RESEARCH ARTICLE

Uropathogens and their antimicrobial susceptibility pattern: A retrospective study in a district level hospital in Western Nepal [version 1; peer review: 2 approved with reservations, 2 not approved]

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Abstract

Background: Urinary tract infection (UTI) is a common cause of hospital visits. There is an increasing trend of resistance of uropathogens to antibiotics worldwide. The aim of this study was to identify the common uropathogens, along with their antimicrobial susceptibility.

Methods: This retrospective cross-sectional study was conducted from April 2018 to April 2020 at Beni hospital. All patients with urinary tract infection visiting Beni hospital during this time and who had urine culture sensitivity tests done were included in this study. Urine samples were first cultured on cystine lactose electrolyte-deficient agar by a semi-quantitative technique, and then incubated aerobically for 18–24 h at 37 °C. The identified bacterial isolates were tested for antimicrobial susceptibility by the Kirby–Bauer disc diffusion technique.

Results: Of the 1173 samples, 164 (14%) samples showed significant growth. *Escherichia coli* (74%) was the most common causative organism. *E. coli* was sensitive in 113 cases (95%) out of 119. Amikacin was tested in 87 isolates that showed 99% sensitivity. Other commonly used antimicrobial agents had lower sensitivity rates: gentamicin (83%), ciprofloxacin (75%), ceftriaxone (59%), cefixime (56%), cotrimoxazole (55%), cefotaxime (41%), and ampicillin (38%).

Conclusions: *E. coli* is the most common pathogen associated with urinary tract infection. Nitrofurantoin and amikacin can be good empirical agents for treating UTI in patients coming to Beni hospital.

Open Peer Review

Approval Status

	1	2	3	4
version 1				
11 May 2021	view	view	view	view

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2. **Piyush Rhajbhandari**, Patan Academy of Health Sciences, Lalitpur, Nepal
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4. **José Molina-López**, National Autonomous University of Mexico (UNAM), Mexico City, Mexico

Any reports and responses or comments on the article can be found at the end of the article.

Keywords

antimicrobial susceptibility; Escherichia coli; urinary tract infection; uropathogens

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Author roles: **Ghimire M:** Conceptualization, Data Curation, Formal Analysis, Supervision, Writing – Original Draft Preparation; **Adhikari S:** Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Ghimire K:** Data Curation, Project Administration, Supervision, Writing – Review & Editing; **Tiwari B:** Project Administration, Supervision, Writing – Review & Editing; **Koju S:** Data Curation, Writing – Original Draft Preparation; **Poudel S:** Conceptualization, Data Curation, Project Administration; **Khanal S:** Data Curation, Methodology

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Introduction

Urinary tract infection (UTI) is one of the common health problems affecting people of all ages, race/ethnicity, sex, and circumcision status. It is caused mostly by bacteria, but viruses and fungi have also been implicated in rare cases.^{1,2}

Several studies have reported that there is an increased resistance of uropathogens to a number of common broad-spectrum antibiotics worldwide.³⁻⁵ As the resistance patterns differ across different areas, every hospital should formulate their own anti-microbiogram for common infections, so as to guide the appropriate treatment.

The objective of this study is to find the prevalence of common uropathogens and, secondly, to identify antimicrobial sensitivity and resistance patterns to those pathogens.

Methods

Study setting, design, population and sampling techniques

A retrospective cross-sectional study was carried out in Beni hospital. A convenient sampling technique was used and all patients with urinary tract infection visiting Beni hospital from April 2018 to April 2020 and had urine culture sensitivity tests done were included in this study.

Urine sample collection

15–30 mL of urine was collected in a sterile leak-proof urine container. A midstream clean-catch specimen was taken. Patients were first asked to cleanse the urethral area before collecting the specimen. Specimens received in the laboratory were processed within 2 hours. Transport medium for urine specimens, such as 1.8% boric acid, sodium chloride or polyvinylpyrrolidone, was used.

