most common microorganism isolated in a cvll age-groups and both sexes followed by *Klebsiella* and *Enterococcus faecalis*

Table 3: Distribution of isolated bacteria in patients with UTI by sex

Organism	Number and percentage of isolates		
	Male No. %	**Female No. %**	**Total No. %**
E. coli	58	163	221
	37.91	60.15	52.12
Klebsiella	3	40	72
pneumoniae	220.92	14.76	16.98
Enterococcus	25	31	56
faecalis	16.34	11.44	13.21
Pseudomonas	16	14	30
aeruginosa	10.46	5.17	7.1
Citrobacter spp	13	12	25
	8.50	4.43	5.9
Staphylococcus	9	11	20
aureus	5.88	4.1	4.72
Total	153	271	424

Organism / age group	10-19	No. %	20-29	No. %	30-39	No. %	40-49	No. %	50-59	No. %	>60	No. %	Total
E. coli	28	70	22	59.46	47	52.81	61	68.54	33	29.46	30	52.63	221
Klebsiella pneumoniae	3	7.5	9	24.32	6	6.74	9	10.11	30	26.79	15	26.32	72
Enterococcus faecalis	5	12.5	2	5.41	17	19.10	5	5.62	22	19.64	5	8.77	56
Pseudomonas aeruginosa	2	5	0	0	14	15.73	7	7.87	6	5.36	1	1.75	30
Citrobacter spp	1	2.5	2	5.41	1	1.12	4	4.49	13	11.61	4	7.02	25
Staphylococcus aureus	1	2.5	2	5.41	4	4.49	3	3.37	8	7.14	2	3.51	20
Total	40		37		89		89		112		57		424

In the present study the most prevalent organism isolated causing UTI was *E. coli* in all age group. while the others organisms recorded different frequency in different age group as in **Table 4**.

Table 4: Distribution of UTIs isolates in various species on the basis of age group

Antibiotics	Concentration mcg	E. coli (221)	K. pneumoniae (72)	P. aeroginosa (30)	E. faecalis (56)	C. spp (25)	S. aureus (20)	Mea
Rifampicin	5	88* (39.82%)	12 (16.67%)	10 (33.33%)	24 (43.00%)	7 (28.00%)	2 (10.00%)	(28.4
Nalidixic acid	30	127 (57.47%)	7 (9.72%)	12 (40.0%)	18 (31.5%)	9 (36.0%)	8 (40.00%)	(35.7
Tetracycline	30	95 (42.53%)	41 (57.00%)	10 (33.33%)	39 (69.16%)	7 (28.00%)	9 (45.00%)	(45.8
Ceftizoxime	30	101 (45.70%)	9 (12.5%)	10 (32.1%)	34 (61.00%)	3 (12.00%)	7 (35.00%)	(33.5
Gentamicin	10	19 (8.6%)	9 (12.9%)	4 (13.33%)	9 (16.10%)	4 (16.3%)	2 (10.12%)	(12.8
Ciprofloxacin	5	25 (11.3%)	3 (4.17%)	5 (16.66%)	8 (14.20%	4 (16.3%)	1 (5.00%)	(11.2

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Amikacin	30	192 (86.88%)	39 (54.17%)	22 (73.33%)	24 (43.7%)	8 (30.3%)	18 (90.00%)	(63.0
Nitrofurantoin	300	101 (45.70%)	37 (51.4%)	28 (93.33%)	11 (20.1%)	18 (73.08%)	4 (20.00%)	(50.6
Norfloxacin	10	40 (18.1%)	37 (51.4%)	15 (50.00%)	11 (19.8%)	11 (44.0%)	12 (60.00%)	(40.5
Amoxicillin	10	185 (83.71%)	46 (64.00%)	24 (80.00%)	28 (50.00%)	13 (51.8%)	13 (65.3%)	(65.8
Ampicillin	10	198 (89.59%)	59 (81.94%)	22 (73.33%)	52 (92.00%)	19 (74.1%)	15 (75.4%)	(81.0
Trimethobrim-sulfamethaxazole	25	168 (76.01%)	41 (57.94%)	16 (53.33%)	27 (48.00%)	13 (52.6%)	13 (65.00%)	(58.8

Antibacterial activity of 12 antibiotics were tested against all bacterial pathogens identified. In **Table 5**, the highest resistance was shown to ampicillin with (81.32%) and amikacin with (62.97%) of all uropathogens isolated, while ciprofloxacin and gentamicin shown a high antimicrobial activity, (13.62%) and (14.62%) respectively.

