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, 1 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.
Keywords
antimicrobial susceptibility; Escherichia coli; urinary tract infection; uropathogens

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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.\textsuperscript{1,2}

Several studies have reported that there is an increased resistance of uropathogens to a number of common broad-spectrum antibiotics worldwide.\textsuperscript{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: \textit{E. coli}, \textit{Staphylococcus aureus}, \textit{Klebsiella} spp., \textit{Acinetobacter} spp. The most common pathogen isolated was \textit{E. coli} (74%) followed by \textit{Klebsiella} spp. as shown in Table 1.

\textit{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
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%). Variou...
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
Kalpana Ghimire- Data Curation, Project Administration, Supervision, Writing – Review & Editing
Bishal Tiwari- Project Administration, Supervision, Writing – Review & Editing
Soni Poudel- Data Curation, Writing – Original Draft Preparation
Sajana Poudel- Conceptualization, Project Administration, Data Curation
Sulab Khanal- Data Curation, Methodology

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Reviewer Report 17 August 2021

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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
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
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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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