

Bacterial profiling and antibiotic-resistant pattern among cancer patients

Abstract

The study aimed to assess the bacterial profile and antibiotic resistance pattern among cancer patients attending B.P Koirala Memorial Cancer Hospital, Bharatpur, Chitwan. Hospital-based cross-sectional studies were carried out on 384 samples collected from cancer patients and were processed. After the growth of the organism on different media, Grams staining was done for primary identification and later identified by biochemical characteristics and was processed for antibiotic susceptibility by Kirby-Bauer method of disk diffusion and was reported sensitive or resistant by measuring zone of inhibition following the CLSI guideline (2020). The collected data were analyzed using SPSS V20.0.

Out of 384 participants, 55.4% were male and 44.6% were female. Growth was found in 43.5% of specimens. Hematogenous cancer growth was 40.7% while 45.5% of growth was among non-hematogenous cancer patients. *Escherichia coli* were reported 38.9%, *Klebsiella spp.* 20.4%. *Pseudomonas spp.* 19.2%, *Citrobacter spp.* with 9.0%, and *Acinetobacter spp.* with 4.8%. Like-wise *Staphylococcus aureus* and *Enterobacter aerogenes* contributed 3.6% and 3.0% respectively. *Proteus spp* and CoNS were 0.6% each. *E.coli* was found to be highly resistant to Amoxycillin (98.5%), followed by Ciprofloxacin (73.9%) and then Cotrimoxazole (67.7%) while low resistance was found to Amikacin. *Staphylococcus aureus* was the most prevalent Gram-positive found to be 100% resistant to Amoxycillin, Ciprofloxacin, Cloxacillin, and Cephalexin i.e; 66.7% each and fully sensitive to Amikacin and Tigecycline. Gram-negative bacteria were more frequently isolated than gram-positive bacteria. Many opportunistic pathogens including MDR strains may readily infect patients with cancer.

Keywords: Antibiotic resistance, Antimicrobial stewardship, Bacterial profile, Cancer patients

Introduction

Cancer is a disease that starts with the uncontrolled proliferation of abnormal cells beyond their usual limit to invade adjoining parts and spread to other parts of the body. In 2018, 9.6 million deaths were due to cancer, which is the second leading death cause globally. Lung cancer, colorectal, prostate, and liver cancer are the most common types of cancer in men while breast, colorectal, lung, cervical, and thyroid cancer are most common among women.^[1] They showed that a rapid increase in incidence was among the 45-49 age groups in males and 30-34 age groups in females, the highest incidence rate was between age groups 70-74 years and 65-69 age groups in females. By 2020 the incidence rate of cancer per 100,000 is estimated to be 41.4 in females and 38.5 in males.^[2]

Cancer patients are immunocompromised because of the nature of the disease itself and also due to intervention in the form of

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chemotherapy.^[3] Despite these advances; cancer patients are prone to serious infection complications with substantial morbidity and mortality. Cancer patients who have solid tumors might tend to undergo surgery to remove the tumor or sometimes due to other medical reasons.^[4] This increases the high chances of obtaining bacterial infection by normal flora or due to nosocomial infections such as air in the hospital, hospital staff, inanimate objects, and medical equipment, as a result of their frequent contact. Therefore, patients with both types of cancer (solid tumors and hematological malignancies) are highly susceptible to almost any type of bacterial infection. Among Gram-positive cocci (GPC) genus *Staphylococcus* and from Gram-negative bacilli (GNB): *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are frequently associated.^[5, 6]

The pattern of bacteria causing infections and their antibiogram vary widely from one country to another as well as from one

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hospital to another and even among the intensive care unit in one hospital.^[7] Therefore, if the clinician has adequate information on the spectrum of microorganisms and the AMR patterns prevalent in that particular setting, appropriate empiric antibiotic therapy can be started.^[8]

Frequent hospitalizations, exposure to invasive procedures, use of broad-spectrum antibiotics, and chemotherapy have been indicated as the most important risk factors for infections in cancer patients.^[9, 10]

Therefore, this study was designed to assess the bacterial profile and antibiotic resistance pattern among cancer patients attending B.P Koirala Memorial Cancer Hospital, Bharatpur, Chitwan.