Table 5: Percentages of uropathogens sensitive to antibiotics tested

*****The antibiotics sensitivity of uropathogens are compared with standard antibiotics table[15] .

Discussion

Urinary tract infection are a major public health problem[3]. It should be noted that the vast majority of the samples collected from patients correspond to clean-catch midstream urine[16]. Laboratory diagnosis of UTI using urine cultures is based on semi-quantitative counts of organisms and identification of the isolates. A known quantity of urine is streaked on solid agar media and incubated at 37°C for 24-48 hr's and the bacterial colonies counted. The reliability of this methods, however, is strongly depended on the time taken to send the urine specimens to the laboratory and the transport conditions because urine is an excellent culture medium and delayed plating may result in false-positive results[17].

In our study that was conducted on (856)patients, 424 patients (49.53%) developed UTIs. 63.9% were females and 36.1% were males **(Table 1)**. This results reflect that UTIs are more common in female and the male to female ratio was approximately 1:2, this figure is in agreement with others studies, Stamm[18] showed that the risk of development UTI in females exceeds the risk in males by two folds in each decade of life, other workers[19] observed that the number of female patients were significantly higher than the number of male patients i.e. UTI is more prevalent in females. Schaeffer et al, confirm that UTIs was frequently caused in female (63%)than male (37%)[20]. It is stated that UTI is predominantly a disease of the females due to anatomical predisposition[20] short urethra and proximity to the anal opening and urothelial mucosa adherence to the mucopolysaccharide lining or other host factors.

The demographic data**(Table 2)** indicates that women of reproductive age group formed the main group of adult patients with UTI presenting to the general practice clinics(46%of all UTI detected were from women age 10-49 years). The rates we report in the present study agree with those reported by Keah et al[19]. Other workers recorded 54%of all UTI detected were from women age 10-49 years[21].

A high percentage of UTI in men were found in ages (50->60)years, it is worthy of note that the high rate of UTI of older males due to commonly complicated by presented principally to secondary or tertiary health care facilities.

Among the uropathogens the most frequent causative agent of UTI in this study was found to be *E.coli* (52.12%) followed by *Klebsiella pneumonia*e (16.98%), *Enterococcus faecalis* (13.21%), *Pseudomonas aeruginosa* (7.1%), *Citrobacter* spp (6%) and *Staphylococcus aureus*(4.72%), as shown in **(Table 3)**. In other studies Khan[22] reported a uropathogens prevalence of (45.6%) for E.coli. this was followed by *Enterococcus faecalis* (10.2%) while other workers[23]reveled a prevalence of (42%) for *E. coli*, (16%) *Pseudomonas aeruginosa*, (11%) *Klebsiella pneumonia*e, (4%) *Enterococcus faecalis*, (3%) *Citrobacter* spp. and (0.5%) *Staphylococcus aureus*. On the other hand, Shah[4]recorded *E. coli* was the most abundant (40%) followed by *Klebsiella pneumoniae*

(20.5%) and (7.5%)*Citrobacter* species. prevalence of different pathogens is dependent on several population attributes, sample size and hygienic conditions of the patients. Therefore, a stable pattern in this regard cannot be predicated. This is why data vary when different studies are compared. It is interesting to note that in our study *E. coli* is more common in females(60.15%) than in males (37.91%) this figure is in agreement with Manzoor and co-workers[24] ,also agree with Salma[25] reported that *E. coli* grew in (78.6%) of the specimens from females, but only (51.2%)of those from males. Uropathogenic *E. coli* exhibits specific virulence which allow them to adhere to vaginal and uroepithelial cells, resist bacterial activity of human serum, prevent phagocytosis by leukocyte and production of specific cytotoxins for tissue invasion[17].

