

Analysis of Bacterial Biofilm Formation in Patients with Malignancy Undergoing Double J Stent Indwelling and Its Influencing Factors

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Objective: To analyze the bacterial biofilm (BF) formation in patients with malignancy undergoing double J stent indwelling and its influencing factors.

Methods: A total of 167 patients with malignant tumors who received double J stent indwelling in the hospital from January 2018 to January 2021 were included in the study. The urine and double J stent samples were collected for bacterial identification and observed for BF formation on the surface of the urinary catheter under a scanning electron microscope (SEM). Univariate and multivariate logistic regression analyses were used to analyze the influencing factors of BF.

Results: The BF formation rate was 34.73% (58/167). The BF formation rate of positive specimens cultured in urine and double J stent was significantly higher than that of negative ones ($P<0.05$). Staphylococcus was the main BF bacteria in double J stent and urine culture specimens, followed by Enterococcus, Pseudomonas, Enterobacter, and Acinetobacter. Compared with the non-BF group, the number of viable bacteria in the double J stent and urine and the catheterization time in the BF group rose markedly ($P<0.05$). Advanced age, chemotherapy, anemia, indwelling time ≥ 90 d, and urinary tract infection were risk factors for BF formation in patients with malignancy undergoing double J stent indwelling ($P<0.05$).

Conclusion: There is a high rate of BF formation in patients with malignancy undergoing double J stent indwelling, with Staphylococcus as the dominant species. Treatment requires enhanced urinary catheter management and nutritional status to inhibit BF formation and lower the rate of urinary catheter-related infections.

Keywords: malignant tumor, double J stent indwelling, biofilm, catheter-related infection, etiology, influence factor

Introduction

Double J ureteral stent, frequently used for indwelling ureter, can effectively relieve ureteral obstruction and prevent ureteral stenosis.¹ Consequently, preoperative or intraoperative indwelling double J stent is a common measure to prevent ureteral injury² as well as postoperative upper urinary tract obstruction due to surgery or metastasis of tumor in patients with malignant tumors, especially gynecological and urinary malignant tumors.³ However, its therapeutic effect is undermined by complications, such as urinary tract infection and urinary sepsis in severe cases, which threatens the life and health of patients.⁴ It has been confirmed by relevant research that bacteria biofilm (BF) formation, membrane-like substances formed by bacteria adhering to the surface, can enable bacteria to develop drug resistance against

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antimicrobial agents.⁵ As a result, it is considered that the formation of BF may be a key contributory factor to the relapse and intractability of urinary tract infections.⁶ Therefore, the analysis of BF formation in patients with malignancy undergoing double J stent indwelling and the exploration of factors affecting urinary tract infection in patients are of great significance for clinical treatment. The results are reported as follows.

Data and Methods

Clinical Data

A total of 167 patients with malignant tumors who received double J stent indwelling in the hospital from January 2018 to January 2021 were included in the study. Inclusion criteria: ① 18–80 years old; ② malignant tumors confirmed by pathology; ③ the double J stent was indwelling for the first time, with the indwelling time no less than 30 d; ④ COOK(UFH-626, USI-626-CE-B) double J stent was used; ⑤ Estimated survival time ≥ 6 months. Exclusion criteria: ① urinary tract infection existed before the double J stent indwelling; ② double J stent indwelling contradiction; ③ failure in double J stent indwelling; ④ Infection occurred due to other reasons after operation; ⑤ urological tumors such as epithelial tumors of the upper urinary tract. The study was approved by the Hospital Ethics Committee of First people's hospital of Zigong city and the patients signed an informed consent form. The study was in accordance with the Helsinki Declaration as revised in 2013 for experiments involving humans.

Methods

General Information Collection

General information, including the patients' age, gender, height, weight, highest educational level, hypertension, diabetes mellitus, hormone therapy, chemotherapy, radiotherapy, tumor clinical stage, tumor type, anemia, double J stent indwelling time, the presence of urinary tract infection, and the type of urinary tract infection was recorded. Body Mass Index (BMI) = Weight (kg) divided by the square of Height (m).

