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Clinical and microbiological profile of patients with urinary tract infection visiting general outpatient department, National Referral Hospital

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ABSTRACT

Background: Urinary Tract Infection (UTI) is one of the most common infections encountered in outpatient settings. The random use of antibiotics, particularly in resource-limited settings, has contributed to the emergence of antibiotic resistance. This study aimed to identify common uropathogens and assess their antibiotic sensitivity patterns among patients with symptoms of UTI attending the General Outpatient Department (GOPD) at Jigme Dorji Wangchuck National Referral Hospital in Thimphu. **Method:** A cross-sectional study was conducted from September 2021 to August 2022, involving patients over the age of 13 years with symptoms of UTI. Midstream urine collected via proper technique was analyzed by the laboratory. Once there was growth on the urine culture media, antibiotic sensitivity testing was performed against the commonly used antibiotics in the GOPD. **Result:** Amongst 351 participants, 77.8% were female, and the most common symptoms reported were dysuria (93.4%) and increased urinary frequency of micturition (92.3%). Patient's age, sex and previous episodes of UTI were significantly associated with the occurrence of UTI ($p < 0.05$). The overall urine culture positivity rate was 50.53%, with *Escherichia coli* (84.7%) identified as the predominant uropathogen, followed by *Klebsiella pneumoniae* (7.3%). Among the antibiotics tested, gentamicin and nitrofurantoin exhibited the highest sensitivity, while amoxicillin and ampicillin were the least effective antibiotics against *Escherichia coli*. **Conclusion:** UTI predominantly affected females. *Escherichia coli* was the commonest causative agent and nitrofurantoin could be the potential drug of choice for UTI treatment. The irrational use of antibiotics, as a contributing factor to resistance should be addressed.

Keywords: *Antibiotic sensitivity; Culture; Microscopic examination; Urinary tract infections.*

INTRODUCTION

Urinary Tract Infections (UTIs) are the most prevalent bacterial infectious diseases encountered in both community and hospital settings, imposing huge impact on global health[1]. UTIs are a widespread health concern in developing countries, and Bhutan is no exception. According to Bhutan's Annual Health Report of 2020, there were 35,657 cases related to kidney and urinary tract diseases, of which 1,901 were specifically identified as cystitis[2]. These statistics emphasize the need for a comprehensive understanding and effective management of UTIs.

UTIs present with symptoms like dysuria, increased frequency, urgency, altered urine color, flank pain and, in some cases, fever[3]. Apart from clinical symptoms, UTIs are diagnosed by urinalysis, either with a urine dipstick or microscopy, and urine culture. However, the gold standard for confirming uropathogens is urine culture with antibiotic sensitivity testing[4,5].

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The emergence of antibiotic resistance is a global concern, varying across regions based on distinct antibiotic regimens. Inaccurate diagnoses and the use of antibiotics based solely on symptoms contribute to the rising rates of antibiotic resistance in microbes causing UTIs[4]. This emergence of antibiotic resistance not only complicates disease management but also poses a significant public health concern including increased healthcare expenditures. Complications such as renal scarring, often observed after UTIs in infancy, can lead to hypertension, end stage renal disease, and proteinuria, further exacerbating the health burden[6].

As UTIs are a significant health challenge, this study aimed to provide updates on the local antibiotic sensitivity patterns of uropathogens, which could guide healthcare workers towards the effective therapeutic management of UTIs in low resource settings where culture facilities are not available.

METHODS

Study Design and Setting

This cross-sectional descriptive study was conducted at the

General Outpatient Department (GOPD) of Jigme Dorji Wangchuck National Referral Hospital in Thimphu, from September 2021 to August 2022. The GOPD has five clinicians who offer clinical services to patients of all ages and specialties. Ethical clearance was obtained from the Institutional Review Board (IRB) [Ref. No. IRB/Approval/PN21-015/2021/521] dated 21/09/2021.

Study Population and Sample Size

The study included patients aged 13 years and above with UTIs symptoms. Patients with urinary symptoms who were already undergoing antibiotic treatment were excluded. Records maintained at the GOPD revealed that 1000 new cases with UTI symptoms were treated over a period of six months from January to June 2020. Therefore, we estimated around 2000 patients with UTI symptoms to visit the GOPD during our study period. A sample size of 355 was calculated by using Open Epi, Version 3, taking into consideration a 95% confidence interval.

