

Prevalence of urinary pathogen and antimicrobial susceptibility of isolates in cervical cancer patient attending BPKMCH Bharatpur, Chitwan

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Abstract

Introduction: This study aimed to identify urinary tract infections in cervical cancer patients treated at BPKMCH Bharatpur, Chitwan, and test isolates for antibiotic susceptibility. Cervical cancer, a disease with abnormal cell development, can spread to other parts of the body. Treatment modalities like surgery, chemotherapy, radiation, and anticancer medication increase the risk of infections.

Methodology: Urine samples from cervical cancer patients at BPKMCH, Chitwan, were processed using standard guidelines in the Microbiology Laboratory. Isolates were identified using biochemical tests and techniques. Pure colonies were tested for antimicrobial susceptibility on Mueller Hinton agar (MHA) using Kirby-Baur disc diffusion method, following CLSI guideline 2017.

Results: A study of 350 cervical cancer specimens revealed that 43.1% contained significant growth of microorganisms, with *Escherichia coli* having the highest predominance at 35.9%. *Staphylococcus aureus* was the most prevalent Gram positive organism, with most isolates resistant to Ampicillin, Cotrimoxazole, and Cefixime. Polymyxin B and Imipenem were most effective for Gram Negative bacilli, while Vancomycin and Azithromycin were most effective for Gram Positive cocci.

Conclusion: Out of 350 patients, 43.1% had a UTI. Cervical cancer patients are more likely to get UTI as a result of immunosuppressive medication therapy and invasive surgery. As a result, they should be evaluated on a regular basis for the development of UTI, and their treatment should include AST. Such hospital-based study should be conducted to establish empirical therapy of UTI among cervical cancer patients with UTI, as well as to ensure adequate treatment and care.

Keywords: Antimicrobial Susceptibility Testing; Cervical Cancer; Uropathogen; UTI

1. Introduction

Cancer is a leading cause of mortality globally, with more than half of cases occurring in poorer nations. The leading causes of mortality from cancer include lung, liver, colorectal, stomach, breast, cervical, and leukemia (1). New advancements in treatment choices have enhanced the survival rates of cancer patients in recent decades (2). However, significant immunosuppression as a side effect of various treatment techniques raises the risk of opportunistic infections (3). Infections are one of the most severe consequences and the major cause of morbidity and death in cancer patients (4). Neutropenia, stem cell transplantation, prolonged catheterization, and the widespread use of medical devices such as stents, shunts, and central venous catheters are some risk factors for infection acquisition (4).

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Patients with genital cancers may experience both vaginal and uterine infection at the same time. Urinary tract infections can occur as a consequence of surgery, catheterization, or cystoscopy in patients who have had surgery (for cancer or other causes) (5). In addition to surgery, which is a common treatment for these tumors, radiotherapy may also be a factor that raises the incidence of UTIs since it results in infection alterations to the bladder's epithelium (6). Urinary tract infections (UTIs) are among the most prevalent illnesses among cancer patients (7). Urinary tract infections have been linked to a variety of bacteria, with Enterobacteriaceae being the most common. Particularly in cancer patients, the rise of multiple-drug-resistant (MDR) strains of Gram-negative bacteria that cause UTIs has raised severe concerns (8). Globally, the prevalence of illnesses brought on by multi-drug resistance (MDR) bacteria has been rising (9). New, more potent immunosuppressive regimes may increase the number and MDR range of microbial infections (3). Recent research on cancer patients primarily focuses on bloodstream infections, with little knowledge about bacteria linked to urinary tract infections. This study aims to evaluate the prevalence of UTIs and antibiotic resistance in Nepalese cervical cancer patients receiving treatment at BPKMCH Bharatpur, Chitwan, aiming to better understand the antibacterial susceptibility of uropathogen species.

2. Material and methods

The study was carried out at B.P Koirala Memorial Cancer Hospital in Bharatpur, Chitwan. A prospective, hospital-based cross-sectional research design was used. Study population Throughout the trial period, all cervical cancer patients were included in the study population. Clinical data was acquired using a standardized questionnaire, while pertinent clinical data was collected by clinicians.