Materials and Methods

A quantitative hospital-based cross-sectional study was carried out from December 2020 to May 2021 on 384 cancer patients who were OPD or admitted to the hospitals at B.P Koirala Memorial Cancer Hospital, Bharatpur, Chitwan, Nepal. Samples were collected and processed in the Department of Microbiology at B.P Koirala Memorial Cancer Hospital, Bharatpur, Chitwan, Nepal. Patients under antibiotic therapy were excluded except for cancer therapy. The protocol was approved by PURC, Pokhara University (Ref. no.: 56/077/078). Oral and written consent was taken from the participants and the objective of the study was explained. Sociodemographic characteristics like age, sex, educational level, Occupational status, Marital Status, Ethnicity, and Religion were collected by questionnaires.

All the samples were collected from standard guidelines by the American Society of Microbiology (ASM).^[11] Various samples like blood, urine, sputum, pus, ulcer material and skin specimens were collected by standard guidelines and were transported to the microbiology department. The samples were processed by following standard guidelines. Then isolates were distinguished in the laboratory for various organisms using techniques like catalase, coagulase, oxidase, Sugar utilization (TSI), motility, indole and H₂S production in SIM media, and citrate utilization, and urea hydrolysis, in addition to Gram's stain. The isolated colonies were tested for antimicrobial susceptibility on Mueller Hinton agar (MHA) and the outcome was recorded.

The antimicrobial susceptibility of isolated organisms from the clinical specimen was done on Mueller Hinton Agar (MHA) (Hi-media, India) by standard disk diffusion technique of modified Kirby-Bauer method as per CLSI recommendation. After mixing, the suspension was adjusted to match the 0.5 McFarland's turbidity standard (1.5x10⁸ bacterial load per ml). Bacterial isolates were inoculated in

the agar plates and 5 disks of different antimicrobials were placed no less than 24 mm apart, center to center, and slightly pressed. Then the plates were incubated aerobically at 37°C overnight.^[12, 13]

The zone of inhibition (ZOI) of each disk was measured (including the diameter of the disk) and recorded in millimeters and the results were compared with the Standard chart developed by Kirby-Bauer to determine the bacterial susceptibility in terms of 'sensitive', 'resistant', and 'intermediate'.¹² In-house control strain resembling *S. aureus* ATCC 25923 was also tested, in every set of experiments, in parallel, as a part of quality control.¹² In this study, the organisms resistant to at least three or more groups of first-line antimicrobials are considered to be resistant.^[14]

Descriptive analysis was done by calculating frequencies and percentages. Different parametric and non-parametric tests were based on data scale. Data were presented with a 95% confidence interval (CI) and p values of < 0.05 were considered statistically significant.

Results and Discussion

Out of the total 384 samples processed, pus constituted 155 (40.4%), followed by urine 148 (38.5%), blood 56 (14.6%), and sputum 25 (6.5%). The total number of participants was 384, out of which 213 were males and 171 were females. Both the males and females in the age group 41-60 were the highest in number. Out of the total 384 samples processed, 57% (n=217) reported no growth, while 43% (n=167) had growth respectively. *Escherichia coli* was reported highest of all contributing 65 (38.9%) of total growth followed by *Klebsiella* spp. 34 (20.4%). With *Pseudomonas*, it was 19.2%, *Citrobacter* spp. with 9.0%, and *Acinetobacter* spp. with 4.8%. Like-wise *Staphylococcus aureus* and *Enterobacter aerogenes* contributed 3.6% and 3.0% of the total sample processed respectively. *Proteus* spp and CoNS are the least; 0.6 %.

Among the total isolates, Gram-positive bacteria were 4.2% (n=7) while gram-negative bacteria were 95.8% (n=160). Among the different samples processed; 31.6%, 70.9%, 87.5%, and 56.0% of pus, urine, blood, and sputum respectively had no growth as shown in **Table 1**. Among the two categories of cancer, Hematogenous and Non-hematogenous cancer, 59.2% and 54.5% had no growth respectively as shown in **Table 2**. Relationship between organism and socio-demographic variables like age, gender, ethnicity, religion, and marital status as shown in **Table 3**. Out of total 167 isolates, 65.3% (n=109) were multi-drug resistant while 34.7% (n=58) were non-MDR. The distribution of MDR and Non-MDR organisms is shown in **Table 4**.