Sampling technique and data collection

All the clinicians in the GOPD were sensitized on the study's aims, inclusion and exclusion criteria. Data collection took place on Mondays and Thursdays via convenience sampling until the required sample size was met. On the designated data collection days, clinicians in various chambers of the GOPD directed potential participants to the principal investigator. Participants were enrolled once they provided informed written consent.

After enrollment, the principal investigator gathered data using an interviewer administered questionnaire. The questionnaire consisted of three sections. The first section gathered demographic and clinical details including age, sex, marital status, history of previous UTIs, and the presence of any comorbidities. The second section focused on symptoms of UTI, while the third section included the results of urine microscopic analysis, urine culture and antibiotic sensitivity, which were recorded once the results were available. All participants were instructed to collect a clean catch midstream urine sample in two sterile wide-mouth containers, which were submitted to the laboratory for microscopic examination and culture.

Sample processing

a) Microscopic examination

Urine samples were processed within 2 hours of receipt at the lab, whenever feasible. Otherwise, they were stored in the refrigerator at 2°C to 8°C for up to 4 hours before processing. For processing, 10 mL of urine was centrifuged for 3 minutes at 1800 revolutions per minute. One drop of the centrifuged mixed sediment was poured onto a clean, labeled slide for examination. Microscopic examination showing White Blood Cells (WBCs) of 0-5 cells/high power field (HPF) were considered normal, while the presence of more than 5 cells/HPF indicated infection.

b) Sample culture processing

Urine culture samples submitted to the microbiology unit were processed within two hours. When not feasible, the samples were stored at 2°C to 8°C in a refrigerator until processed. About 1µL of urine was streaked in a cone-shaped manner using a standard loop on a culture plate containing cystine-lactose-electrolyte-deficient (CLED) media. The plates were immediately incubated at 37°C under aerobic conditions overnight, and the presence of growth, extent of growth, and types of colonies were observed. Results were interpreted as follows:

- 'Negative' if no growth was seen after 24 hours of incubation.
- 'Insignificant growth' if less than 10 Colony-Forming Unit (CFUs)/microliter.
- 'Probably significant growth' if 10-100 CFUs/microliter and pure growth.
- 'Significant growth' if ≥ 100 CFUs/microliter and pure growth.
- 'Mixed growth' if two or more bacteria were seen.

Urine culture plates showing growth were further subjected to the Triple Sugar Iron Agar (TSIA) test for the identification of organism.

c) Antibiotic sensitivity testing (ABST)

Bacterial isolates then underwent ABST using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guideline. Antibiotics disks containing commonly used antibiotics in the OPD like amoxicillin 10mcg, ampicillin 10mcg, nitrofurantoin 300mcg, norfloxacin 10mcg, trimethoprim-sulfamethoxazole 25mcg, ceftriaxone 10mcg, and gentamicin 10mcg, were used. Higher generations of antibiotic disks were used when atypical organisms were isolated.

Statistical analysis

Data entry and analysis were performed with Microsoft Access and Statistical Package for Social Science (SPSS) version 25 respectively. Descriptive analysis was used to characterize sociodemographic features, clinical symptoms, uropathogens isolated, and antibiotic sensitivity. The Pearson Chi-square test was used to assess associations between culture positive UTI and associated factors. Binomial logistic regression was used to calculate Odds Ratio (OR) at a 95% confidence interval. Adjusted Odds Ratio (AOR) was calculated for statistically significant variables. A significance level of $p < 0.05$ was established.

RESULTS

Socio-demographic characteristics

Of the 355 participants clinically diagnosed with UTI, statistical analysis was conducted on 351 participants who provided urine samples for microscopy and culture sensitivity tests. Participants in the age range of 21-45 years comprised the largest proportion (241, 68.7%). The majority of participants were female (273, 77.8%) and married (242, 69%). As depicted in Table 1, most participants did not have a prior documented history of UTI (219, 62.4%) and did not have comorbidities (303, 86.3%).

Common clinical presentations among participants

Dysuria (328, 93.4%), increased frequency of micturition (324, 92.3%), and lower abdominal pain or flank pain (323, 92%) were reported as the most common symptoms. Fever was the least common symptom, reported by only 29.9% (105) of the enrolled participants. The frequency of occurrence of other symptoms are as depicted in Figure 1.

