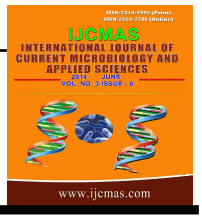
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 876-885**
http://www.ijcmas.com



Original Research Article

Prevalence of Microorganisms isolates from Urinary Tract Infections at Some Hospitals in Sana'a City, Yemen

Salwa H. Alkhyat[1] and Maher Ali Al.Maqtari[2*]

[1]Department of Biology, Faculty of Science, Sana'a University, Yemen
[2]Department of Chemistry, Faculty of Science, Sana'a University, Yemen
Corresponding author

A B S T R A C T

Urinary tract infection (UTI) was one of the most common medical problems. This study is designed to determine the prevalence of UTI among males and females. A total of 140 midstream urine specimens are collected from patients (females and males) and cultured on blood agar, cystine lactose electrolyte deficient (CLED) agar and MacConkey agar. The isolated bacteria identified by morphological and biochemical characters. Bacteria are determined too from 140 urine samples, 104 (74.3%) had UTI, out of 50 males 29 of them (20.7%) had UTI, and 90 are females 75 of them (53.5%) had UTI. The prevalence of UTI is higher in the age group >40 years old as (58.62%) in males and (40%) of them are females. *Escherichia coli* was the most common bacterial isolated, in males (46.6%) and in females was (41.3%), *Staphylococcus aureus* in males (3.4%) and in females was (4%), *Streptococci* in males (6.9%) and in females was (1.3%), *Pseudomonas aeruginosa* in males (24.1%) and in females was (8%), *Klebsiella pneumoniae* in males (17.2%) and in females was (28%), *Proteus vulgaris* in males (6.9%) and in females was (8%) and *Condida albicans* in males (0%) and in females was (4%).

Keywords

Urinary tract infection, microorganisms, biochemical parameters, humans

Introduction

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. Infection of the urinary tract is one the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Kibret and Abera, 2014; Swetha et al., 2014). It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually (10 Amin et al., 2009; Razak and Gurushantappa, 2012; Swetha et al., 2014).

UTI is an extremely common condition that occurs in both male and female of all the ages. The prevalence and incidence of UTI is higher in women than in men, nearly half of whom will experience a UTI during their lifetime (Nordstrom et al., 2013; www.lef.org, 2014), due to several

clinical factors including anatomic differences, hormonal effects and behavioral pattern (Prakasam et al., 2012; Nordstrom et al., 2013).

Urinary tract infections (UTI) are caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the uroepithelium. Proliferation of bacteria in the urinary tract is the cause of urinary tract infection (Prakasam et al., 2012; Swetha et al., 2014).

UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:- bladder [cystitis], kidney [pyelonephritis], or urine [bacteriuria] (Swetha et al., 2014). Signs and symptoms include fever, dysuria, and urinary urgency, cloudy or malodorous urine (Prakasam et al., 2012). Infections of the lower urinary tract (ie, bladder and urethra) commonly cause urinary urgency, pain during urination, or cloudy, pink, or red-colored urine. Less common and potentially more severe are infections of the upper urinary tract, which comprises the kidneys and ureters, kidney infection (pyelonephritis) is associated with fever, vomiting, and flank pain (Jarvis et al., 2014; www.lef.org, 2014).

Furthermore, untreated asymptomatic bacteriuria leads to development of cystitis in approximately 30% of cases, and can lead to the development of pyelonephritis in about 50% of cases. Thus it is important to identify and treat UTI to avoid such complication (Prakasam et al., 2012).

There are many different bacteria that can cause UTIs, with Gram-negative bacteria like *Escherichia coli* being the most common (accou nts for 80–90% of infections), *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Serratia* spp. and Gram-positive bacteria such as *Enterococcus* spp. and *Staphylococcus* spp. (5–10%) (Prakasam et al., 2012, Jarvis et al., 2014; Kibret and Abera, 2014; Swetha et al., 2014). Less commonly, fungi (esp. *Candida* species) may cause UTIs; this is more frequent in hospital settings or individuals with predisposing diseases and/or structural abnormalities of the urinary tract (www.lef.org, 2014).