Table 1. Sample-wise distribution of isolates

Isolation	Sample type							
	Pus		Urine		Blood		Sputum	
	N	%	N	%	N	%	N	%
<i>Escherichia coli</i>	36	34.0	24	55.8	2	28.6	3	27.3
<i>Klebsiella spp.</i>	18	17.0	8	18.6	3	43.0	5	45.4
<i>Pseudomonas spp.</i>	26	24.5	3	7.0	0	0.0	3	27.3
<i>Citrobacter spp.</i>	11	10.4	3	7.0	1	14.2	0	0.0
<i>Acinetobacter spp.</i>	3	2.9	5	11.6	0	0.0	0	0.0
<i>Staphylococcus aureus</i>	5	4.7	0	0.0	1	14.2	0	0.0
<i>Enterobacter aerogenes</i>	5	4.7	0	0.0	0	0.0	0	0.0
<i>Proteus spp.</i>	1	0.9	0	0.0	0	0.0	0	0.0
CoNS	1	0.9	0	0.0	0	0.0	0	0.0
Total	106		43		7		11	

Table 2. Distribution of organisms in different cancer

Organisms isolated	Type of cancer				p-value
	Hematogenous Cancer		Non-hematogenous Cancer		
	N	%	N	%	
<i>Escherichia coli</i>	33	50.0	32	31.7	0.218
<i>Klebsiella spp</i>	12	18.2	22	21.8	
<i>Pseudomonas spp</i>	12	18.2	20	19.8	
<i>Citrobacter spp</i>	4	6.1	11	10.9	
<i>Acinetobacter spp</i>	1	1.5	7	6.9	
<i>Staphylococcus aureus</i>	1	1.5	5	5.0	
<i>Enterobacter aerogenes</i>	2	3.0	3	2.9	
<i>Proteus spp</i>	0	0	1	1.0	
CoNS	1	1.5	0	0	
Total	66		101		

Table 3. Relationship between organisms and socio-demographic variables (Age, gender, ethnicity, religion, and marital status)

Independent variable	Organism isolated								
	<i>E. coli</i>	<i>Klebsiella spp</i>	<i>Pseudomonas spp</i>	<i>Citrobacter spp</i>	<i>Proteus spp</i>	<i>Acinetobacter spp</i>	CoNS	<i>Enterobacter spp</i>	<i>S. aureus</i>
Age	≤20	7 (35.0%)	8 (40.0%)	4 (20.0%)	1 (5.0%)	0	0	0	0
	21-40	17 (47.2%)	16 (16.7%)	5 (13.9%)	2 (5.5%)	1 (2.8%)	1 (2.8%)	1 (2.8%)	0
	41-60	30 (40.5%)	11 (14.9%)	12 (16.2%)	9 (12.1%)	0	6 (8.1%)	0	4 (5.4%)

