Research Article

Biofilm formation and antimicrobial resistance of *Enterobacteriaceae* isolated from cancer patients with urinary tract infections.

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Abstract

Introduction: The rise of multi-drug resistant (MDR) uropathogenic *Enterobacteriaceae* in cancer patients is of significant concern, and biofilm formation further increases the risk of MDR infections. This study was designed to determine the association between MDR and biofilm formation in coliforms isolated from cancer patients with urinary tract infections (UTIs).

Methods: The study was conducted over 50 consecutive days, using coliform isolates obtained from cancer patients with UTI. Bacteria were identified using routine biochemical tests. Antibiotic susceptibility data were obtained from laboratory records. Relevant clinical data of the patients were collected from inpatient clinical records (BHTs). Biofilm formation was assessed by performing tube method (TM) and tissue culture plate (TCP) method.

Results: A total of 67 coliform isolates were included in the study. The majority of the isolates were *Escherichia coli* followed by *Klebsiella pneumoniae*. 64.2% of the coliforms were MDR. Of the isolates, 62.7% and 67.2% were identified as biofilm formers according to the TM and TCP methods respectively. Biofilm formation was significantly higher in coliforms isolated from cancer patients with urinary catheters (20/24, 83.3%) and those with diabetes mellitus (28/36, 77.8%). *Klebsiella* sp. had a significantly high ($p<0.05$) ability (21/26, 80.8%) to form biofilms. Of the biofilm formers, 64.4 % (29/45) were identified as MDR bacteria.

Conclusion: *E. coli* and *Klebsiella pneumoniae* were the predominant uropathogens. A significant association between biofilm formation and multi-drug resistance was not demonstrated in this study ($p>0.05$). Urinary catheterisation, type of *Enterobacteriaceae* species and diabetes mellitus were significantly associated with biofilm formation ($p<0.05$).

Keywords: *Biofilm, antimicrobial resistance, UTI, cancer patients, Enterobacteriaceae*

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Introduction

Cancer patients are at a high risk of developing infections due to immunodeficiency induced by their treatments.[1] Managing urinary tract infections (UTIs) in cancer patients is a public health priority due to the high morbidity and mortality and their rapid onset.[2] An Indian study showed that UTIs are the third most prevalent type of infection among cancer patients.[3]

UTIs have been linked to a wide variety of bacteria, the most common of which are *Enterobacteriaceae.*[2] *E. coli, Klebsiella* sp. and *Citrobacter* sp. are the most frequently isolated uropathogens from cancer patients with UTI[1] followed by *Proteus, Pseudomonas* and Gram-positive cocci.[4]

Despite the widespread availability of antibiotics, UTIs continue to be a global health concern.[4] Cancer patients often require prolonged and repeated antibiotic treatment for infections and are more likely to develop infections due to antibiotic-resistant bacteria.[1] This results in high healthcare costs due to the use of broad-spectrum antibiotics, prolonged hospital stays that lead to other hospital-acquired infections, and an increased mortality rate.[1] Management of UTI has become complicated and challenging due to the emergence of multi-drug resistant uropathogenic coliforms.[5]

In establishing an infection, pathogen virulence factors are crucial in helping microbes successfully infect the host. A key virulence factor among bacteria is their ability to form biofilms. Biofilms promote persistence in the urinary tract by protecting bacteria from being flushed out by hydrodynamic forces and from the lethal activities of host immunity and antibiotics.[6] Antimicrobial agents are inherently less effective against microorganisms developing in biofilms than planktonic cells.[7] High antibiotic use for the treatment of such infections consequently promotes the emergence of multidrug-resistant bacteria.[8]

Due to the scarcity of information on biofilm formation among uropathogenic isolates in cancer patients, finding out the impact of biofilm formation is crucial for understanding pathogenesis and developing future therapeutic strategies in the face of increasing antimicrobial resistance. This study was conducted to determine the antibiotic susceptibility pattern and biofilm forming capacity of uropathogenic *Enterobacteriaceae* isolated from cancer patients with UTIs.