Specimen Collection

After routine disinfection, draping, and local anesthesia, 3 mL of urine was collected by a disposable sterile syringe by urethral catheterization, and the double J stent was removed under a cystoscopy or ureteroscopy. The bladder pelvis segment was divided into two segments for observation, each about 3 cm long. One segment was observed under Scanning Electron Microscope (SEM) on the BF

morphology on the surface of the double J stent. The other section of the double J tube was placed in the inoculation bottle, added with 30 mL of physiological saline, and eluted by the vortex shaker at 3000 R/min for 1 minute. Subsequently, the precipitate was inoculated into blood agar plate medium and MacConkey agar medium, and placed in a constant temperature incubator at 37°C for 16 to 24 hours. The formation of sterile colonies was observed, and the bacterial species and drug sensitivity were identified.

Observation on BF Morphology on Double J Stent Surface and Counting of Viable Bacteria Colony

In the groups of 30–60 days, 61–90 days, 91–120 days, 121–150 days, and more than 150 days, 5 specimens of double J stent were selected from each group by random number table method. The double J stent was rinsed with PBS solution three times, 10 min each. The samples were fixed with osmic acid for 1 h and rinsed with PBS solution three times, 10 min each. The dehydration was carried out in gradient with 30%, 50%, 70%, 80%, 90%, and 100% trimethyl methanol, respectively, and repeated for three times. After drying with a high-vacuum coating apparatus for 20–30 min, the specimens of double J stent were scanned by a SEM (S3000N, Hitachi, Japan) after gold spraying, and six fields of 100 μ m were randomly selected for each specimen. The formation of BF on the surface of the double J stent was observed and photographed, and the viable bacterial colonies were counted. It is defined as BF if meeting the following morphological characteristics: water channel, three-dimensional structure, spherical or elliptical corpuscle coated with matrix.

Viable Bacteria Counting and Bacterial Identification

1 mL each of clean urine and double J stent rinsing fluid were collected, and viable bacteria counts were analyzed with a UF-1000i automatic urine analyzer from SYSMEX. Urine specimens and double J stent specimens were inoculated on blood agar plates (Zhengzhou Antu Company). All the specimens were identified in accordance with the national clinical inspection operating procedures. Vitek 2 compact automatic bacteria identification drug susceptibility instrument (Mérieux Company, France) was used to identify the bacteria, and the reagents were the matching GN, GP identification cards and GN13, GP67 drug susceptibility cards.⁷

Table 1 General Data of Malignant Tumor Patients with Indwelling Double J Stent (n=167)

Item	Number of Cases	Ratio (%)
Age		
< 60 years old	78	46.71
≥60 years old	89	53.29
Gender		
Male	71	42.51
Female	96	57.49
BMI		
18.5~22.9kgm ²	57	34.13
23.0~27.4kgm ²	86	51.50
≥27.5kgm ²	24	14.37
Highest level of education		
Junior secondary and below	44	26.35
Senior high school and technical Secondary school	69	41.32
College and above	54	32.34
High blood pressure		
Yes	96	57.49
No	71	42.51
Diabetes		
Yes	65	38.90
No	102	61.10
Hormone therapy		
Yes	43	25.75
No	124	74.25
Chemotherapy		
Yes	113	67.66
No	54	32.34
Treat by radiation therapy		
Yes	56	33.53
No	111	66.47
Clinical stages		
I-II	79	47.31
III-IV	88	52.69
Malignant tumor type		
Digestive system	44	26.35
Urinary system	62	37.13
Gynecology	38	22.75
Others	23	13.77
Anaemia		
Yes	63	37.72
No	104	62.28
Stenting duration		
30~60d	21	12.57

(Continued)

Table 1 (Continued).

Item	Number of Cases	Ratio (%)
61~90d	48	28.74
91~120d	56	33.53
121~150d	28	16.77
≥151d	14	8.38

Diagnosis Criteria⁸

The occurrence of urinary infection within 48 h after indwelling or removal of double J stent was considered as urinary infection associated with double J stent. Urinary infection was diagnosed with urine bacterial culture containing Gram-positive cocci ≥ 104 CFU/mL and Gram-negative bacilli ≥ 105 CFU/mL.

