Pakistan J. Zool., vol. 40(4), pp. 295-301, 2008.

Prevalence and Antibiogram of Uncomplicated Lower Urinary Tract Infections in Human Population of Gilgit, Northern Areas of Pakistan

KHALIL AHMED AND IMRAN

Department of Biological Sciences, Karakoram International University, Gilgit, Northern Areas, Pakistan
e-mail: khalil.a.kiu@gmail.com

Abstract.- A study was conducted during April, 2006 to March 31, 2007 to investigate the prevalence and antibiogram of urinary tract infections in human population of Gilgit, Northern Areas of Pakistan. Two hundred and fifty eight urine specimens of lower urinary tract infected patients referred to District Headquarter Hospital, Gilgit for bacteriological investigation, were cultured on Cystine Lactose Electrolyte-Deficient (CLED) agar (Oxoid) with the help of celebrated sterilized loop urine (approximately 0.01 ml) and incubated aerobically at 37°C for 24 hours. More than 10 Gram staining. The Gram positive organisms were identified by coagulase, catalase and DNase tests, whereas Gram negative strains were identified by various biochemical tests. Out of 258 urine specimens, 113 (43.80%) were found infected with the pathogenic bacteria. The commonest isolates were *Escherichia coli* 55 (48.67%), *Staphylococcus saprophyticus* 34 (30.1%), *Pseudomonas aeruginosa* 8 (7.2%), *Proteus vulgaris* 5 (4.43%), *Proteus mirabilis* 2 (1.77%), *Proteus* sp. 3 (2.66%), *Pseudomonas* sp. 1 (0.3%) and *Staphylococcus* sp. 3 (2.66%). The incidence of urinary tract infection was high in females 68 (60.18%) as compared to males 45 (39.83%) and in the age group 21-50 years. The highest prevalence was found in Magini Muhala, Basin, Kashrote, Amphary and very few from Nagaral and Jutial. The incidence was found higher in summer as compared to other seasons. Most of the isolated strains were highly resistant to the antibiotics used except *Proteus*, which was 100 per cent sensitive to ciprofloxacin.

Key words: Prevalence, antibiogram, urinary tract infection.

INTRODUCTION

Urinary tract infections are the most common infections in clinical practice (Noor *et al*., 2004). Normal urine does not contain any bacteria although it contains fluids, salts and waste products (Meyhoff *et al*., 1996). Numerous reports have suggested the urinary tract infections can occur in both male and female patients of any age with bacteria count as low as 100 colonies in per milliliters of urine (Akinyemi *et al*., 1997). The most common pathogenic organisms of UTI are *Escherichia coli, Staphylococcus saprophyticus* and less common organisms are *Proteus* sp., *Klebsiella pneumoniae, Pseudomonas aeruginosa*, enterococci and *Candida albicans* (Saelk, 1992; Walker *et al*., 1999) in their study found more than 80% UTI are due to *E. coli* in humans. In an another study conducted by Sabir *et al*. (2004) *E. coli* was reported to be the most frequent organism (53%) and its resistance increasing against most of the antibiotics. Farooqui *et al*. (1989) in their study cultured 9892 mid-stream urine samples from patients attending the Aga Khan Hospital, Karachi and found 23.5% samples infected with different pathogenic bacteria and 40% of them infected with *E. coli*, 16% with *Pseudomonas aeruginosa*, 13% with *Proteus* sp. and 11% with *Klebsiella pneumoniae*.

In view of alarming increase in drug resistance, the pharmaceutical industries are shifting away from traditional strategies to newer approaches (Nwanze *et al*., 2007; Jabra-Rizk *et al*., 2006). The treatment of *E. coli* infections is a growing problem which is probably due to over use of antibiotics and also due to the use of growth promoters in food animals (Johnson *et al*., 2006).