UTIs are a common burden in patients with diabetes mellitus. Cystitis, ascending infection leading to pyelonephritis, impaired leucocyte function, recurrent vaginitis, emphysematous complications and renal/perinephric abscesses are well recognized in this group of patients if glycemic control is poor (Mahesh et al., 2011; Prakasam et al., 2012). Indwelling catheters are a common cause of bacterial colonisation and urinary tract infections (Jarvis et al., 2014; 4 www.lef.org, 2014).

The etiological agents and their susceptibility patterns of UTI vary in regions and geographical location. Besides, the etiology and drug resistance change through time. Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made (Kibret and Abera, 2014).

The aim of the present study is to isolate and identify microorganisms responsible for urinary tract infections, and to determine the prevalence of urinary tract infections among males and females in Sana'a city, Yemen.

Materials and Methods

Study population

Urine samples were collected from a total of 140 urine samples (50 males and 90 females) between the ages of 1 to >40 years. All these persons were outpatients attending some hospitals in Sana'a city. The urine samples were obtained by informed consent of the patients and the permission to that effect was obtained from the ethical committee of the hospitals.

Urine collection

Clean catch midstream urine (MSU) samples were collected inside sterile disposable universal bottles from patients. They were instructed on how to collect samples and the need for prompt delivery to the laboratory. The samples were labeled and transported to the Microbiology laboratory of Sana'a University, Faculty of Science and were analyzed within 30 minutes to 1 hour of collection (Amin et al., 2009; Nerurkar et al., 2012; Kibret and Abera, 2014).

Media

The media used were: nutrient agar (NA) from Biotec Limited, while nutrient broth (NB), MacConkey agar (MCA), blood agar (BA) and cystine lactose electrolyte deficient (CLED) agar were supplied by Oxoid Limited. Media were prepared according to the manufacturer's specifications and sterilized by autoclaving at 121℃ for 15 min (Amin et al., 2009; Kibret and Abera, 2014).

Microscopy

The urine samples were mixed and aliquots centrifuged at 5000 rpm for 5 min. The deposits were examined using both 10X and 40X objectives. Samples with 10 white blood cells/mm^3 were regarded as pyuric (Smith et al., 2003). A volume of the urine samples were applied to a glass microscope slide, allowed to air dry, stained with Gram stain, and examined microscopically (Kolawole et al., 2009; Nerurkar et al., 2012).

Culturing of urine sample

This was carried out as described by Cheesbrough (2002; 2004; 2006), Prescott et al (2008) and Amin et al (2009). Ten-fold serial dilutions were made by transferring 1.0 ml of the sample in 9.0 ml of sterile physiological saline. One ml was then poured into molten nutrient agar in Petri dishes and rotated gently for proper homogenization. The contents were allowed to set and the plates were then incubated at 37 °C for 24 h.

Bacterial colonies appearing on the plates after the incubation period were enumerated to determine urine samples with significant bacteriuria. A loopful of each urine sample was also streaked on MacConkey agar and blood agar plate for the isolation of the bacteria present in the urine. After incubation, plates with pure growth were selected, the colonies were isolated using inoculating loop and subsequently sub cultured on agar slants for use in further tests.

Identification of isolates

The methods used in the identification and characterization of isolated bacteria include Gram stain followed by microscopic examination, motility test and biochemical tests according to Cheesbrough (2002; 2004 and 2006). The isolates were identified by Bergey's Manual for determinative bacteriology (Buchanan and Gribbons, 1974).

Biochemical test

Oxidase test: A portion of a colony from the test organism was smeared by the inoculating needle onto an oxidase disk. The oxidase test is positive if the cell mass turns dark purple in 5-10 seconds.

Urease test: Urea agar base (Himedia-India) was inoculated heavily over the entire surface of the slants in bijou bottle, incubated at 37ºC for 24 hours.