2.1. Urine sample collection and processing

On the same day of enrollment, 5 ml of mid-stream clean catch urine (MSU) was collected using lick-proof, sterile plastic containers. All specimens were processed within one hour of collection. Bacterial isolation Using a calibrated wire loop [0.001 ml], samples were inoculated into Cysteine Lactose Electrolyte Deficient medium (CLED) agar. After an overnight incubation, To assess substantial development, colonies were counted after 24-48 hours at 37°C. Colony counts reveal bacterial growth of 10^5 /mL [A UTI was diagnosed when The presence of at least 10^5 colony forming units (CFU) per milliliter of urine was considered significant for bacteriuria(10). CLED colonies were subcultured into blood agar and MacConkey agar. Plates were incubated at 37°C for 24-48 hours. Identification of Bacteria were performed utilizing colony characteristics, gram reaction of the organisms, and biochemical assays. Following conventional bacteriological procedures (11).

The antibiotic susceptibility of isolated organisms from clinical specimens was determined on Mueller Hinton Agar (MHA) (Hi-media, India) using the standard disk diffusion technique of the modified Kirby-Bauer method in accordance with CLSI recommendations(12). Four to five isolated 18 to 24 hour old colonies from blood agar plates were suspended in 1.0 ml of saline using a sterile inoculating loop. Following mixing, the solution was adjusted to meet the 0.5 McFarland's turbidity criterion (1.5×10^8 bacterial load per mL) (12). The antibiotic susceptibility of isolated organisms from clinical specimens was assessed on Mueller Hinton Agar (MHA) (Hi-media, India) using the standard disk diffusion technique of the modified Kirby-Bauer method, as per CLSI recommendations (12). Using a sterile inoculating loop, four to five isolated colonies from CLED plates 18 to 24 hours old were suspended in 1.0 ml of saline. After mixing, the solution was adjusted to satisfy the 0.5 McFarland turbidity criteria (1.5×10^8 bacterial load per mL) (13). To eliminate any surplus fluid that could shelter bacteria, a sterile cotton swab was dipped into the solution and swirled over the tube walls. The study involved testing six disks of different antimicrobials on a plate, spaced at least 25 mm apart. The disks were gently squeezed and left for fifteen minutes to ensure diffusion. The plates were incubated overnight at 37°C in an aerobic environment. The zone of inhibition (ZOI) was measured and reported in millimeters(13). The data was compared with Kirby-Bauer's Standard chart to identify bacterial susceptibility to antimicrobial agents. The results were compared with an in-house control strain, *S. aureus* ATCC 25923, as part of quality control. Organisms resistant to at least three or more than three groups of first-line antimicrobials were considered as multi drug resistance (14). Data from the questionnaire and laboratory registration book were entered into excel version 6 and exported to SPSS version 25 for analysis.

3. Results

3.1. Frequency of different uropathogens

The overall number of organisms identified from the 350 cervical cancer patients was 43.1% (n=151). *Escherichia coli* is the most prevalent of them, accounting for 35.9% (n=54), followed by *Klebsiella spp.* The number of *Klebsiella* species is equivalent to the total number of gram-positive bacterial isolates, whereas almost half of *Escherichia coli* accounts for 17.3% (n=26). There are four times as much *Staphylococcus aureus* as CONS. CONS is equivalent to 2.6% of *salmonella* and *citrobactor* species (n=4). is the organism that accounts for the least amount of overall growth.

Table 1 Frequency of different uropathogens

SN.	Organism	Frequency (n)	Percentage (%)
1	<i>Escherichia coli</i>	54	35.9
2	<i>Klebsiella spp.</i>	26	17.3
3	<i>Pseudomonas aeruginosa</i>	18	11.9
4	<i>Staphylococcus aureus</i>	16	10.6
5	<i>Proteus spp.</i>	14	9.3
6	<i>Enterococcus spp.</i>	6	3.9
7	<i>Candida spp.</i>	5	3.3
8	<i>Citrobactor spp.</i>	4	2.6
9	CONS	4	2.6
10	<i>Salmonella spp.</i>	4	2.6
11	Total	151	100