Culture, identification technique and antimicrobial susceptibility testing

The sample received was inoculated in the cysteine lactose electrolyte deficient agar media with a 1-mL calibrated loop of internal diameter, 0.001 mL volume of urine specimen using a semi-quantitative method. After inoculation, it was incubated at 37°C overnight for visible growth. A growth of $>10^5$ colony forming unit/mL was considered as significant bacteriuria. Bacterial identification was done using standard bacteriological techniques. The antibiotic susceptibility tests of the isolates against different antibiotics were done using Mueller Hinton agar (MHA) by the standard disk diffusion technique of modified Kirby–Bauer method as recommended by the Clinical & Laboratory Standards Institute. In this study, if the isolates were resistant to at least one agent in three or more antimicrobial categories, they were regarded as multi-drug resistant (MDR) organisms.

Potential biases

This study is subject to selection bias as samples were taken conveniently and confirmation bias as observer might be familiar with common drugs that are resistant to pathogens

Data processing and analysis

Data entry and analysis were done by using SPSS version 25.0 (RRID:SCR_019096); JASP (RRID:SCR_015823) is an open-source alternative to SPSS. Descriptive statistical methods were carried out for data analysis.

Ethical consideration

Ethical clearance was obtained from the national ethical review board, Nepal Health Research Council. The reference number of the ethical letter was 1886 dated 20th January, 2021.

Consent

Hospital data were reviewed, and patients were not directly involved in the research; approval from the hospital was taken and consent from the patient had been waived by the Nepal Health Research Council

Results

Urine samples from 1173 symptomatic patients were received for urine culture during the study period. Out of 1173 samples, 164 urine samples (14%) showed significant growth of at least one of the uropathogens tested in this study: *E. coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter* spp. The most common pathogen isolated was *E. coli* (74%) followed by *Klebsiella* spp. as shown in Table 1.

E. coli was sensitive in 113 cases (95%) out of 119. Amikacin was tested in 87 isolates that showed 99% sensitivity. Other commonly used antimicrobial agents had lower sensitivity rates: gentamicin (83%), ciprofloxacin (75%), ceftriaxone

Table 1. Uropathogens isolated in patients' urine samples in this study.

Gender	E.coli	S. aureus	Klebsiella	Acinetobacter	Total
Male	24	1	3	3	31
Female	98	4	18	13	133
Total (percentage)	122 (74)	5 (3)	21 (13)	16 (10)	164

Table 2. Antimicrobials sensitivity and resistance pattern of uropathogens.

No of urinary pathogen isolates sensitive and resistant to specific antimicrobial agents					
Antimicrobial agent		<i>E. Coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Acinetobacter spp</i>
Ampicillin	Sensitive	30	1	5	3
	Resistant	49	1	9	7
Cefixime	Sensitive	59	1	16	4
	Resistant	47	3	2	11
Ciprofloxacin	Sensitive	77	3	16	8
	Resistant	25	0	0	3
Cotrimoxazole	Sensitive	58	3	17	5
	Resistant	48	1	1	10
Ceftriaxone	Sensitive	69	2	17	4
	Resistant	47	0	14	11
Amikacin	Sensitive	86	3	14	7
	Resistant	1	0	0	3
Cefotaxime	Sensitive	48	2	13	0
	Resistant	65	0	8	16
Gentamicin	Sensitive	87	5	16	7
	Resistant	17	0	1	4
Nitrofurantoin	Sensitive	113	4	16	11
	Resistant	6	0	4	4
Multi- Drug Resistance		52	1	5	13

(59%), cefixime (56%), cotrimoxazole (55%), cefotaxime (41%), and ampicillin (38%). The sensitivity pattern of other microbes is shown in [Table 2](#).

Discussion

As with other infections, UTIs are managed initially with empirical antibiotics till the urine culture reports become available. In Nepal, most hospitals in rural areas do not have proper microbiology laboratories performing bacterial culture, so treatment is solely empirical in such cases. Our study showed *E. coli* as the most common pathogen (74%) causing UTI, followed by *Klebsiella* spp. (13%) and *Acinetobacter* spp. (10%). Similar findings have also been reported in different studies conducted in Nepal and other countries.⁴⁻⁷ So, empirical antibiotics to treat UTI should target *E. coli*.