Citrate utilization test: This test is based on the ability of an organism to use citrate as its only source of carbon. Simmons citrate agar (Himedia- India) were prepared in bijou bottles. The slopes were then stabbed and incubated at 37ºC for 48 hours.

Indole test: The bacteria were sub-cultured in nutrient broth or sulphate indole motility (SIM medium) and incubated for 24 hours, 3 drops of Kovac's indole reagent was added and mixed gently. Some isolates of enterobacteria break down the amino acid tryptophan with the release of indole.

Kligler iron agar test: The medium (KIM) was inoculated with colonies of bacteria isolate thoroughly the surface of slant and then stab down into the center of the butt. These were then incubated at 37ºC for 24 - 36 hours. Blackening of the medium strip indicates hydrogen sulphide production (Al-Afifi, 2009).

Motility: A sterile straight wire loop was used to incubate sulphate indole motility (SIM) medium with bacterial isolate and incubated overnight at 37ºC. Motility was shown by diffused turbidity in the medium.

Diagnostic Test

Catalase test: A sterile wire loop was used to pick some colonies of bacterial isolates and mixed with 2-3 drops of hydrogen peroxide on clean grease free slide.

Coagulase test: A pair of the pure isolates was emulsified in two drops of physiological saline. A loopful of citrated human plasma was added and examined after 2 minutes,

Results and Discussion

Prevalence of UTI and non-UTI among males and females

A total of 140 urine samples (50 were from males and 90 samples from females) are collected in this study through the period from June and July 2013 from patients in Sana'a city. The prevalence of UTI and non-UTI among males and females which shown in table 1. Pathogenic microorganisms were isolated in 104 samples with prevalence rate of 74.2%, (the prevalence in males were 20.7% and in females were 53.5%).

Distribution of UTI according to the patients age

The distribution of UTI among males and females according to their age groups which shown in table 2. It is found that the highest prevalence of UTI in males and female are in the age group >40 years 58.6% in males and 40% in females. The lowest prevalence of UTI in males found in the age groups 11 to 20 years 3.4% . While, the lowest prevalence of UTI in females are in the age group 1 to 10 years which equal 4% .

Table.1 Distribution of isolated microorganism from UTI patients

Type	Male		Female		Total	
	No.	%	No.	%	No.	%
UTI	**29**	**20.7**	**75**	**53.5**	**104**	**74.2**
Non- UTI	**21**	**15**	**15**	**10.7**	**36**	**25.7**
Total	**50**	**35.7**	**90**	**64.2**	**140**	**100**

Table.2 Distribution of UTI according to the age of patients

Patients age group (years)	UTI					
	Male		Female		Total	
	No.	%	No.	%	No.	%
1-10	0	0	3	4	3	2.9
11-20	1	3.4	9	12	10	9.6
21-30	3	10.3	15	20	18	17.3
31-40	8	27.6	18	24	26	25
>40	17	58.6	30	40	47	45.2
Total	29	100	75	100	104	100

Table.3 Distribution of isolated microorganism from UTI patients

Isolated Microorganism	Number of isolates	%
Staphylococcus aureus	4	3.8
Escherichia Coli	47	45.2
B-Hemolytic Streptococci	3	2.9
Pseudomonas aeruginosa	13	12.5
Klebsiella pneumoniae	26	25
Proteus vulgaris	8	7.7
Candida albicans	3	2.9
Total	104	100

Table.4 Distribution of isolated microorganisms in male and female UTI

Microorganism	Male		Female		Total	
	No.	%	No.	%	No.	%
Staphylococcus aureus	1	3.4	3	4	4	3.8
Escherichia coli	12	41.3	35	46.6	47	45.2
B-Hemolytic Streptococci	2	6.9	1	1.3	3	2.9
Klebsiella pneumoniae	5	17.2	21	28	26	25
Proteus vulgaris	2	6.9	6	8	8	7.7
Pseudomonas aeruginosa	7	24.1	6	8	13	12.5
Candida albicans	0	0	3	4	3	2.9
Total	29	100	75	100	104	100

Table.5 Distribution of the etiological agents of UTI according to the age groups of patients