The present study was aimed at assessing common pathogenic bacteria causing urinary tract infections in this community and to determine antimicrobial susceptibility pattern of these isolates

0030-9923/2008/0004-0295 $ 8.00/0

through the disc diffusion method (Bauer *et al*., 1966).

MATERIALS AND METHODS

Collection of samples

Mid-stream urine from the suspected uncomplicated lower UTI patients referred by physicians of District Headquarter Hospital, Gilgit was collected in open mouthed clean and sterilized containers. The patients were instructed on how to collect urine sample and its prompt delivery to laboratory for culture. The name, age, sex and address of the patients was also recorded.

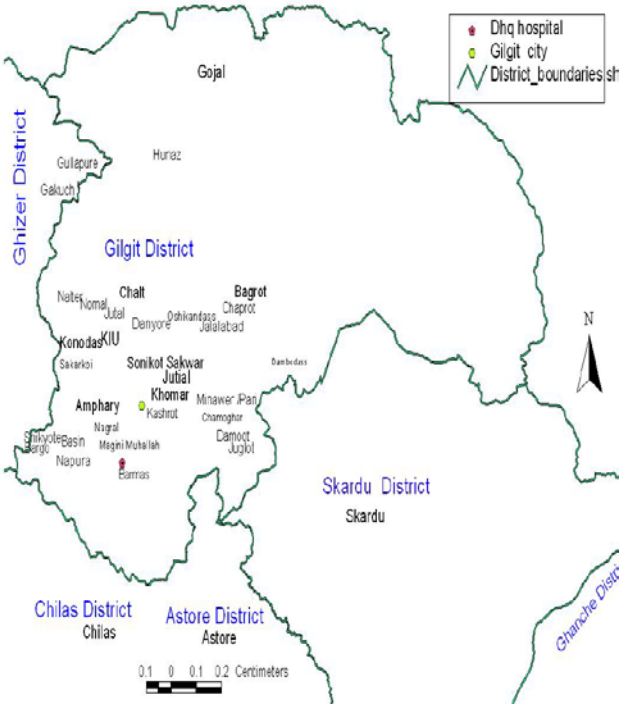

Fig 1. Map of the Northern Area showing the places from where urine specimens from suspected uncomplicated lower urinary tract infected patients were referred to the Laboratory of District Headquarter Hospital, Gilgit for investigation and were found to be infected with pathogenic bacteria.

Processing of samples and culture growth

The collected urine samples were cultured on CLED agar plates with the help of celebrated sterilized loop delivering approximate 0.01 ml urine (Theodore, 2007) by rotating the container. The cultured plates were kept on the bench for some time, to allow the urine to be absorbed into the cultured medium and incubated aerobically at 37°C for overnight. The quality of the medium and techniques was confirmed by culturing the standard reference strain *E. coli* ATCC 25922.

The urine culture plates were examined for pure growth determined by morphologically same type of colonies and colony counts for determination of significant ($>10^2$ colonies) and insignificant growth ($<10^2$ colonies). Gram staining was performed to differentiate the Gram positive and Gram negative organisms. The gram positive colonies were characterized by coagulase, DNase, catalase tests and Gram negative colonies characterized according to WHO Manual (1987) for Laboratory Investigations of Acute Enteric Infections.

Antimicrobial sensitivity test

The antimicrobial sensitivity of the confirmed micro-organisms was done by disc diffusion method (Bauer *et al*., 1966) on Muler Hinton agar. The discs used were quinolone group (Ciprofloxacin 5 µg, Nalidixic acid 30 µg), tetracycline group (Minocyclin 30 µg), cephalosporin group (Cefaclor 30 µg, Ceftazidim 30 µg), miscellaneous (Chloramphenicol 30 µg, Pipamidic acid 30 µg) and semisynthetic (Amoxicillin 30 µg). To maintain the quality of the sensitivity test in each batch, *E. coli* ATCC 25922 was also processed.