3.2. Antimicrobial susceptibility pattern of gram negative isolates

Of the 54 *E. Coli* isolates, 41 were resistant to ampicillin and 13 were sensitive, whereas 36 isolates were resistant to cotrimoxazole and 18 were susceptible. Forty-four isolates were susceptible to ciprofloxacin, whereas ten were resistant. Nitrofurantoin exhibits sensitivity to 39 isolates and resistance to 15. Imipenem and Polymixine B had the highest reported sensitivity (100%) of any drug. Amikacin and gentamycin exhibit nearly identical patterns of sensitivity and resistance. When it comes to *Klebsiella species*, cefixime and levofloxacin were the least resistant (12.5% and 8.33%, respectively), while gentamycin, amikacin, and ciprofloxacin showed the maximum resistance. Imipenem and polymixine B are 100% sensitive. All eighteen *Pseudomonas aeruginosa* isolates exhibited resistance to both ampicillin and cotrimoxazole. Ciprofloxacin was 16.66% resistant, and nitrofurantoin was 33.33%. Imipenem and Poymixin B have 100% sensitivity. All 14 *Proteus spp.* isolates are resistant to drugs, with the exception of imipenem and ciprofloxacin, which were 100% responsive. Cotrimoxazole and Gentamycin were 100% resistant among the four isolates of *Citrobactor spp.*, whereas every other antibiotic tested was 100% responsive. Ampicillin, ciprofloxacin, and cotrimoxazole were 50% resistant and 50% sensitive among the four isolates of *Salmonella*. While Gentamycin was quarterly sensitive and Cefixime and Levofloxacin were quarterly resistant, Polymyxin B and Imipenem were 100% sensitive. The majority of the isolates exhibited resistance to ampicillin, which was followed by gentamycin and cotrimoxazole, respectively. Ciprofloxacin was the most effective treatment for Gram-negative bacteria, followed by Polymixin B and Imipenem.

Table 2 Antimicrobial susceptibility pattern of gram negative isolates

Antibiotics	<i>E. coli</i>		<i>Klebsiella spp.</i>		<i>P. aeruginosa</i>		<i>Proteus spp.</i>		<i>Citobactor spp.</i>		<i>Salmonella spp.</i>	
	R	S	R	S	R	S	R	S	R	S	R	S
Ampicillin	41	13	-	-	18	0	14	0	-	-	2	2
cotrimoxazole	36	18	11	15	18	0	14	0	4	0	2	2
Ciprofloxacin	10	44	13	13	3	15	0	14	0	4	2	2
Nitrofurantoin	15	39	6	20	6	12	14	0	0	4	0	4
Amikacin	26	28	13	13	5	13	14	0	0	4	0	4
Cefixime	8	46	3	23	2	16	14	0	0	4	1	3
Levofloxacin	11	43	2	24	3	15	14	0	0	4	1	3
Gentamicin	28	26	13	13	5	13	14	0	4	0	3	1
Polymixin B	0	54	0	26	0	18	-	-	0	4	0	4
Imipenem	0	54	0	26	0	18	0	14	0	4	0	4
Total	175	365	61	173	57	120	98	28	8	28	11	28

3.3. Antimicrobial susceptibility pattern of gram positive bacterial isolates

Of the 16 *Staphylococcus aureus* isolates, gentamycin had the highest resistance (56.25%), whereas tecoplanin had the lowest resistance (12.5%). The most sensitive (100%) was vancomycin. Among the six *Enterococcus* spp isolates. The most resistant antibiotic is ciprofloxacin (83.3%), which is followed by ampicillin (66.66%). 50% of them were resistant to cefixime. While Vancomycin, Azithromycin, Erythromycin, and Linezolid were 100% sensitive, Teicoplanin was the least resistant antibiotic. Of the four CONS isolates The most resistant drugs were cefixime (100%) and gentamycin (75%) and cotrimoxazole (75%). Teicoplanin and Erythromycin were 25% resistant, whereas Amikacin was the least resistant antibiotic. Levofloxacin, Vancomycin, and Linezolid were all 100% sensitive. The majority of the isolates exhibited resistance to cefixime, gentamycin, and cotrimoxazole. In that order. For gram-positive cocci, the most effective antibiotics were vancomycin and azithromycin, followed by erythromycin.