Age groups / Pathogenic isolate	(1-10)		(11-20)		(21-30)		(31-40)		(> 40)	
	NO	%	NO	%	NO	%	NO	%	NO	%
S. aureus	0	0	0	0	1	5.5	3	11.5	0	0
E. coli	2	66.6	7	70	9	50	11	42.3	18	38.3
B-Hemolytic Streptococci	0	0	0	0	0	0	1	3.8	2	4.2
P. aeruginosa	0	0	0	0	0	0	5	19.2	8	17
Proteus vulgaris	0	0	1	10	0	0	2	7.7	5	10.6
K. pneumoniae	1	33.3	2	20	8	44.4	4	15.3	11	23.4
Candida albicans	0	0	0	0	0	0	0	0	3	6.3
Total	3	100	10	100	18	100	26	100	47	100

Distribution of isolated microorganisms for UTI patients

Distribution of isolated microorganisms which found in patients with UTI are shown in table 3. Out of 104 isolated microorganisms, 101 of them (97.1%) are bacteria and only three microorganism (2.9%) is fungi which identified as *Candida albicans***.** While *Escherichia coli* (45.2%)was the most common bacteria. Followed *Klebsiella pneumoniae* (25%), then by *Pseudomonas aeruginosa* (12.5%). Other species of bacteria such as *Proteus vulgaris***,** (7.7%), *Staphylococcus aureus,* (3.8**%**) and B-Hemolytic *Streptococci* which found in lowest number (2.9%).

Distribution of isolated microorganism according to the male and female of UTI patients

The distribution of isolated microorganism from males and females with UTI which shown in table 4. In males, *Escherichia coli* was the most common isolated bacteria (41.3%), followed by other species of microorganism which isolated with lower number, such as *Pseudomonas aeruginosa.* (24.1%), *Klebsiella pneumoniae* (17.2%) *Proteus vulgaris* (6.9%) and also B-Hemolytic *Streptococci* (6.9%). *Staphylococcus aureus* was the lowest number in the UTI males patients.

In females, *Escherichia coli* was the most common isolated bacteria (46.6%), followed by *Klebsiella pneumoniae* (28%), then other species of bacteria which found in lower number such as *Pseudomonas aeruginosa* (8%), also *Proteus vulgaris* **(8%**) and *Staphylococcus aureus* (4%). B-Hemolytic *Streptococci* was the lowest found in females UTI samples (1.3%). Fungi *Candida albicans* which found in female UTI reach to 4%.

The relationship between the etiological agents of UTI and the age groups

The relationship between etiological agents and age groups of patients are shown in table 5. *E. coli and K. pneumoniae* were the most common pathogenic isolated in all age groups**.** *E. coli* in group (1-10) was 66.6% and in age group (11-20) was 70%, while ***K.*** *pneumoniae* in age group (31-40) reached to 44.4%.

S. aureus, B- Hemolytic *Streptococci, P. aeruginosa* and *Proteus vulgaris* were the lowest numbers of pathogenic isolates found in the age groups. While, *Candida albicans* was found only in the age group >40 as equal to 6.3%.

Urinary tract infections are one of the most common diseases diagnosed worldwide. UTIs account for seven million office visits and 100,000 hospitalizations yearly, making them the most common bacterial infections inoutpatient settings (Amin et al., 2009; Razak and Gurushantappa, 2012; Swetha et al., 2014).

Approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40% to 50% of women will have a UTI during their lifetime. The estimated annual cost of UTIs is $1.6 billion for evaluation and treatment. Despite advances in antimicrobial therapy, UTIs remain a significant cause of morbidity (Amin et al., 2009).

The study observes that the prevalence of UTI was high among the females (53.5%) than males (20.7%). This correlates with other studies by Swetha et al (2014); Kibret and Abera (2014). Physiological and anatomical differences are accounted for the differences in males and females.