Methods

This study was conducted at Apeksha Hospital, Maharagama which is the main tertiary care cancer institute in Sri Lanka. The study included adult cancer patients (>18 years) who had a positive urine culture and were diagnosed with a UTI. Patients admitted to medical and surgical wards, as well as those from outpatient clinics (OPD) were selected. Uropathogenic *Enterobacteriaceae* isolates were obtained from the microbiology laboratory of the hospital and were sub-cultured on blood agar for species identification and to study biofilm formation. Antibiotic susceptibility of each isolate was obtained from the laboratory data. Relevant clinical data of the patients were collected from inpatient clinical records (BHTs) using a data extraction sheet.

The study commenced on May 5th, 2022. Over a consecutive period of 50 days, a total of 186 positive urinary cultures were received by the laboratory. Among them, 83 were positive for *Enterobacteriaceae* and 67 of these isolates met the inclusion criteria for this study.

Bacterial identification
Each isolate was given a unique identification number. Species identification was performed using biochemical tests according to the Manual for the Identification of Medical Bacteria.[9] The identification of organisms up to species level was done based on identification charts in the Laboratory Manual in Microbiology[10] and Textbook of Diagnostic Microbiology.[11] API 20E was used for isolates that could not be speciated using routine biochemical tests.

Antibiotic susceptibility tests
Antimicrobial susceptibility of the coliforms was obtained from laboratory records. ABST according to the disk diffusion method as per the guidelines of the Clinical Laboratory Standards Institute (CLSI) was used by the laboratory.[12] Multi-drug resistance (MDR) was defined as 'resistant to three or more antimicrobial classes'.[13]

Determination of biofilm formation
Isolates were tested for biofilm-forming ability using the tube method and tissue culture plate method.

Tube method (TM):
Enterobacteriaceae isolates from 24-hour cultures on blood agar were incubated in 5ml brain heart infusion (BHI) broth with 2% sucrose. 0.1% crystal violet stain was used, and the experiment was performed in triplicate.[14] Positive biofilm formation was defined as the presence of a visible film lining the wall and bottom of the tube. A tube with sterile BHI broth with 2% sucrose was used as the negative control and a known biofilm former, *Pseudomonas aeruginosa* ATCC 27853 was used as the positive control.[15] The biofilm formation was rated as none/weak/moderate/strong.[16]

Tissue culture plate method (TCP method):
BHI broth containing 2% sucrose was used to inoculate the isolates. Wells with sterile BHI broth containing 2% sucrose were used as the negative control and a known biofilm former, *Pseudomonas aeruginosa* ATCC 27853 was used as the positive control.[15] The method was adapted from Dilrukshi GN *et al*.[14] and Swagatika PP *et al*.[17] Each strain was tested in triplicate and the final solution's absorbance was measured at 550 nm.[18]

Data analysis
Data was entered into an excel sheet and data analysis was performed by SPSS software (version 25.0). To detect associations between variables and to determine significance (p-values), Chi-square and Fisher's exact tests were conducted. Conclusions were made at 5% significance.

Results

During the study period, 67 coliform isolates were obtained from positive urine cultures in cancer patients, 50.7% (n=34) of whom were females. The age range of the study population was 20 to 86 years. Of these patients, 53 (79.2%) had solid organ cancers, while others (n=14, 20.8%) had haematological cancers. All patients with haematological cancers were inpatients, while 14 patients with solid organ cancers were outpatients (from the OPD).

Enterobacteriaceae species isolated from urine specimens of cancer patients are shown in Table 1.