Table 1. Socio-demographic profile of patients clinically diagnosed as UTI during the study period N=351

Characteristics	n (%)
Age (years)	
13 – 20	25 (7.1)
21 – 45	241 (68.7)
46 – 65	58 (16.5)
>65	27 (7.7)
Gender	
Male	78 (22.2)
Female	273 (77.8)
Marital status	
Married	242 (69.0)
Unmarried	57 (16.2)
In a relationship	42 (12.0)
Others	10 (2.8)
Documented previous UTI	
Yes	132 (37.6)
No	219 (62.4)
Documented comorbidities	
Yes	48 (13.7)
No	303 (86.3)

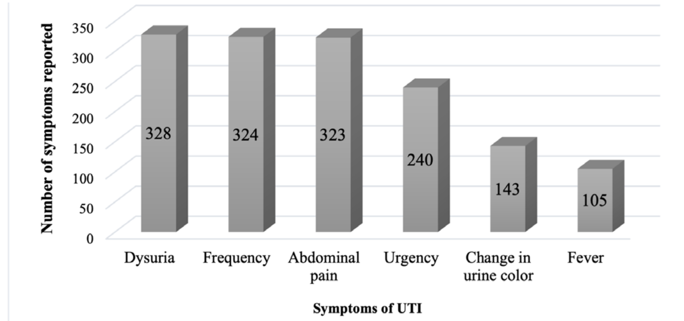

Figure 1. Symptoms reported by enrolled participants during the study period

Culture positive UTIs

The overall urine culture positivity rate among the 351 patients was 50.53%. The highest culture positivity was observed in females (164, 60.7%) and those in the age group of 46 – 65 years (35, 60.34%). The majority (94, 71.21%) of participants with a documented history of previous UTI had positive urine cultures. Participants with comorbidities exhibited higher culture positivity (28, 58.33%) compared to those without comorbidities.

Associations of culture positive UTIs

Univariable analysis demonstrated that the participants above the age of 21 years, females, married participants, and those with a previous history of UTI had a statistically significant association ($p < 0.05$) with culture-positive UTI. Further analysis with binomial logistic regression revealed that patients aged between 46 to 65 years had higher odds of having a culture-positive UTI (AOR 1.93, CI 1.30 – 2.86, $p \leq 0.001$). As depicted in Table 2, females had an eight times higher odds of culture positive UTI compared to males (AOR 7.99, CI 3.90 – 16.4, $p \leq 0.001$), and participants with a previous history of UTI had nearly three times higher odds of culture positive UTI (AOR 2.77, 1.68 – 4.56, CI 95%, $p \leq 0.001$).

Comparing urine microscopy and culture in diagnosing UTI

Sensitivity, specificity, positive predictive value (PPV) and

Table 2. Predictors of culture positive UTI amongst participants presenting to the general OPD during the study period n=351

Characteristics	Positive urine culture	Negative urine culture	Crude OR	P value	aOR	P value (95% CI)
Age group (years)						
13 – 20	3	22	Ref			
21 – 45	124	117	7.4	0.001		
46 – 65	35	23	10.45	0.001	1.93	0.001 [1.30 – 2.86]
>66	15	12	8.48	0.001		
Gender						
Male	13	65	Ref			
Female	164	109	7.42	0	7.99	0.000 [3.90 – 16.40]

Continued...						
Marital status						
Unmarried	18	39	Ref			
Married	135	107	2.71	0.001	1.08	0.687 [0.73 – 1.61]
In a relationship	18	24	1.62	0.25		
Others	6	4	3.15	0.08		
Previous history of UTI						
No	83	136	Ref			
Yes	94	38	4.03	0	2.77	0.000 [1.68 – 4.56]
Co-morbidities						
No	149	154	Ref			
Yes	28	20	1.44	0.238		

negative predictive value (NPV) were calculated to compare urine microscopy with urine culture results. Of the 202 positive urine microscopy samples, 156 (77.2%) turned out to have a positive urine culture. Of the remaining 128 negative urine microscopy samples, 21 (16.4%) had a positive urine culture as depicted in Table 3. Urine microscopy had a sensitivity of 88.1% and specificity of 73.6% in diagnosing UTIs. The PPV and NPV of urine microscopy were 77.2% and 86.0% respectively.