RESULTS

Age-wise distribution and prevalence of pathogenic bacteria

Table I shows age-wise distribution of suspected urinary tract infected patients referred and investigated for different pathogenic organisms. The highest numbers of cases were investigated in age group 21–30 years (67) followed by 31–40 years (65), 41–50 years (54), 51-60 years (29), 11–20 years (26), 1–10 years (7), 61–70 years (6), 71–80 years (3) and 81-100 years (1). The highest prevalence of pathogenic bacteria was found in age group 21–30 years (32, 47.5%) followed by 41–50 years (26, 50%), 31–41 years (25, 41%), 51–60 years (11, 37.54%), 1–10 years (3, 42%), 11–20

years (10, 38.12%), 61–70 years (3, 50%), 71–80 years (2, 66.2%) and 81–100 years (1, 100%).

Table I.- Age-wise distribution of suspected uncomplicated lower urinary tract infected patients referred for bacteriological investigation and prevalence of pathogenic bacteria.

Age (years)	Specimens investigated	Total (% infestation)	*E. coli*	*Staphylococcus*		*Pseudomonas*		*Proteus*			*Klebsiella pneumoniae*
				sap.	sp.	*aerug.*	sp.	*mira.*	*vulg.*	sp.	
01–10	7	3 (42)	1	1					1		
11–20	26	10 (38.12)	4	2		2		1	1		
21–30	67	32 (47.5)	11	11	2	4			1	2	1
31–40	65	25 (41)	11	10		1	1			1	1
41–50	54	26 (50)	17	4	1	1		1	2		
51–60	29	11 (37.24)	7	4							
61–70	06	3 (50)	2	1							
71–80	3	02 (66.2)	1	1							
81–100	1	01 (100)	1								
Total	258	113	55	34	3	8	1	2	5	3	2

sap, saprophyticus; sp., species; *aerug*., *aeruginosa*; *mira*., *mirabilis.*

Area wise distribution and prevalence of pathogenic bacteria

Table II shows area wise distribution of suspected uncomplicated lower urinary tract infected patients referred for laboratory investigation of different pathogenic bacteria and prevalence of pathogenic bacteria in each locality. The highest number of specimens 161 (62.41%) were received from different localities of the municipal area of the district Gilgit and prevalence of the different pathogenic bacteria was 69 (61.06%) which is higher as compared with other areas. Out of the municipal area, from different localities of tehsil Gilgit 74 (28.7%) specimens were received and prevalence of different pathogenic bacteria was 50%. From other parts of the Northern Areas, the district Ghizer (villages Gakuch and Gullapure) 7 specimens were received and prevalence of pathogenic bacteria was 28.7%. From Tehsils Hunza and Gogal 4 and 1 specimens were received, respectively, and prevalence of pathogenic bacteria was 100% in both. From the other three districts *i.e.* Chilas, Skardu and Astore 4, 2 and 2 specimens were received, respectively, and the prevalence of pathogenic bacteria was 0% in all the samples.

Seasonal distribution and prevalence of pathogenic bacteria

Table III shows seasonal distribution and prevalence of pathogenic bacteria of the suspected uncomplicated lower UTI patients referred to laboratory for investigation in different seasons of the year. The highest number of cases were referred to the laboratory in summer *viz.*, 80 (31.01%), followed by autumn (76, 29.46%), spring (60, 23.26%), and winter (42, 16.29). The highest prevalence of pathogenic organisms was also found during summer (53.57%) followed by autumn 36 (47.3%), winter (38.0%) and during Spring (30.0%).

Monthly distribution and prevalence of pathogenic bacteria

Table IV shows month-wise distribution of suspected uncomplicated lower UTI patients referred for bacteriological investigation and prevalence of pathogenic bacteria in each month. The highest number of specimens were referred to the laboratory in the month of September (41) followed by April (37), August (32), June (31), May (21), November (20), December (19), October (15), January (12) and February (11). The highest prevalence was found during the month of August (62.5%) followed by December (57.17%), July (52.6%), November (50.0%), September (48.7%), May (42.8%), June (41.9%), October (33.3%),

February (27.28%), January (25.0%) and April (24.3%).