Table 1: *Enterobacteriaceae* species isolated from urine specimens of cancer patients

Organism	Frequency	%
Escherichia coli	26	38.8
Klebsiella pneumoniae	15	22.4
Klebsiella sp.	11	16.4
Citrobacter freundii	4	6
Citrobacter sp.	4	6
Enterobacter sp.	3	4.5
Morganella morganii	2	3
Providencia sp.	2	3
Total	**67**	**100**

Antibiotic susceptibility pattern of the isolated bacteria

Antibiotic resistant rates of the *Enterobacteriaceae* isolates are shown in Table 2. Of the 67 isolates, 43 (64.2%) were MDR, while *Klebsiella* sp. accounted for the highest proportion of MDR isolates (20/67, 29.9%).

Table 2: Antibiotic resistant rates of the isolated bacteria

Antibiotic	*Klebsiella* sp. (n=26)	*E coli* (n=26)	Others (n=15)
	R (%)	R (%)	R (%)
Amikacin	23.1	15.4	33.3
Amoxicillin-Clavulanate	73.1	42.3	60.0
Ampicillin	92.3	73.1	86.7
Cefepime	57.7	46.2	46.7
Cefotaxime	61.5	53.8	53.3
Cefuroxime	69.2	53.8	66.7
Ciprofloxacin	88.5	57.7	86.7
Gentamicin	46.2	23.1	40.0
Imipenem	30.8	30.8	40.0
Nalidixic acid	84.6	65.4	66.7
Nitrofurantoin	80.8	23.1	40.0
MDR	**76.9**	**61.5**	**46.7**

R – Resistant

Assessment of biofilm formation

Biofilm formation among urinary coliforms according to the TM and TCP method are shown in Table 3.

Table 3: Proportion of biofilm forming *Enterobacteriaceae* using the TM and TCP methods

Biofilm Formation	Tube method (TM)		Tissue culture plate method (TCP)	
	Frequency	%	Frequency	%
Non-biofilm producer	25	37.3	22	32.8
Weak biofilm producer	21	31.3	17	25.4
Moderate biofilm producer	11	16.4	22	32.8
Strong biofilm producer	10	14.9	6	8.9
Total no. of biofilm producers	**42**	**62.7**	**45**	**67.2**

Association between patient factors with isolation of MDR coliforms and biofilm forming coliforms in UTIs.

Selected demographic and clinical characteristics of cancer patients with isolation of MDR coliforms in UTIs are shown in Table 4.

Table 4: Selected demographic and clinical characteristics of cancer patients with UTI and isolation of MDR coliforms in urine culture

Characteristics	MDR(n=43,64%)		Non MDR(n=24,35.8%)		P-value
		%		%	
Gender					
Male	23	53.5	11(%)	45.8	0.547
Female	20	46.5	13(%)	54.2	
Age					
<60 years	23	53.5	15(%)	62.5	0.475
>60 Years	20	46.5	9(%)	37.5	
Hospitalised/Clinic					
Hospitalised	37	86	16	66.7	0.061
Clinic	6	14	8	33.3	
Type of malignancy					
Solid	33	76.7	20	83.3	0.520
Hematological	10	23.3	4	16.7	
Treatment type					
Chemotherapy only	18	41.9	11	45.8	0.809
Radiotherapy only	6	14.0	5	20.8	
Both	10	23.2	4	16.7	
Not under therapy	9	20.9	4	16.7	
Infection type					
Hospital acquired	22	51.2	16	66.7	0.210
Community acquired	21	48.8	8	33.3	
Previous exposure of antibiotics within past 14 days					
Yes	32	74.4	10	41.7	**0.007***
No	11	25.6	14	58.3	
# Urinary catheterisation					
Yes	18	42.9	6	28.6	0 .271
No	24	57.1	15	71.4	
Diabetes mellitus					
Yes	24	55.8	12	50.0	0.064
No	19	44.2	12	50.0	

*-Significant P-value, #- Total sample size was 63 due to missing data

Selected demographic and clinical characteristics of cancer patients with isolation of biofilm forming coliforms in UTIs are shown in Table 5.