Among 119 samples of *E. coli* tested for nitrofurantoin, it was sensitive in 113 cases (95%). Amikacin was tested in 87 isolates that showed 99% sensitivity. Other commonly used antimicrobial agents had lower sensitivity rates: gentamicin (83%), ciprofloxacin (75%), ceftriaxone (59%), cefixime (56%), cotrimoxazole (55%), cefotaxime (41%), and ampicillin (38%). Various studies done in Nepal have also shown lower sensitivity of the pathogen to these agents.^{3,14} Our study suggests that nitrofurantoin would be a good first-choice oral antibiotic for managing lower UTI such as cystitis. However, in pyelonephritis or prostatitis, nitrofurantoin is not recommended as it does not attain sufficient concentration in these

tissues. Based on our study results, aminoglycoside amikacin (parenteral) can be used empirically for pyelonephritis, or when a patient does not tolerate oral medicine. In a prospective cohort study conducted in Singapore from 2015 to 2016, *E. coli* was sensitive to amikacin in 100% of the cases.⁶

Other commonly used oral antimicrobial agents like ciprofloxacin, cotrimoxazole and cefixime had lower sensitivity to *E. coli*. They had been used rampantly in Nepal in the past, mainly for typhoid fever. This explains the increase in resistance to these agents. Several studies done in Nepal and abroad have also shown higher resistance of *E. coli* to these antibiotics.^{3,4,7-10} Ceftriaxone one of the most commonly used antibiotic in hospitalized patients in Nepal, however its sensitivity to *E. coli* was only 59%, hence its empirical use in hospitalized UTI patients should be discouraged.

Klebsellia spp. was found to be more sensitive to antimicrobial agents as compared with *E. coli*. It was sensitive in all 16 samples tested for ciprofloxacin, 14 samples tested for amikacin, 17 out of 18 samples tested for cotrimoxazole (94%) and 16 out of 17 tested samples tested for gentamycin (94%). However, a study done at KIST Medical College from March 2013 to April 2014 showed that *Klebsellia* spp. obtained from all urine samples were multidrug resistant and extended spectrum beta lactamase producers.¹⁵

Acinetobacter spp. was more resistant compared with other microbes isolated in this study. It was resistant in all 16 cases tested for cefotaxime, 11 out of 15 samples tested for ceftriaxone (73%), 10 out of 15 samples tested for cotrimoxazole (67%), four out of seven samples for gentamycin (36%) and three out of seven samples for amikacin (30%). Similar to this study, a study published in The Pan African Medical Journal also showed *Acinetobacter* spp. resistant to commonly administered antibiotics with high susceptibility to amikacin.¹⁶

Multidrug-resistant organisms are resistant to at least one agent in three or more classes of antimicrobial agents. The rising incidence of a multidrug resistance phenotype of extended-spectrum beta-lactamase (ESBL) genes and fluoroquinolones resistance, has become a global concern because of their potential cause of serious infections which are difficult to treat.¹² In this study, MDR was isolated in 52 out of 122 cases of *E. coli* (43%), 13 out of 16 cases of *Acinetobacter* spp. (81%) and five out of 21 cases of *Klebsiella* spp. (24%). Significant proportions of MDR uropathogens were seen in other studies too, done in different hospitals of Nepal.^{5,13} Antimicrobial resistance (AMR) is a major concern in both developed and developing countries as various studies have shown its rising incidence. It has posed a major challenge for successful treatment of infectious diseases. With increased prevalence of irrational and injudicious use of antimicrobial agents and inadequate antibiotic stewardship programs, it is a major burden for Nepal.¹⁴ Even in rural areas like Beni, Myagdi, all classes of antimicrobial agents are easily available. In our experience antibiotics have been sold mostly without proper diagnostic evaluation of patients and prescription of physicians in both major cities and rural areas of Nepal.

In Beni hospital, culture and sensitivity were not routinely sent in all clinically suspicious cases of urinary tract infections. Also, sensitivity was not tested on all appropriate classes of antimicrobial agents. Had it been done, we would have a broader view of sensitivity and resistance pattern. As we are collecting more data on this matter, we will have analysis of more data in the future which would give a more accurate prospect of the antimicrobial susceptibility pattern in this hospital.

The susceptibility patterns of antimicrobial agents to microbes vary from country to country and also in different regions of the same country. The guidelines used in Western countries may not be useful in Nepal. It is necessary to identify the sensitivity pattern in a particular location and to develop the treatment protocol accordingly. Very few studies have been published regarding AMR in the Gandaki province of Nepal.¹⁴ This study will help for selection of appropriate empirical antimicrobial agents for treatment of UTI in this region.