Table II.- Area-wise distribution of suspected uncomplicated lower urinary tract infected patients referred for bacteriological investigation and prevalence of pathogenic bacteria.

District Gilgit Municipal area	No. of specimens investigated	Total No. of infestation	Percentage
Magini muhallah	41	19	46.34
Kashrote	16	6	37.5
Nagral	7	3	42.8
Barmas	5	1	20.0
Amphary	9	5	55.5
Konodas	11	6	54.5
Sakarkoi	2	0	00.0
SoniKot	3	2	000
Jutial	18	9	50.0
Basin	34	12	35.2
Napura	2	1	50.0
Khore	1	0	00.0
Khomar	6	4	66.6
KIU	3	0	00.0
Sakwar	3	1	33.3
Sub. Total	**161(62.41%)**	**69**	**61.06**
Tehsil Gilgit			
Danyor	12	6	50.0
Minawer/pari	5	2	40.0
Chamoghar	9	6	66.6
Juglot	7	3	42.8
Oshikhandass	3	1	33.3
Chaprot	3	0	00.0
Nomal	8	4	50.0
Jalalabad	12	8	66.6
Damoot	1	1	100.0
Bargo	4	2	50.0
Chalt	2	0	00.0
Nalter	1	1	100.0
Jutal	1	1	100.0
Bagrot	4	0	00.0
Shikyote	1	1	100.0
Dambodas	1	1	100.0
Sub. total	**74 (28.7%)**	**37**	**50.0**
Tehsil Hunza			
Hunza	4	4	100.0
Tehsil Gogal			
Gojal	1	1	100.0
District Ghizer			
Gakuch	3	0	0.0
Gullapure	7	2	28.5
District Chilas			
Chilas	4	0	00.0
District Skardu			
Skardu	2	0	00.0
District Astore			
Astore	2	0	00.0
Sub. Total	**23 (8.92%)**	**7**	**30.44**
G. total	**258**	**113**	**43.80**

Table V shows the frequency and percentage antibiotic resistance pattern of the isolated microorganisms from uncomplicated lower UTI patients. Out of 55 *E. coli* isolates 36 (65.45%) were resistant to Pipamidic acid, 38 (69.1%) to Nalidixic acid, 25 (45.45%) to Ciprofloxacin, 44 (80.0%) to Minocyclin, 30 (54.55%) to Cefaclor, 46 (83.64%) to Chloramphenicol, 40 (72.73%) to Ceftazidim and 40 (72.73%) to Amoxicillin. Out of 34 *Staphylococcus saprophyticus* isolates 12 (35.29%) were resistant to Pipamidic acid, 25 (73.53%) to Nalidixic acid, 20 (58.82%) to Ciprofloxacin, 28 (82.35%) to Minocyclin, 30 (88.24%) to Cefaclor, 30 (88.24%) to Chloramphenicol, Ceftazidim and 25 (73.53%) to Amoxicillin. Out of 8 *Pseudomonas aeruginosa* 2 (25.0%) isolated strains were resistant to Pipamidic acid, 7 (87.50%) to Nalidixic acid, 2 (25.0%) to Ciprofloxacin, 8 (100%) to Minocyclin, Cefaclor, Chloramphenicol, Amoxicillin and 4 (50%) to Ceftazidim. Out of 2 isolates of *Klebsiella pneumonia* 1 (50.0%) isolated strains was resistant to Pipamidic acid, 0 (0%) to Nalidixic acid, 1 (50%) to Ciprofloxacin, 1 (50%) to Minocyclin, 2 (100%) to Cefaclor, 1 (50%) to Chloramphenicol and 2 (100%) to Ceftazidim and Amoxicillin. Out of 5 *Proteus vulgaris* isolates 5 (100%) strains were resistant to Pipamidic acid, 3 (60%) to Nalidixic acid, 0 (0%) to Ciprofloxacin, 3 (60%) to Minocyclin, 5 (100%) to Cefaclor and Chloramphenicol, 4 (80%) to Ceftazidim and 5 (100%) to Amoxicillin. Out of 2 *Proteus mirabilis* isolates 2 (100%) strains were resistant to Pipamidic acid and Nalidixic acid, 0 (0%) to Ciprofloxacin, 2 (100%) to Minocyclin and Cefaclor, 1 (50%) to Chloramphenicol, 2 (100%) to Ceftazidim and Amoxicillin.