Table 5: Selected demographic and clinical characteristics of cancer patients with UTI and isolation of biofilm forming coliforms in urine culture

Characteristics	Biofilm forming(n=45,67.2%)		Non-biofilm forming(n=22,32.8%)		P value
		%		%	
Age					
<60 Years	25	55.6	13	59.1	0.783
>60 Years	20	44.4	9	40.9	
Gender					
Male	23	51.1	10	45.5	0.664
Female	22	48.9	12	54.5	
Infection type					
Community acquired	21	46.7	17	77.3	**0.018***
Hospital acquired	24	53.3	5	22.7	
Hospitalised/Clinic					
Hospitalised patients	37	82.2	16	72.7	0.369
Clinic patients	8	17.8	6	27.3	
Type of malignancy					
Solid	35	77.8	18	81.9	0.702
Haematological	10	22.2	4	18.1	
Treatment type					
Chemotherapy only	16	35.6	13	59.1	0.303
Radiotherapy only	9	20.0	2	9.1	
Both	10	22.2	4	18.2	
Not under therapy	10	22.2	3	13.6	
MDR					
Yes	29	64.4	14	63.6	0.280
No	16	35.6	8	36.4	
Isolated organism					
E. coli	12	26.7	14	63.7	**0.014***
Klebsiella sp.	21	46.6	5	22.7	
Others	12	26.7	3	13.6	
#Urinary catheterisation					
Yes-(24/63)	20	46.5	4	20	**0.044***
No-(39/63)	23	53.5	16	80	
##Days of catheterisation					
<6 days-(16/24)	15	93.8	1	6.2	0.091**
>6 days-(8/24)	5	62.5	3	37.5	
Diabetes mellitus					
Yes	8	62.2	8	36.4	**0.046***
No	17	37.8	14	63.6	

*-Significant P-value,**- P value from fisher exact test #- Total sample size was 63 due to missing data,##-Total samples according to catheterised patients.

The majority of organisms isolated from cancer patients with hospital-acquired UTIs (24/45, 53.3%) were biofilm-formers. Among these biofilm-forming isolates, a significantly higher proportion ($p<0.05$) was *Klebsiella* sp. (46.6%) followed by *E. coli* (26.7%) and other *Enterobacteriaceae* species (26.7%). Among the 24 catheterised patients, 83.3% were found to have infections with biofilm-forming coliforms ($p<0.05$). Furthermore, 77.8% (28/36) of coliforms isolated from cancer patients with diabetes mellitus were biofilm-formers ($p<0.05$).

DISCUSSION

The present study included cancer patients at Apeksha Hospital, Sri Lanka who had UTIs caused by *Enterobacteriaceae* (coliforms). This study showed that *E. coli* and *Klebsiella pneumoniae* were the most frequently isolated coliforms, which is consistent with previous studies.[1,2]

According to the results of the present study, the lowest resistance rate for all isolates was seen with amikacin. Chathuranga *et al.* have observed a similar high susceptibility rate for amikacin.[19] Thana *et al.* have reported higher % sensitivity to amikacin in coliforms isolated from non-cancer patients as well.[20] The majority of urinary coliforms isolated in the present study were MDR (62.7%), highlighting the increased vulnerability of cancer patients to infections with MDR organisms. In a study conducted by Sime *et al.* on UTIs among cancer patients, 33.3% of the isolated organisms were MDR.[2] However, we speculate that their relatively small sample size (n=18) might have influenced this comparatively low result. In contrast to cancer patients, another study reported a lower prevalence of MDR infections among non-cancer patients (19%).[20] This disparity raises concerns about the limited treatment options available for cancer patients with MDR infections.

A significant proportion of patients from whom MDR coliforms were isolated had a documented history of antibiotic exposure within the preceding 14 days ($p<0.05$, Table 4). This observation is consistent with findings from multiple studies[20,21], which indicate that prior antibiotic exposure is a major factor in the isolation of MDR organisms.

The presence of a urinary catheter was found to be an independent risk factor for MDR bacteraemia in cancer patients (Gudiol *et al*.,2011) .[21] However, we did not observe a significant association between catheterisation and MDR infections. We believe this discrepancy could be due to our inclusion of both inpatients and outpatients in the study, while Gudiol *et al*.,2011 included only hospitalised patients over a longer period.