Table 3 Antimicrobial susceptibility pattern of gram positive isolates

Antibiotics	<i>S. aureus</i>		<i>Enterococcus SPP</i>		CONS		Total	
	R	S	R	S	R	S	R	S
Ampicillin	-	-	4	2	0	0	4	2
cotrimoxazole	7	9	-	-	3	1	10	10
Ciprofloxacin	-	-	5	1	0	0	5	1
Nitrofurantoin	-	-	-	-	0	0	0	0
Amikacin	6	10	-	-	1	3	7	11
Cefixime	7	9	3	3	4	0	14	12
Levofloxacin	3	13	-	-	0	4	3	17
Gentamicin	9	7	-	-	3	1	12	8
Vancomycin	0	16	0	6	0	4	0	26
Teicoplanin	2	14	2	4	1	3	5	21
Linezolid	-	-	0	6	0	4	0	10

Azithromycin	-	0	6	-	0	6	
Erythromycin	-	0	6	1	3	60	124

3.4. Distribution of MDR and Non MDR

A UTI was identified in 151 of the 350 cervical cancer patients, or 43.14% of the total. Only 146 isolates, or 41.7% (n=146) of the total infected bacterial uropathogens, underwent antibiotic susceptibility testing since 5 of them were *Candida* spp. *Salmonella* spp. accounted for 75% (n=3) of MDR cases, with *Pseudomonas aeruginosa* accounting for 100% (n=14) of MDR cases. The respective MDRs for *Pseudomonas aeruginosa* and *Staphylococcus aureus* were 66.67% (n=12) and 56.25% (n=9). MDR 53.70% (n=29) *E. Coli* isolates were the most prevalent, followed by *Klebsiella* spp. Of the 11 isolates, 42.30% were MDR. The CONS of the gram-positive isolates were 50% MDR (n=2). *Enterococcus* accounted for 33.33% of the MDR isolates (n=2), while *Citrobacter* spp. isolates were 100% Non-MDR.

Table 4 Distribution of MDR and Non MDR

Bacteria	MDR (%)	Non MDR (%)	Total (%)
<i>Escherichia coli</i>	29 (53.70)	25 (46.30)	54 (100)
<i>Klebsiella</i> spp.	11 (42.30)	15 (57.70)	26 (100)
<i>Pseudomonas aeruginosa</i>	12 (66.67)	6 (33.33)	18 (100)
<i>Staphylococcus aureus</i>	9 (56.25)	7 (43.75)	16 (100)
<i>Proteus</i> spp.	14 (100)	0	14 (100)
<i>Enterococcus</i> spp.	2 (33.33)	4 (66.67)	6 (100)
<i>Citrobacter</i> spp.	0	4 (100)	4 (100)
CONS	2 (50)	2 (50)	4 (100)
<i>Salmomella</i> spp.	3 (75)	1 (25)	4 (100)
Total	82(56.12)	64(43.88)	146 (100)

4. Discussion

The study was cross-sectional and quantitative in nature, conducted at a hospital BPKMCH. The samples were gathered and processed at BPKMCH's Microbiology Laboratory in Bharatpur, Chitwan, Nepal. The samples were taken from cervical cancer patients who visited the outpatient department or were admitted to the hospital. In our investigation, out of 350 processed samples, 86.9% (n=304) showed no growth, whereas 13.1% (n=46) showed growth. In this study, the total frequency of UTIs among patients with cervical cancer was 13.1%. *Escherichia coli* has the largest preponderance, accounting for 41.3%, followed by the gram-positive bacteria *Staphylococcus aureus* (19.6%), and *Proteus* (4.3%).

In our investigation, *E. coli* was the most prevalent uropathogen. Similar outcomes were reported by Prasad et al. Prasad et al. (1995) discovered that cervical cancer may have an effect on UTI, with a higher percentage (33.3%) of infections in patients with stage III cervical cancer compared to patients with stage II disease (16.7% UTI) in patients with stage I B cervical cancer, four with stage III B cervical cancer, and one with stage IV cervical cancer (15). *E. coli* was the most commonly identified causal agent. The second most prevalent species was *E. faecalis* strains. One of the 11 isolates of *E. coli* was resistant to five drugs, another to three, and three to two. *E. coli* strains were grouped into nine sensitivity patterns, whereas *E. faecalis* strains were divided into four. UTI was found in four persons. According to Pradhan et al.'s study in Kathmandu, the most prevalent pathogenic bacterium recovered was *E.coli* (79.1%), followed by *Klebsiella* (11.7%), *Citrobacter* (3.34%), and *Proteus* (2.92%) (16).