DISCUSSION

Urine is one of the sterile body fluids (Kass, 1962), but the presence of bacteria in urine is called bacteriuria (Whalley, 1997). In this study 43.80% urine specimens from suspected uncomplicated lower UTI patients gave significant growth. The absence of bacterial growth in 145 (56.20%)

suspected uncomplicated lower UTI patients may be due to the fact that the patients were already undergoing antibiotics therapy which have inhibited or destroyed the bacterial growth (Okonofua *et al*., 1989).

In this study, most predominant organisms isolated among the community of Gilgit attending the District Headquarter Hospital were *E. coli* (48.67%). This finding agreed with other reports, which indicated that the Gram negative bacteria mostly *E. coli* was the commonest bacteria isolated in patients with UTI (Nwanze *et al*., 2007; Farooqi *et al*., 1989; Theodore, 2007; Khattak *et al*., 2006).

The second most frequent organism was *Staphylococcus saprophyticus*. Same results were obtained by Khattak *et al*. (2006). The referred fecal specimens for the laboratory investigation were 52.33% females and 47.68% males and frequency of isolation of pathogenic bacteria was also higher in females (60.18%) than in males (39.83%). Same results have been obtained in many other studies

Table III.- Season-wise distribution of suspected uncomplicated lower urinary tract infected patients referred for bacteriological investigation and prevalence of pathogenic bacteria.

Spring (March - May)		Summer (June- August)		Autumn (September – November)		Winter (December - February)	
Specimens investigated	Specimens infected & % prevalence	Specimens investigated	Specimens infected & % prevalence	Specimens investigated	Specimens infected & % prevalence	Specimens investigated	Specimens infected & % prevalence
60 (23.26%)	18 (30.0 %)	80 (31.01%)	43 (53.5%)	76 (29.46%)	36 (47.3%)	42 (16.29%)	16 (38.0%)

Table V.- Frequency and percentage of *in vitro* antibiotic resistance pattern of isolated microorganisms.

Drugs	*E. coli* (55)	*Staphylococcus saprophyticus* (34)	*Pseudomonas aeruginosa* (08)	*Klebsiella pneumoniae* (02)	*Proteus vulgaris* (05)	*Proteus mirabilis* (02)
Pipamidic acid (30 µg)	36 (65.45)	12 (35.29)	2 (25.0)	1 (50.0)	5 (100.0)	2 (100.0)
Nalidixic acid (30 µg)	38 (69.1)	25 (73.53)	7 (87.5)	0 (0.0)	3 (60.0)	2 (100.0)
Ciprofloxacin (5 µg)	25 (45.45)	20 (58.82)	2 25.0)	1 (50.0)	0 (0.0)	0 (0.0)
Minocyclin (30 µg)	44 (80.0)	28 (82.35)	8 (100.0)	1 (50.0)	3 (60.0)	2 (100.0)
Cefaclor (30 µg)	30 (54.55)	30 (88.24)	8 (100.0)	2 (100.0)	5 (100.0)	2 (100.0)
Chloramphenicol (30 µg)	46 (83.64)	30 (88.24)	8 (100.0)	1 (100.0)	5 (100.0)	1 (50.0)
Ceftazidim (30 µg)	40 (72.73)	30 (88.24)	4 (50.0)	2 (100.0)	4 (80.0)	2 (100.0)
Amoxicillin (30 µg)	40 (72.73)	25 (73.53)	8 (100.0)	2 (100.0)	5 (100.0)	2 (100.0)