Table 3. Comparison of urine microscopy and urine culture

Urine Microscopy	Urine Culture		Total
	Positive	Negative	
Positive	156	46	202
Negative	21	128	149
Total	**177**	**174**	**351**

Types of uropathogens identified in urine culture

The most common isolated pathogen was *Escherichia coli* (150, 84.7%). As depicted in Figure 2, *Klebsiella pneumoniae, Streptococcus saphrophyticus, Streptococcus pneumoniae, and Enterococcus sp.*, constituted the other uropathogens identified via urine culture.

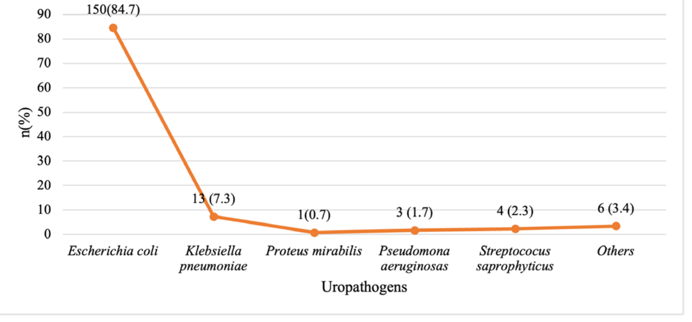

Figure 2. Common Uropathogens isolated in the urine culture of study participants

Antibiotic Sensitivity Pattern

Most of the *Escherichia coli* isolates were sensitive to nitrofurantoin and gentamicin while the least number of isolates were sensitive to amoxicillin (37.3%) and ampicillin (29.3%). *K. pneumoniae* was 100% sensitive to gentamicin and 84.6% to nitrofurantoin, with varying sensitivities to other antibiotics. Pseudomonas aeruginosa showed varying sensitivity to different antibiotics. Table 4 depicts the resistance patterns of the various uropathogens isolated to the commonly used antibiotics in the OPD.

Table 4. Antibiotic sensitivity pattern of uropathogens isolated from participants presenting with UTI symptoms to the general OPD during the study period

Uropathogens	Antibiotics n(%)							
	AMP	AMX	TMT-SMX	NOR	NIT	CTX	GEN	Remarks
Escherichia coli (n=150)	44(29.33)	56(37.33)	85(56.67)	110(73.33)	143(95.33)	92(61.33)	143(95.33)	1 isolate was sensitive to piperacillin, amikacin & meropenem
Klebsiella pneumoniae (n=13)	X	X	10(76.92)	9(69.23)	11(84.61)	10(76.92)	13(100)	
Proteus mirabilis (n=1)	1(100)	1(100)	1(100)	1(100)	X	1(100)	1(100)	

Continued...

Pseudomonas aeruginosas (n=3)	X	X	X	1(33.33)	1(33.33)	X	1(33.33)	All isolates sensitive to piperacillin, cefazolin, ceftazidime
Streptococcus saprophyticus (n=4)	X	X	3(75)	4(100)	4(100)	X	X	All isolates sensitive to penicillin G, cefotaxime & polymyxin
Enterococcus Sp. (n=1)	X	X	X	X	1(100)	X	X	Sensitive to penicillin G
Streptococcus pneumoniae(n=1)	X	X	X	1(100)	1(100)	X	1(100)	
Others (n=4)	X	X	X	X	X	X	X	Mixed growth

AMP=Ampicillin, AMOX=Amoxicillin, TMT-SMX=trimethoprim-sulphamethoxazole, NOR=Norfloxacin, NIT=Nitrofurantoin, CTX=Ceftriaxone, GEN=Gentamicin, X=Either not sensitive or ABST not tested

DISCUSSION

The highest proportion (68.7%) of patients diagnosed with UTI in this study were in the age group of 21-45 years, which is similar to the findings reported in studies from Nepal and India[7,8]. The higher prevalence in this age group might be attributed to increased sexual activity and a higher proportion of married individuals within this age group[9]. However, a study conducted in Japan reported that majority of the participants with UTI belonged to the older age group[10]. This discrepancy in age distribution between regions could be due to demographic variations, especially with the higher percentage of elderly individuals in Japan.

Our findings of females outnumbering males with UTI (77.8% vs. 22.2%) are consistent with findings from studies in Nepal and India[11,12]. Anatomical and hormonal differences between males and females may have contributed to this gender disparity[13,14]. Married participants constituting a majority (68.9%) of UTI cases in our study is similar to the findings of studies from India and Palestine where females comprised 72.5% and 65.9% of affected participants, respectively[7,15]. Implicating factors such as high sexual activity, contraceptive use, and pregnancy could be potential contributors to UTI amongst married people[9,16].