The limitation of the study is external validity. As data were collected from a single hospital, our findings cannot be generalized and multicenter studies with larger sample size are needed to find out the real scenario of antimicrobial resistance pattern and formulation of treatment guidelines accordingly. Also patient information like genitourinary malformations, prior exposure to antibiotics, recent hospitalization or prior history of UTI were not taken into consideration, which can be important risk factors for resistant uropathogens.

Conclusions

E. coli is the most common pathogen associated with urinary tract infection in Beni hospital. It is resistant to broad-spectrum penicillin, third-generation cephalosporins and fluoroquinolones. Resistance to nitrofurantoin is low and could be the antibiotic of choice for uncomplicated cystitis. Amikacin showed promise as a suitable intravenous agent but needs further studies with adequate sample size

Data availability

Underlying data

Harvard Dataverse: Underlying data for 'Uropathogens and their antimicrobial susceptibility pattern: A retrospective study in a district level hospital in Western Nepal', <https://doi.org/10.7910/DVN/HTQELY>.

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

Author Contributions:

Manoj Ghimire- Conceptualization, Data Curation, Formal Analysis, Supervision, Methodology, Writing – Original Draft Preparation

Sudeep Adhikari- Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Kalpna Ghimire- Data Curation, Project Administration, Supervision, Writing – Review & Editing

Bishal Tiwari- Project Administration, Supervision, Writing – Review & Editing

Soni Koju- Data Curation, Writing – Original Draft Preparation

Sajana Poudel- Conceptualization, Project Administration, Data Curation

Sulab Khanal-Data Curation, Methodology

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Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 28 September 2021

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José Molina-López

Unidad Periférica de Investigación Básica y Clínica en Enfermedades Infecciosas, Departamento de Salud Pública, División de Investigación, Facultad de Medicina, National Autonomous University of Mexico (UNAM), Mexico City, Mexico

The manuscript, "Uropathogens and their antimicrobial susceptibility pattern: A retrospective study in a district level hospital in Western Nepal by Ghimire M, Adhikari S, Ghimire K, Tiwari B, Koju S, Poudel S and Khanal S", is an interesting study where the authors show the identification of several bacterial species associated with urinary tract infections and determine their susceptibility to different antimicrobial agents. They concluded that *Escherichia coli* (*E. coli*) is the most common pathogen found.

I have a lot of suggestions for the authors:

- If *E. coli* is associated with urinary tract infections (UTIs) worldwide in high percentages and it's known that several serogroups (O1, O2, O4, O6, O7, O8, O15, and O25) of *E. coli* cause UTIs, the authors could search the main serogroups for a stronger study.
- There are also a lot of publications about the virulence factors in *E. coli* (FimH, sat, iutA, papC, cnf1 and hlyCA) that promote the damage in the patients suffering UTIs. That information will be important to characterize the *E. coli* strains.
- On the other hand, Clermont *et al.*¹ published the phylogenetic classification of associated *E. coli* UTIs.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bacteriologist: antimicrobial drug resistant

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 17 August 2021

<https://doi.org/10.5256/f1000research.55454.r90903>

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Adhi Kristianto Sugianli

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General comment: Interesting topic about the antimicrobial susceptibility pattern in uropathogens, which needed to establish the guideline for empirical treatment. However, there is some specific concern regarding the article, as follows:

Introduction

- In the Introduction section, the authors need to show the importance of performing local antimicrobial susceptibility data, e.g elaborate with WHO surveillance program (GLASS) as the source of information. This is an important point to strengthen the rationale of the article.

Methods

- In the Methods section, information regarding microbiology procedures has been written properly. However, the authors need to add more about the study population, data collection, and variable definition, for example:
 - (1) Describe the site of data collection, e.g Beni Hospital;
 - (2) How do the authors collect the data retrospectively, e.g using medical records and/or laboratory information systems? If both of them are used as the source of the data, then how do the authors elaborate on both of the data? Please clarify and explain in the Methods section;
 - (3) Lastly, the authors need to define the definition of a patient with a UTI in this study.
- The important information that is also needed is the antibiotic that is used in this study. This needs to be explained and described clearly in the Methods section, as well as the quality control of the AST procedure.
- Please indicate and clarify that no technical method and/or supplies (e.g reagent, instrument, etc) was changed between 2018 – 2020.
- Please provide references in the Methods section, as well as the version of CLSI.