	≥61	11 (29.7%)	9 (24.3%)	11 (29.7%)	3 (8.1%)	0	1 (2.7%)	0	1 (2.7%)	1 (2.7%)
Gender	Male	43 (42.1%)	15 (14.7%)	22 (21.6%)	15 (14.7%)	0	3 (2.9%)	0	1 (0.9%)	3 (2.9%)
	Female	22 (33.8%)	19 (29.2%)	10 (15.3%)	0	1 (1.5%)	5 (7.7%)	1 (1.5%)	4 (6.1%)	3 (4.6%)
Ethnicity	Brahmin	29 (41.4%)	14 (20.0%)	16 (22.8%)	2 (2.8%)	0	5 (7.1%)	1 (1.4%)	2 (2.8%)	1 (1.4%)
	Chhetri	13 (43.3%)	5 (16.7%)	6 (20.0%)	3 (10.0%)	0	2 (6.7%)	0	0	1 (3.3%)
	Janajati	11 (28.2%)	12 (30.8%)	5 (12.8%)	6 (15.3%)	1 (2.6%)	1 (2.6%)	0	2 (5.1%)	1 (2.6%)
	Dalit	9 (40.9%)	2 (9.0%)	5 (22.7%)	3 (13.6%)	0	0	0	1 (4.5%)	2 (9.0%)
Religion	Others	3 (50.0%)	1 (16.7%)	0	1 (16.7%)	0	0	0	0	1 (16.7%)
	Hindu	60 (39.2%)	30 (19.6%)	32 (20.9%)	11 (7.1%)	1 (0.6%)	8 (5.2%)	1 (0.6%)	5 (3.3%)	5 (3.3%)
	Muslim	3 (50.0%)	1 (16.7%)	0	1 (16.7%)	0	0	0	0	1 (16.7%)
Marital status	Christian	2 (25.0%)	3 (37.5%)	0	3 (37.5%)	0	0	0	0	0
	N/A	3 (33.3%)	3 (33.3%)	2 (22.2%)	1 (11.1%)	0	0	0	0	0
	Married	58 (40.2%)	24 (16.7%)	27 (18.7%)	14 (9.7%)	1 (0.7%)	8 (5.5%)	1 (0.7%)	5 (3.4%)	6 (4.1%)
	Un-married	4 (28.5%)	7 (50.0%)	3 (21.4%)	0	0	0	0	0	0

Table 4. Distribution of MDR and Non-MDR organisms

Organisms isolated	Multi-Drug Resistant			
	Non-MDR		MDR	
	N	%	N	%
<i>Escherichia coli</i>	13	20.0	52	80.0
<i>Klebsiella spp</i>	13	38.2	21	61.8
<i>Pseudomonas spp</i>	12	37.5	20	62.5
<i>Citrobacter spp</i>	11	73.3	4	26.7
<i>Proteus spp</i>	0	0	1	100
<i>Acinetobacter spp</i>	4	50.0	4	50.0
<i>CoNS</i>	1	100	0	0
<i>Enterobacter aerogenes</i>	3	60.0	2	40.0
<i>Staphylococcus.aureus</i>	1	16.7	5	83.3

The resistant pattern of *E. coli* isolates was amoxicillin with the highest 98.5% followed by ciprofloxacin at 73.9%. Among the drugs, isolates were resistant to cotrimoxazole 67.7%, levofloxacin 50.8%, gentamicin 44.7%, piperacillin-tazobactam 32.4%, tigecycline 18.5% and amikacin 17.0% as shown in **Figure 1**.

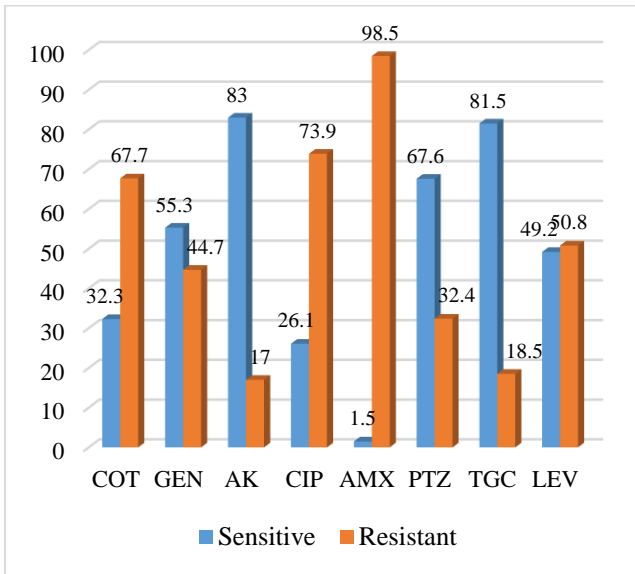


Figure 1. Antibiotic pattern of *E. coli*

The resistant pattern of *Klebsiella* spp. isolates from different samples were obtained as; amoxicillin 100.0%, cotrimoxazole 70.6%, gentamicin 55.9%, ciprofloxacin 47.1%, amikacin 35.3%, piperacillin-tazobactam 32.4%, levofloxacin 29.5%, and tigecycline 14.8%. The resistant pattern of *Pseudomonas* spp. isolates from different samples were obtained as; cotrimoxazole 100.0%, amoxicillin 93.8%, gentamicin 37.5%, tigecycline 34.4 %, ciprofloxacin 25.0%, levofloxacin 18.8%, amikacin 15.7%, and piperacillin-tazobactam 12.5%.