Statistical Methods

SPSS20.0 software was used for statistical analysis. Measurement data conforming to normal distribution were expressed as ($\bar{X} \pm s$). Independent sample *t*-test was used for comparison between groups, paired sample *t*-test for intra-group comparison, one-way analysis of variance for comparison among groups, and Sink-q test for pairwise comparison. The enumeration data were expressed by frequency or ratio and analyzed using the Karman non-correction method, with the total cases no less than 40 and the minimum theoretical frequency over 5. Logistic regression was used to analyze the risk factors. $P < 0.05$ indicated that the difference was statistically significant.

Results

General Information About Double J Stent Indwelling Patients with Malignant Tumors

Patients' general information is presented in Table 1.

Formation of BF of Urine and Double J Stent Specimens

The BF formation rate in 167 malignant tumor patients with double J stent indwelling was 34.73% (58/167). Positive specimens cultured in urine and double J stent showed a remarkably higher formation rate of BF than those negative specimens ($P < 0.05$). See Table 2.

Table 2 Formation of Urinary Stent BF in Double J Stent Indwelling Patients with Malignant Tumors

Item	Result	Number of Cases	BF Formation		χ^2	P
			Number of Cases	Formation Rate (%)		
Urine culture	Positive	45	11	24.4	12.01	<0.001
	Negative	122	5	4.09		
Double J stent culture	Positive	78	58	74.36	13.22	<0.001
	Negative	89	3	3.37		

The Changes of Double J Tube Electron Microscope Monitoring of Patients in Each Group

BF formation is observed on various surfaces. After 30 days, the bacteria on the surface of the double J stent were few and scattered, and covered by a large number of fibrous membranes. Inconsistent amounts of inflammatory attachments and crystals were observed on the surface of the double J stent (Figure 1A). At 90 days, piles of colonies on the surface of the double J stent were observed, and most were cocci (Figure 1B). The number of bacterial colonies in the ducts was (1.73 \pm 0.59), (2.26 \pm 1.19), (4.68 \pm 1.31), (5.71 \pm 1.06), (7.76 \pm 1.37) CFU/cm² in each group, respectively (P < 0.05). It shows that bacterial colonies, inflammatory attachments, crystals, and bacterial biofilm on the surface of the double J stent increase with the extend of the double J tube indwelling time.

Bacterial Identification of BF in Double J Stent of Patients

A total of 58 strains of biofilm-positive bacteria were screened out from 78 positive specimens cultured in a double J stent. The results showed that *Staphylococcus* was the main type of bacteria in double J stent, followed by *Enterococcus*, *Pseudomonas*, *Enterobacter*, and *Acinetobacter*. See Table 3.

Bacterial Identification of BF in the Urine of Patients

Eleven strains of biofilm-positive bacteria were screened out from 45 positive urine-cultured specimens. After identifying the bacterial genus, it was found that the biofilm bacteria in the urine were mainly *Staphylococcus*, followed by *Enterococcus*, *Pseudomonas*, and *Enterobacter*, *Acinetobacter*, see Table 4.

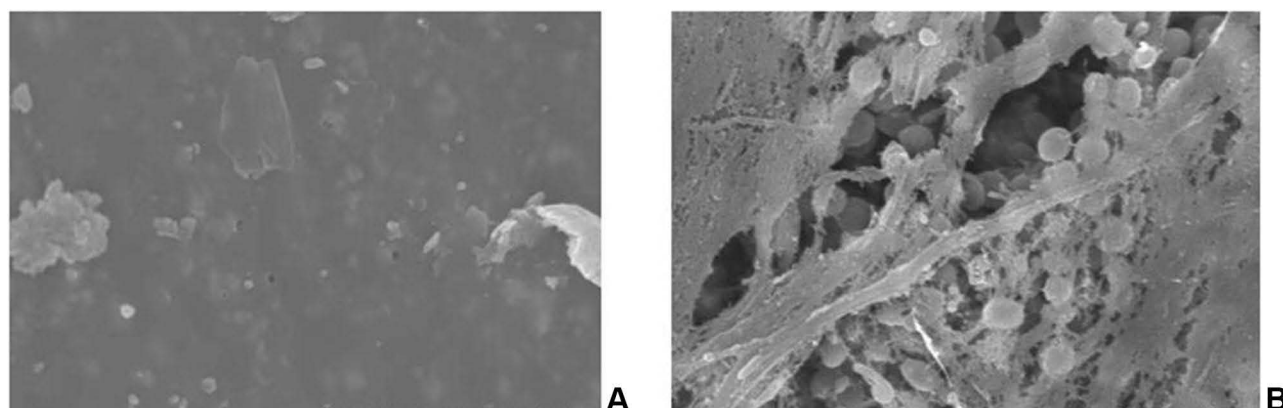


Figure 1 BF morphology on the surface of double J (SEM, \times 600).