This is because of the fact that compared to females, the drier environment in the urethra prevents the optimal growth of bacteria. The antimicrobial activity of prostate secretions and longer distance between the anus and urethra meatus are among the factors responsible for the differences in prevalence between the two genders (Hooton, 2000; Dielubanza and Schaeffer, 2011; Prakasam et al., 2012; Kibret and Abera, 2014). The anatomical relationship of the female's urethra and vagina makes it liable to trauma during sexual intercourse as well as bacteria been massaged up the urethra into the bladder during pregnancy and child birth (Kibret and Abera, 2014).

In the present study when the data were visualized in different age groups for both genders, it appeared that Higher proportions of patients were in the age group >40 years, in females 40% and in males 58.6%. The incidence of UTI increases in males as the age advances probably because of prostate enlargement and other related problems of old age (Mahesh et al., 2010; Prakasam et al., 2012; Swetha et al., 2014).

In contrast to our finding**,** Swetha et al (2014) who found that bacteriuria was commonest in the age group 21-40 years. While, Prakasam et al (2012) who found that bacteriuria among females were 42% in the age group (31-40) and among males an increased prevalence of UTI was recorded among the elderly age group, 51-60 (54.28%), than among young age patients (10%). On the other hand, Krieger et al (1993) reported that the prevalence of UTI increase with increasing age for both sexes.

Gram negative bacteria (90.4%) were more responsible for UTI than Gram positive bacteria (6.7%) and this finding is in agreement with the findings of previous studies (Nerurkar et al., 2012; Prakasam et al., 2012; Kibret and Abera, 2014). Bacterial etiologies of UTI can show geographic variations and may even vary over time within a population (El-Mahmood et al., 2009; Demile et al., 2012).

E. coli (45.2%) was the most commonly isolated urinary pathogen in our study. This was in correlation with other studies by Khan and Ahmed (2001), Bashir et al (2008), Sibi et al (2011), Nerurkar et al (2012) and Swetha et al (2014). The second most common isolated pathogen was *K. pneumoniae* (25%). This correlates with the studies of Khameneh and Afshar (2009) and Chin et al (2011) and Swetha et al (2014). This was not agreement with the study of Prakasam et al (2012) in which 83.8% was *E. coli* and 9.6% was *Klebsiella* species.

The similarities and differences in the type and distribution of uropathogens in present study and other studies conducted in different countries may result from different environmental conditions and host factors, and practices such as healthcare and education programmers, socioeconomic standards and hygiene practices in each country (Bashir et al., 2008; Amin et al., 2009).

The prevalence of UTI is higher among females (53.6%) compared to males (20.7%). The higher prevalence of UTI is found in the age group >40 years old in both males and females. Gram negative bacilli (Enterobacteriaceae) were responsible for urinary tract infections and the most common of isolates were *E. coli* and *K. pneumoniae***,** followed by *P.aeruginosa* and *Proteus vulgaris* (12.5%) and (7.7%) respectively of each.

Acknowledgments

The authors are very grateful to Biology Department for help in analysis and to the faculty of sciences, Sana'a University for providing infrastructure facilities.

References

Al-Afifi AA. 2009. The hygienic importance of brucella in milk in Thamar province. Thamar University. Faculty of Agriculture and Veterinary Medicine. Master degree.

Amin M, Mehdinejeh M and Pourdangchi Z. 2009. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishapur J Microbiol*, 2(3): 118-123.

Bashir MF, Qazi JI, Ahmad N and Riaz S. 2008. Diversity of urinary tract pathogens and drug resistant isolates of *Escherichia coli* in different age and gender groups of Pakistanis. *Trop J Pharmaceut Res*, 7 (3): 1025-1031.

Buchanan RE and Gribbons NE. 1974. Bergey's manual of determinative bacteriology (8th edition). Williams & Wilkins Co. Baltimore USA.

Cheesbrough M. 2002. Medical laboratories manual for tropical countries. Cambridge University Press. pp. 479.

Cheesebrough M. 2004. District laboratory practice in tropical countries. Cambridge University Press. pp. 357.