The common symptoms observed in our study including dysuria, frequency, and flank pain, are consistent with findings from a study in India[17]. However, a study in Ethiopia reported fever and urgency to urinate as the most common symptoms[18]. This difference could be due to subjective interpretation of reporting symptoms.

The proportion of positive urine cultures (50.4%) in our study is higher than the 14.3% that was reported in a retrospective study conducted in Bhutan in 2013[19]. The rates are higher even in comparison to studies conducted in India, Nepal and Pakistan where the positivity rates range from 10.1% to 27.4%[7,11,20] The prior study in Bhutan included urine culture samples from both inpatient and outpatient settings as well as pediatric and gynecology patients. The varying rates in other studies may be attributed to the thoroughness of participant screening.

This study noted that older age groups, females and those with a prior history of UTI had higher odds of culture positive UTI, consistent with findings from studies conducted in countries within our region as well as in Ethiopia and Switzerland[8,12,18,20-22]. Pregnancy, hormonal influences and anatomical differences in females and high sexual activity may have contributed to the increased risk of UTI in females and adult age groups. Poor hygiene and inadequate antibiotic treatment in previous UTIs could have contributed to the increased risk of recurrent UTI[6,23].

Escherichia coli dominated urine cultures, consistent with global findings[11,19,24,25]. In fact, the prevalence of Escherichia coli was higher than the rates reported by studies in Bangladesh, Japan, and Taiwan. Contrary to findings from studies in these countries, Klebsiella isolates were lower in our study. The potential for pathogenic transformation of Escherichia coli to a virulent form in the urinary tract, and urinary tract's proximity to the perianal region explains the predominance of Escherichia coli in causing UTIs[26].

Most isolates of Escherichia coli showed equal sensitivity to nitrofurantoin and gentamicin at 95.33%, suggesting their higher efficacy in treatment. Gentamicin's high sensitivity towards Escherichia coli aligns with global trends[18,24,27]. Escherichia coli's sensitivity of 95.3% is lower than the sensitivity of 97.4% reported in the 2013 study in Bhutan. This decrease in nitrofurantoin sensitivity towards E. coli may be attributed to its widespread use, as it is available in tablet form for oral therapy and is accessible even in the primary health care centers[19]. It is concerning to note that Escherichia coli was least sensitive to commonly used antibiotics like amoxicillin (37.33%) and ampicillin (29.33%). These sensitivity rates are lower than those reported in a study in India where the sensitivity of amoxicillin to Escherichia coli was 69.6% and sensitivity to ampicillin was 47.9%[28].

Klebsiella pneumoniae displayed varying sensitivity patterns, with the highest sensitivity to gentamicin (100%). This finding contradicts a study in India but was similar to a study conducted in Bangladesh[24,28]. Pseudomonas aeruginosa demonstrated equal sensitivity to piperacillin-tazobactam, cefazolin, and ceftazidime (100%) but had lower sensitivity to commonly used antibiotics. Although a similar pattern was observed in a study in India, it was comparatively lower than in this study[17]. Although antibiotic resistance is an evolving process, irrational use of antibiotics and local epidemiological factors are postulated reasons for the differences in antibiotic sensitivity patterns in Bhutan and other countries.

LIMITATIONS

The study's single-site design, conducted amongst adult patients visiting GOPD with pediatric and gynecology patients excluded, limits its generalizability to the general population. The convenience sampling method has the potential for introducing bias and inter-observer variability during manual microscopic examination can affect the results.

CONCLUSION

This study provides crucial information regarding UTIs among adults seeking services at the general OPD. UTI symptoms are most prevalent in individuals in the age group of 21-45 years, predominantly affecting females. Escherichia coli emerges as the predominant causative agent of UTIs, and the drug of choice in resource constraint centers is nitrofurantoin. While these findings are valuable, future research should consider more diverse samples from different hospitals and departments.

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AUTHORS CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

UT: Concept, design, data collection and analysis, manuscript writing and review

DS: Concept, design, data collection and analysis and review

CL: Concept, design, data collection and analysis, manuscript writing and review

ST : Manuscript writing, review and submission

ND: Manuscript writing, review and submission

Authors agree to be accountable for all respects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

None

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None