In this study as seen in **(Table 4)**, in age 10 to 60 and above years the most prevalent organism was *E. coli*, in all age group. For other uropathogens in 10-19 age the most prevalent organism was *Enterococcus faecalis,* 5 out of 40 (12.5%), in 20-29 *Klebsiella pneumoniae* out of 37(24.32%)was the most prevalent organism, in 30-39 *Enterococcus faecalis* predominant out of 89(19.1%), in 40 to 60 and above *Klebsiella pneumoniae* again predominant organism off course after *E.coli,* similar results have been obtained by other workers*,* [4]reported that *E. coli* was the most prevalent organism in all age groups while *Klebsiella pneumoniae* was most common in the age group of 50-60 years. Also similar to [18]they have reported that overall, *E. coli* was the most prevalent isolate in all age group *Klebsiella pneumoniae,* although with a significant prevalence, was commonly isolated in the population 40 to 60 and above years old.

The prevalence of antimicrobial resistance among microorganisms that cause UTI is increasing world wide and is a major factor in selecting antibiotics for treatment[26]. In the present study as seen in **(Table 5)**, uropathogens were resistant to ampicillin (81.32%). Similar results have been by other workers, Tankhiwate et al [27]observed resistant to ampicillin was (79.6%), but are lesser than those reported by Tambekar[19] et al they reported that uropathogens were resistant to ampicillin(87%). On the other hand, in our study *E. coli*, Staphylococcus aureus and

Pseudomonas aeruginosa strains were highly sensitive to ciprofloxacin and gentamicin. This figure is in agreement with Tambekar[20]and co-workers and Selma[25]. In present study a significant increase in resistance of Uropathogenic strain to Trimethobrim-sulfamethaxazole this is agree with other studies[28,29,30]. Older agents like gentamicin and Rifampicin, that still show high efficiency against UTI pathogens, should be considered as options for treatment.

In the present study the percentages of isolates demonstrating a multi drug resistant(MDR) phenotype was extremely high. The most frequently observed multi drug resistance phenotypes agree with those already, in special for ampicillin and Trimethobrim-sulfamethaxazole [29,31]. The isolated bacteria were resistant to ampicillin. Amoxicillin, amikacin, tetracycline, Trimethobrim-sulfamethaxazole and nalidixic acid. This observed resistance to their drugs is a probable indication of earlier exposure of the isolates to these drugs, which may have enhanced resistant development[32].most of isolated of uropathogens showed multiple antibiotics resistance in this area may be due to large portion of the bacterial isolates being previously exposed to several antibiotics. The present study data gives idea about the common trend of increased antibiotics resistance of uropathogens in this region, which may be due to geographic variation or discriminate or sub lethal use of antibiotic. This data not only help in proper treatment of UTI patients but also discourage the indiscriminate use of antibiotics and prevent further development of bacterial drugs resistance.

Many factors may have contributed to such high rates of resistance including misuse of antibiotics by health care professionals or non skilled misuse of antibiotics by the general public antibiotics can be purchased in our country without a prescription, and inadequate surveillance due to lack of information arising from routine antimicrobial susceptibility testing, like report from other developing countries.

Planning to reduce resistance is very important, as organisms resistant to one antimicrobial agent are likely to become resistant to others. Researchers have found that bacteria isolated from UTIs have resistance to common drugs such as ampicillin and

Trimethobrim-sulfamethaxazole in over one third of cause. These drugs have been on the market for several decades[33,34].