The resistant pattern of *Staphylococcus aureus* isolates from different samples were obtained as; amoxicillin 100.0%, cephalexin 66.7 %, cloxacillin 66.7%, ciprofloxacin 66.7%, cotrimoxazole 50.0% gentamicin 50.0%, levofloxacin 16.7%, amikacin 0.0%, and tigecyclin 0.0% as shown in **Figure 2**.

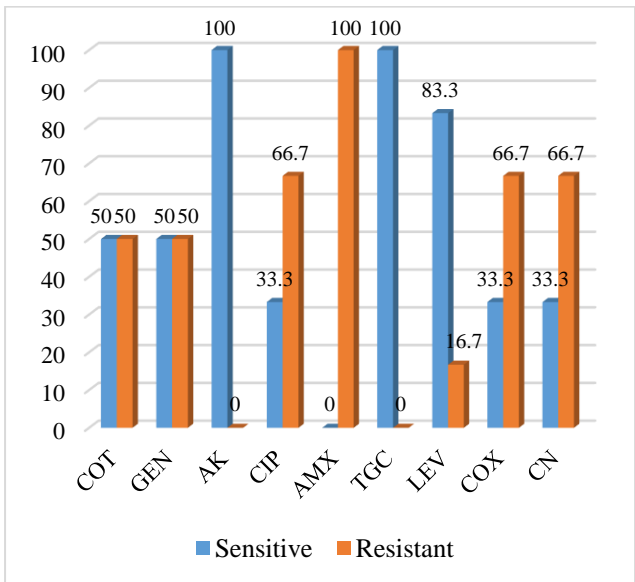


Figure 2. Antibiotic patterns of *Staphylococcus aureus*

A quantitative type hospital-based cross-sectional study was conducted from December 2020 to May 2021 at BPKMCH in order to assess the bacterial profile and AST pattern among isolates in Cancer patients.

In this study, out of a total of 384 samples processed, 57% (n=217) reported no growth, while 43% (n=167) had growth respectively. The overall prevalence of bacterial infection among cancer patients was 43.0%. This finding was comparable with the study conducted by Nurain *et al.* in Sudan at 48.1% [15] and Almaziny in Iraq mean of 44.2%. [16] However, this finding was higher as compared to other studies conducted by Fentie *et al.* in Ethiopia at 19.4% [5] and Eslami Nejad *et al.* in Iran at 24.6%. [17] This variation in prevalence may be due to the differences in geographical location and differences in the study population and the use of different treatment procedures.

In this study among the isolates, 4.2% were gram-positive organisms while 95.8% were gram-negative organisms. GNB was found to be a predominant isolate from cancer patients as similar findings were observed in many studies. [7, 15, 18-21] But in a study conducted by Fentie *et al.* in Ethiopia, GPC was found to be the predominant isolate. [5] In cancer patients, the pattern of infections has shifted from Gram-positive organisms to Gram-negative organisms in recent years. [22, 23] This may be due to the infrequent use of indwelling catheters, less cytotoxic agents for chemotherapy, and decreased use of antibiotic prophylaxis.

The prevalence of various organisms in this study was *E. coli* 38.9%, *Klebsiella* spp 20.4%, *Pseudomonas* spp 19.2%, and *Citrobacter* spp 9.0%. Similar findings were reported in a study done by Sime *et al.* in Ethiopia. [24] Among the gram-negative isolates, *E. coli* 40.6% was the predominant organism followed by *Klebsiella* 21.3% in this study. This was in contrast to Garg *et al.* a study conducted in India, where the predominant organism was *Klebsiella* 34.78% with a low incidence of *E. coli* 18.84%. [8] Nurain *et al.* reported *Proteus* spp. 23.5% as the main microorganism recovered from cancer patients 15 but in this study *Proteus* spp. 0.6% is found to have the least growth among all the isolates. This may be due to differences in antibiotic prescription policies.