Table IV.- Month-wise distribution of suspected uncomplicated lower urinary tract infected patients referred for bacteriological investigation and infestation of common pathogenic bacteria

Name of months	Specimens investigated	Prevalence of pathogenic bacteria	% Prevalence
March	-	-	-
April	37	09	24.3%
May	21	09	42.8%
June	31	13	41.9%
July	19	10	52.6%
August	32	20	62.5%
September	41	20	48.7%
October	15	05	33.3%
November	20	10	50.0%
December	19	11	57.17%
January	12	03	25.0%
February	11	03	27.28%
Total	258	113	43.80%

conducted by Dimitrov *et al*. (2003) in Kuwait, Nwanze *et al*. (2007) in Okaada village and Theodore *et al*. (2007) among prisons in Nigeria. Urinary tract infection occur much less frequently in men at all ages. Antibiotic use changes the vaginal flora and promotes colonization of the genital tract with *E. coli* resulting in subsequent increase risk of UTI. Moreover, females have short urethra due to which bacteria migrate the urethra and cause UTI. Other risk factors associated with UTI include recent sexual activity, new sexual partner, and use of spermicide. Our study results showed that a high percentage of suspected uncomplicated lower UTI patients were referred for bacteriological investigation and prevalence of organisms was also high with in the age groups 21-30 years and 31-40 years and 41-50 years. Almost same results have been obtained by Theodore *et al*. (2007) in their study. Usually majority in this age groups are sexually active peoples and prone to get UTI.

In area-wise distribution more suspected patients were referred from the near and most populated areas of hospital where this study was conducted. The frequency of isolation was high in populated and more congested areas and in those where surface channel water was being used. While some cases referred from far-flung areas have high prevalence of pathogenic bacteria, this may be due to the fact that the physicians may have referred the UTI patients who may not have been cured after antibiotic treatment. In season-wise distribution more patients were referred in summer than in other seasons and the isolation of pathogenic bacteria was also high. This was due to the reason that in the summer the contamination of water becomes very high. Ahmed *et al*. (2007) reported 580 colonies of fecal coliforms in 100 ml of drinking water in Nomal valley of Gilgit. The community uses this highly contaminated water for drinking as well as for hygienic purposes. Moreover, in summer there is more insensible loss of water from the body and urine becomes concentrated which provides better environment for bacteria to grow.

In month wise investigation the referring of suspected uncomplicated lower UTI and prevalence of pathogenic organisms was also high in July and August. It was perhaps due to the fact that during high temperature melting of glaciers and erosion of the land and activities related to water increases and contamination of water becomes very high.

Antimicrobial resistance is a natural biological phenomena of response of microbes to the selective pressure of an antimicrobial drugs. Resistance may be inherent. In this study the antibiogram of antimicrobial agents shows that organisms are highly resistant to all the commonly used antibiotics *i.e.* Pipamidic acid, Nalidixic acid, Ciprofloxacin, Minocyclin, Cefaclor, Chloramphenicol, Ceftazidim and Amoxicillin. This high resistance of antibiotics is attributable partly to self medication, which is very common in the community and partly due to treatment of patients by the physicians with out proper diagnosis in the laboratory.

On the basis of this study we can conclude that the resistance of commonly used antibiotic is very crucial. The antibiotic treatment should be limited to symptomatic urinary tract infections and be initiated after sensitivity testing only.

ACKNOWLEDGEMENT

The authors are grateful to Medical Superintendent and Pathologist of the District Headquarter Hospital Gilgit, for providing their laboratory facilities to conduct this study successfully.

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(Received 21 April 2008, revised 26 June 2008)