Results

- In the Results section, there is the statement: “out of 1173 samples, 164 urine samples (14%) showed significant growth of at least one of the uropathogens tested in this study: *E. coli*, *Staphylococcus aureus*, *Klebsiella spp.*, *Acinetobacter spp.*” This might be better to provide information among 1173 specimens; how many urine specimens have shown growth of the organism? This is confusing since only 164 were included from 1173, which indicates almost half of the population was rejected. Please clarify and write more information about this in the results.
- The authors need to clarify the purpose for tabulating between gender and certain isolate and might be more informative if the data are reported separately between years (looking for trends).

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious Diseases, microbiology, antimicrobial resistance

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 16 August 2021

<https://doi.org/10.5256/f1000research.55454.r89329>

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Piyush Rhajbhandari

Patan Hospital, Patan Academy of Health Sciences, Lalitpur, Nepal

The research article is well written, however:

Methodology:

As a retrospective study, the methods describing sample collection and culture sensitivity do not change the outcome and can be omitted.

Detailed methods on how the reports were stored in the hospital (HMIS vs hardcopy), how it was retrieved (database from server vs data entry from hardcopy), and function used in SPSS needs to be included. This will help in analyzing errors as typo or process errors.

Data analysis:

The authors need to be aware of the intrinsic resistance of organisms to particular drugs (eg: *Klebsiella pneumoniae* vs ampicillin) and CLSI guidelines.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology and infectious disease

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 09 August 2021

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Gianluigi Franci

Department of Medicine, Surgery and Dentistry “Scuola Medica Salernitana”, University of Salerno, Baronissi, Italy

Summary:

This retrospective study highlights an increasingly growing problem: antimicrobial resistance to antibiotics commonly used in hospitals, focusing on urinary infections. The objectives of this study were to identify the most frequent uropathogens, with their pattern of sensitivity to antibiotics, in patients with bacteriuria visiting the Beni hospital from April 2018 to April 2020. The results report an incidence of positivity equal to 14%. *Escherichia coli* (*E. coli*) was the most common isolate (74%), poly sensitive strain in 95% of cases, with high sensitivity towards Amikacin, gentamicin, ciprofloxacin but with a sensitivity of around 50% for the other antibiotics tested (ceftriaxone, cefixime, co-trimoxazole), and less than 40% to cefotaxime and ampicillin. In conclusion, *E. coli* is recognized as the most common pathogen associated with urinary tract infection, and indicating nitrofurantoin and amikacin as efficient antibiotics to be used in empirical therapy for the treatment of urinary tract infections in patients arriving at Beni hospital. The objectives of the study are very interesting, but the statistics and numbers of analyzed microorganisms do not fit

the possibility of having a statistical analysis in terms of species isolated and antibiotic resistance profiles. Moreover, the manuscript needs a deeper reorganization and correction of the text.

Abstract:

- The abstract is missing some information. This is part of the low number of microorganisms isolated. Taking apart the *E. coli*, all the other species are so limited that no assumption and evaluation can be included.

Introduction:

- The introduction should be improved with epidemiological data of similar hospitals in terms of geographical region and assisted person.
- The aims and perspectives of the study should be described better.

Methods:

- Concerning the high potential interest of people around the world to this kind of data, an international guideline in the antimicrobial resistance value should be used. Indeed in the paper, it was not possible to understand the concentration of the antibiotics that have been used. I strongly recommend the use of EUCAST lines.

Results:

- In order to have a sufficient number of isolated microorganisms requested for robust statistical analysis, I recommend increasing the period of time analysed.
- In addition, in the tables, it may be more useful to report the percentages and in parentheses the total number of isolates tested. However, there is no statistical value (*p*-value) to confirm the significance of the information calculated and presented in the manuscript, so statistical calculations are lacking.
- Finally, why did the authors just evaluate the last two years? Moreover, they include a period of analysis pre-COVID-19 and during the pandemic infection. Is it possible that this variable was not included?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology, Clinical Microbiology, Host-Microorganism interaction, Microbial Epigenetic

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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