Similarly, among the gram-positive isolates, *S. aureus* 85.7% was most commonly isolated, followed by CoNS 14.3%. This finding is slightly higher than the study conducted by Naznzen *et al.*; where *S. aureus* was reported at 65.38% among gram-positive isolates. (7) This finding was rather different from the prevalence rate in a study done by Kumar *et al.* [25]

In this study, *E. coli* 34.0% reported the highest growth in the pus sample which is in contrast to many studies where *E. coli* was reported low 13%, [26] 8.3%, [27] 16.5% [28] and 14.0%. [29] Similarly, in the urine sample, the predominant isolate observed was *E. coli* 55.8 % in this study which is similar to a study done by Shrestha *et al.* where *E. coli* was reported at 58.0% in urine samples. [30] But in a study conducted by Fentie

et al. in Ethiopia, the percentage of isolated *E.coli* in the urine samples was 28.1%^[5] which is lower than in this study. In blood culture, the highest growth observed was of *Klebsiella* spp. 43.0% in this study but in a study conducted by Kapoor *et al.* in North India, although the predominant organism in bloodstream infection was found to be *Klebsiella* spp. the percentage is slightly higher 50.0%^[20] as compared to ours.

In this study, the cancer patients with non-hematological malignancies were more likely to be culture-positive than hematological malignancies. This is similar to a study done by Sirkhazi *et al.*, where infections in solid tumor patients 53.77% are higher as compared to patients with hematological malignancies 46.23%.^[31] But in a study done by Al. Mulla *et al.*, infections in solid tumor patients 31.3% is lower as compared to leukemic patients 47.8%.^[32] These differences may be due to undergoing surgical treatment and catheterization.

In this study, the rate of MDR isolates was 65.3%. These results were significantly lower compared to a study done by Fazeli *et al.* where the estimated rate of MDR isolates was 91.5%.^[33] Also, this finding is higher as compared to the studies done by Fentie *et al.* with an MDR rate of 46.5%,^[5] Jiang *et al.* with an MDR rate of 27.6%,^[34] and Cornejo-Juarez *et al.* with an MDR rate of 39.5%.^[35] The frequency of occurrence of MDR-resistant organisms is variable according to the country and continent.^[36]

The resistance in gram-negative organisms could be due to various mechanisms like ESBL production, carbapenemase production, and Metallo-beta-lactamases. For GPC the antibiotic resistance mechanisms could be the development of Methicillin resistance and iMLSb among staphylococcal and enterococcal isolates.

Conclusion

Overall the burdens of bacterial infections among cancer patients are higher in those who are at chemotherapy. Patients with both types of cancer (hematogenous and non-hematogenous) had the highest reported growth of *Escherichia coli* 50.7% and 31.7 % The most prevalent Gram-negative bacteria i.e; *E.coli* was found to be highly resistant to Amoxicillin 98.5%, followed by Ciprofloxacin 73.9% and then Cotrimoxazole 67.7% while low resistance was found to Amikacin. Likewise, the most prevalent Gram-positive bacteria i.e; *Staphylococcus aureus* was found to be 100% resistant to Amoxicillin, followed by Ciprofloxacin, Cloxacillin, and Cephalixin i.e; 66.7% each and fully sensitive to Amikacin and Tigecycline.

The MDR and possible AMR mechanisms (like Methicillin resistance, Extended Spectrum β -lactamases production, inducible clindamycin resistance, Metallo β -lactamases, etc.) should be screened in all samples received for detection of infection by such organisms. Continuous monitoring of the MDR and such AMR should be done in order to prevent infection and transmission.

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Conflict of interest

None.

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None.

Ethics statement

Ethical approval was obtained from the PURC, Pokhara University (Ref. no.: 56/077/078). All participants consented to participate in the survey and provided informed consent.

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