We evaluated biofilm formation using both the tube method (TM) and the tissue culture plate method (TCP). Our findings showed comparable results between the two methods with minor deviations. According to Panda *et al*., (2016), the TCP method is the most sensitive biofilm detection assay and can be considered the gold standard for detecting biofilms.[17] Hassan *et al*. noted that the TM method yielded false negative results compared with the TCP method.[15] Using the TCP method, we detected 45(67.2%) biofilm-forming isolates, while the TM method identified 42(62.7%) biofilm-producing isolates. Due to observer variability, the TM method is not recommended as a universal screening test for identifying biofilm-producing isolates.[15] Therefore; we utilised the results from the TCP method to analyse associations with clinical parameters.

We observed a significant association between the type of coliform species and biofilm formation, corroborating the findings of Gunardi *et al*., (2021).[22] Notably, *Klebsiella* sp. was significantly associated with biofilm formation in our study ($p<0.05$, Table 5). This highlights the importance for clinicians to be vigilant when *Klebsiella* sp. are identified in urine cultures, as biofilm formation can complicate treatment and lead to persistent infections.[22]

The ability of bacteria to form biofilms can lead to increased resistance to antibiotics and complicate the clinical management of these infections. Many studies have found a significant correlation between biofilm formation and increased antibiotic resistance in bacteria causing

infections in non-cancer patients.[23] However, our study did not observe a significant association between these variables. Some researchers have also concluded that the relationship between antibiotic resistance and biofilm formation is not significant.[24] One possible explanation for our observation could be the notable difference in antimicrobial resistance between patients with or without cancer. Over the years, the bacterial and antimicrobial spectra in cancer patients have shifted towards more resistant strains[19] suggesting that cancer patients might exhibit different associations. Assefa M. *et al., (*2022) noted that biofilm formation and MDR prevalence could be influenced by factors such as sample size and study duration.[25] Our relatively small sample size (n=67) might have affected our findings. The majority of biofilm-formers encountered in the present study were multi-drug resistant (64.4 %), which is consistent with most previous studies.[16,22,24]

A significant proportion of cancer patients with diabetes were found to have UTIs with biofilm-forming organisms. This finding is likely attributable to the high concentration of glucose in the body fluids of diabetic patients, which may enhance the ability of bacteria to form biofilms. We also observed that the majority of coliforms isolated from catheterised patients were biofilm producers. This indicates that urinary catheters are a risk factor for biofilm development as shown previously.[22] Presence of an indwelling device enhances the chance of being contaminated by the environment, which may promote biofilm formation.[7] A significantly high proportion of coliforms isolated from patients with hospital-acquired UTIs demonstrated the ability to form biofilms. This observation aligns with the findings of Zhang *et al*.,2019.[26]

Despite the study being conducted in a single hospital, these findings reflect the association between biofilm formation and antibiotic sensitivity patterns in cancer patients in Sri Lanka, as Apeksha Hospital provides treatment to patients from across the country.

Conclusion

No significant association was found between biofilm formation and multi-drug resistance among coliforms isolated from cancer patients with UTI in the present study. Among the tested antibiotics, amikacin was the most effective antibiotic against coliforms. *Klebsiella* sp. demonstrated the highest biofilm-forming ability. Urinary catheterisation, species isolated and diabetes mellitus were significantly associated with biofilm formation in *Enterobacteriaceae* causing UTI in cancer patients. Previous exposure to antibiotics was significantly associated with the isolation of multi-drug resistant bacteria.

Declaration

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Wahalathantry DM	Methodology, Investigation, Data curation, Writing – original draft
Gunasekara SP	Conceptualization, Methodology, Resources, Project administration
Chathuranga BAG	Conceptualization, Methodology, Writing – review and editing, Visualization, Supervision, Project administration
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