References

1. Ram S, Gupta R and Gaheer M.(2000). Emerging antibiotic resistance among the uropathogens. Indian J Med Sci; 54:388-94.
2. Jamal-omidi S, et al.(2008). Nosocomial bacterial infections and their antimicrobial resistance patterns in university hospitals of Hamadan, Iran. Final abstract ;no:64.
3. Butt T, Leghari MJ and Mohmood A.(2004). In-vitro activity of nitrofurantoin in Enterococcus urinary tract infection.JPMA;54:466.
4. Shah A, Hasan F and Hameed A.(2002). Study on the prevalence of Enterobacteriaceae in hospital acquired and community acquired infections. Pakistan J Med Res.vol.41;No.1.
5. Kahlmeter G.(2003). An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS project. J Antimicrobial Chemother;51:69-76.
6. Joklik K et al.(1984). Zinsser microbiology, Appleton-century-Crofts, Norwalk, Conn.P.631-636.
7. Moellering C.(1988). The *Enterococcus*: A versatile pathogen. health mark K.P.3-6.
8. Margaret A and Ingram P.(1982). The role of a-hemolysin in the virulence of *E. coli* for mice. J Med Microbiol;15:23-30.
9. Prabhakar H et al.(1984). A study of pathogenicity of *E. coli* isolated from different sources. Indian J Pathol Microbiol;27:57-62.
10. Siegfried L et al.(1994). Virulence associated factors in *E. coli* strains isolated from children with UTI. J Med microbial;41:127-132.
11. Kass E.(1956). Asymptomatic infections of urinary tract. Trans Assoc Am Physicians;59:56-64.
12. Finegold S and Baron E.(1986). Baily and scott's diagnostic microbiology.17th ed the C.V.Mosby co.st.Louis.
13. Kiieg R and John H.(1986). Bergy's manual of systemic bacteriology.9th ed.Williams &Wikins, Baltimore.vol.11.
14. Lennett H et al.(1985). Manual of clinical microbiology.4th ed .Amer Soc Microbiol.washington.
15. Difco laboratory.1984. Difco manual 10th ed. Difco Lab Inc, Detroit.
16. Carlos k et al.(2007). Antibiotic resistance and trend of urinary pathogens in general outpatients from a major Urban city.Int Braz J Urol;33:42-9.
17. Keah H et al.(2007). Antimicrobial susceptibility of community acquired uropathogens in general practice. Malaysian family physician;2(2):64-69.
18. Stamm E.(1998). Urinary tract infections. In: hospital infection. Edited by Bennett V and Brochman S.4th ed.P.477-425.
19. Bran L et al.(1972). Entrance of bacteria in the female urinary bladder. N Eng J Med;286:626-631.

20. Tambekar H et al.(2006). Antimicrobial susceptibility of some urinary tract pathogens to community used antibiotics. African J Biotech vol.5(17):1562-1565.
21. Azra H et al.(2007). Resistance patterns of urinary isolations in A Tertiary Indian hospital.J Ayub Med coll Abbottabad;19(1).
22. Khan W and Ahmed A.(2001). Uropathogens and their susceptibility patterns: a retrospective analysis. J pak Med Assoc;51:98-100.
23. farooqi J et al.(1989). Urinary tract infection.J Pak Med Assoc;39:129-31.
24. Manzoor S et al.(2004). Antibiotics sensitivity and resistance profile of the microorganisms responsible for urinary tract infection observed in Kashmir, India. Ind J Practising Doctor vol.1,no.1.
25. Selma U.(2006). Antibiotic resistance of coli form organisms from community-acquired urinary tract infections in Zeniac-Doboj conton, Bosnia and Herzegovina.J Antimicrob Chemother;58(2):344-348.
26. Kader A et al.(2004). Antimicrobial resistance patterns of gram-negative bacteria isolated from urine cultures at a general hospitals. Saudi J Kidney Dis Transpl;15:135-9.
27. Tankhiwale et al.(2004). Evolution of extended spectrum of beta lactamase in urinary isolates.Ind J Med Res;120:553-556.
28. Inabo I and Obanibi T.(2006). Antimicrobial susceptibility of some urinary tract clinical isolates to commonly used antibiotics.vol.5(5):487-489.
29. Sahm D et al.(2002). Multidrug-resistant urinary tract isolates of *E. coli*: prevalence and patients demographics in the united states I 2000. Antimicrob Agent Chemother;45:1402=6.
30. Hooton M.(2003). Fluoroquinolones and resistance in the treatment of uncomplicated urinary tract infection. Int J Antimicrob Agents;22:565-572.
31. Karlowsky A et al.(2003). Trends in antimicrobial resistance among urinary tract infection isolates of *E. coli* from female outpatients in the united states. Antimicrob Agents Chemother;46:2540-45.
32. Joseph E.(2003). Antibiotic susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Tropical J Pharmceutical Res.vol.2(2):223-228.
33. Edilene S et al.(2007). Multidrug-resistant urinary tract isolates of *E. coli* from ribirao prect, sao Paulo, Brazil.Braz J Infect Dis;vol.11.no.6.
34. Bashir F et al.(2008). Diversity of urinary tract pathogens and drug resistant of *E. coli* indifferent age and gender group of Pakistanis. Tropical J Pharmaceutical Res;7(3):1025-1031.