In this study 13.1% samples were found culture positive from 350 urine sample whereas the study conducted in Addis Ababa, Ethiopia showed eighteen (6.3%) were culture positive cases out of 292 urine samples tested. The most common isolated uropathogen was *Escherichia coli* (44.4%), followed by *Klebsiella pneumoniae* (22.2%) and *Citrobacter diversus* (16.7%) (17). Shrestha et al. study done in cancer patients who had requested urine culture observed bacterial growth

in 73 (24%) in B.P Koirala Memorial Cancer Hospital, Bharatpur, Nepal between July 2018 to June 2019. Which is higher than findings of the present study (18). Bacteria isolated from urine culture samples of cancer patients in B.P Koirala Memorial Cancer Hospital, Bharatpur, Nepal by Shresth et al. reported that the most common organisms isolated were *E. coli* (58%), *Staphylococcus* (11%) and *Klebsiella* (10%). Which is almost similar to the findings of this study (18).

In this study vancomycin, teioplanin for Gram positive cocci and polymyxin B and imipenem for Gram negative bacilli were found 100% sensitive whereas Millena R.S. et al. study reported same sensitivity with Polymyxin B and only 62.6 % for Imipenem. In this study, the susceptibility of Gram-negative bacteria to imipenem was found 100% whereas the Murugesan et al. study reported only 75% Gram negative bacteria were sensitive to carbapenems (19). This variation may be due to the sample size, study subjects and area of study. Here ampicillin and nitrofurantoin were found least sensitive among other antibiotics which is unlike to the findings reported by Addis Ababa, Ethiopia, shows nitrofurantoin were the most effective antibiotics for *K. pneumoniae* isolates. However, 75% of *K. pneumoniae* isolates were resistant for Trimethoprim-Sulfamethoxazole and ampicillin (20).

Our study is the first of its kind to describe bacterial growth and antibiotic resistance patterns in cervical cancer patients with UTI in Nepal. Out of the 350 patients with urine culture results, 13.1% had bacterial growth, most commonly *E. coli*. Of the 19 samples having bacterial isolates, we observed high levels of resistance to Levofloxacin, Nitrofurantoin, Ampicillin, of antibiotics. My study showed MDR 21.7% of the culture positive samples.

Previous studies from other countries have shown that growth of bacteria among urine samples from cancer patients suspected of UTI ranged from 6% in a hospital in Ethiopia to as high as 72% in a hospital in Egypt (21). Within Nepal, the bacterial growth in urine samples from general patients ranges from 14% to 32% in those who have undergone urine bacterial cultures (22,23). Lastly, due to the irregular pattern of antibiotic testing, the prevalence of resistance to various antibiotics could not be ascertained in this study. This points to the need for optimal and rational use of antibiotics in cancer patients to prevent antibiotic resistance, as well as improvement of quality of antibiotic resistance testing. Currently, no guidance exists on symptoms indicating urine sample culture in cancer patients and antibiotics which require drug susceptibility testing in the cancer hospital.

5. Conclusion

Out of 350 samples, only 46 showed growth, with common organisms being *E. Coli*, *Staphylococcus aureus*, *Klebsilla SPP*, *CONS*, *Pseudomonas aeruginosa*, and *Proteus SPP*. Most patients were 40-60 years old. Nine out of 46 isolated cases were MDR. Vancomycin was the most effective against *CONS* and *Staphylococcus aureus*, followed by levofloxacin, cefixime, and nitrofurantoin. Tecoplanin, imipenem, and polymyxin B were 100% effective against every isolate. Antimicrobial susceptibility testing should be performed before prescribing antibiotics.

Compliance with ethical standards

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The research has complied with all the relevant national regulations and institutional policies and has been approved by the Institutional Research Committee (IRC) of BP Koirala Memorial Cancer Hospital (letter of approval Registration No: (61/2022).

Statement of informed consent

Written informed consent was taken from all the participants involved in the study.

Availability of Data

The datasets of the current study will be available from the corresponding author upon reasonable request.

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