Cheesbrough M. 2006. District laboratory practice in tropical countries. 2^{ed}. Cambridge University Press

Chin BS, Kim MS and Han SH. 2011. Risk factors of all-cause in-hospital mortality among Korean elderly bacteremic urinary tract infection patients. *Archive of Gerontology and Geriatrics*. 52: 5055.

Demile T, Beyene G, Melaku S and Tsegaye W. 2012. Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in North West Ethiopia. *Ethiop J Health Sci*, 22: 121-128.

Dielubanza EJ, and Schaeffer AJ. 2011. Urinary tract infections in women. *The Medical clinics of North America*. 95(1): 2741.

El-Mahmood AM, Atimi AT, Tirmidhi B, Mohammed A. 2009. Antimicrobial susceptibility of some quinolone antibiotics against some urinary tract pathogens in a tertiary hospital, Yola, Adamawa State, Nigeria. *J Clin Med Res*, 1: 26-34.

Hooton TM. 2000. Pathogenesis of urinary tract infections: an update. *J Antimicrob Chemother*, 46(1): 1-17.

Jarvis TR, Chan L and Gottlieb T. 2014. Assessment and management of lower urinary tract infection in adults. *Ustralian Prescriber*. 37(1): 7-9.

Khameneh ZR and Afshar AT. 2009. Antimicrobial susceptibility pattern of urinary tract pathogens. *Saudi J Kidney Dis Transpl*, 20: 251-253.

Khan SW, Ahmed A. 2001. Uropathogens and their susceptibility pattern: a retrospective analysis. *J Pak Med Assoc*, 51:98-100.

Kibret M and Abera B. 2014. Prevalence and antibiogram of bacterial isolates from urinary tract infections at Dessie Health Research Laboratory, Ethiopia. *Asian Pac J Trop Biomed*, 4(2):164-168.

Kolawole AS, Kolawole OM, Kandaki-Olukemi, YT, Babatunde SK, Durowade KA and Kolawole CF. 2009. Prevalence of urinary tract

infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. *Inter J Med and Medi Sci*, 1: 163-167.

Krieger JN, Ross SO and Simonsen RM. 1993. Urinary tract infections in healthy university men. *JUral*, 149: 1046- 1048.

Mahesh E, Medha Y, Indumathi VA, Kumar PS, Khan MW and Punith K. 2011. Community acquired urinary tract infection in the elderly. *Brit J Med Pract*, 4(1): 406.

Mahesh E, Ramesh D, Indumathi VA, Punith K, Kirthi Raj and Anupama HA. 2010. Complicated urinary tract infection in a tertiary care centre in south India, *Al amen journal of medical sciences*, 3(2): 120-127.

Nerurkar A, Solanky P and Naik SS. 2012. Bacterial pathogens in urinary tract infection and antibiotic susceptibility pattern. *J Pharm Biomed Sci*, 21(12): 1-3.

Nordstrom L, Liu CM and Price LB. 2013. Foodborne urinary tract infections: a new paradigm for antimicrobial-resistant foodborne illness. Mini review article no.29, *Frontiers in Microbiology*, 4:1-6.

Prakasam AKC, Kumar KGD and Vijayan M. 2012. A Cross Sectional Study on Distribution of Urinary Tract Infection and Their Antibiotic Utilisation Pattern In Kerala. *Int J PharmTech Res*,4(3):1309-1316.

Prescott M, Harley P and Klein A. 2008. Microbiology 7th edition. McGraw-Hill: New York. pp. 124-126.

Razak SK and Gurushantappa V. 2012. Bacteriology of urinary tract infection and antibiotic susceptibility pattern in a tertiary care hospital in South India. *Int J Med Sci Public Health*, 1:109-112.

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

Smith PJ, Morris AJ and Reller LB. 2003. Predicting urine culture results by dipstick testing and phase contrast microscopy. *Pathol*, 35: 161-165.

Swetha VV, Rao US, Prakash PH and Subbarayudu S. 2014. Aerobic bacteriological profile of urinary tract infections in a tertiary care hospital. *Int J Curr Microbiol App Sci*, 3(3): 120-125.

www.lef.org. 2014. LifeExtension®/ Review / visited 19 April 2014.