Notes: (A) no BF formation. SEM shows normal grooves and depressions, no adhesions on the surface; (B) BF formation. SEM shows the presence of polymorphism in the inner wall of the ureter, partly with single or multiple bacterial adhesions, and visible stereospecific structure formation.

Table 3 Bacterial Identification of BF in Double J Stent of Patients

Strain	Bacterial Name	Number of Plants	Ratio (%)
Enterobacter hormaeche and edwards Enterococcus	Escherichia coli	6	10.34
	Enterococcus faecalis	14	24.14
	Uroenterococcus	5	8.62
Pseudomonas Staphylococcus	Pseudomonas aeruginosa	5	8.62
	Staphylococcus epidermidis	11	18.97
	Staphylococcus haemolyticus	7	12.07
	Staphylococcus aureus	6	10.34
Acinetobacter	Acinetobacter baumannii	4	6.90
Total		58	100.00

Table 4 Bacterial Identification of Urine BF in Patients

Strain	Bacterial Name	Number of Plants	Ratio
Enterobacter hormaeche and edwards Enterococcus	Escherichia coli	6	15.38
	Enterococcus faecalis	16	41.03
	Uroenterococcus	1	2.56
Pseudomonas Staphylococcus	Pseudomonas aeruginosa	1	2.56
	Staphylococcus epidermidis	6	15.38
	Staphylococcus haemolyticus	4	10.26
	Staphylococcus aureus	3	7.69
Acinetobacter	Acinetobacter baumannii	2	5.13
Total		39	100.00

Comparison of Viable Counts of Bacteria - In Double J Stent and Urine of Patients in the BF-Forming Group and Non-BF-Forming Group

The viable count of bacteria in the double J stent and urine of patients of the BF-forming group exceeded that of the non-BF-forming group ($P < 0.05$). See [Table 5](#).

Table 5 Comparison of Viable Counts of BF in Double J Stent and in Urine of Patients Between the BF Forming Group and the Non BF Forming Group (Cells/ μ L, \bar{X} s)

Group	Number of Cases	Double J Stent	Urine
BF forming group	58	1042.54 ± 253.46	836.48 ± 214.32
Non BF forming group	109	234.28 ± 248.14	112.36 ± 123.61
t		19.893	27.701
P		<0.001	<0.001

Comparison of Double J Stent Indwelling Time Between Groups with and without BF Formation

A markedly longer double J stent indwelling time was recorded with patients in the BF-forming group in comparison with the non-BF-forming group ($P < 0.05$). See [Table 6](#).

Table 6 Comparison of Double J Stent Time of Patients in the BF Forming Group and Non BF Forming Group (d, \bar{X} s)

Group	Number of Cases	Stent Setting Time
BF forming group	58	18.93 \pm 2.48
Non BF forming group	109	8.14 \pm 2.31
t		28.011
P		<0.001

Table 7 Comparison of Viable Counts of Biofilm in Double J Stent and Urine of Patients with Different Double J Stent Duration (Cells/ μ L, \bar{X} s)

Tube Setting Time	Number of Cases	Double J Stent	Urine
30~60d	21	349.61 \pm 187.43	138.49 \pm 84.36
61~90d	48	512.43 \pm 195.82	298.69 \pm 80.41
91~120d	56	739.48 \pm 198.84	412.43 \pm 78.95
121~150d	28	993.16 \pm 201.15	647.15 \pm 81.47
\geq 151d	14	1134.14 \pm 214.36	834.18 \pm 85.64
F		59.767	236.852
P		<0.001	<0.001

Comparison of Viable Counts Of Bacteria in Double J Stent and Urine of Patients with Different Double J Stent Indwelling Time

As the stenting duration increased, the viable count of bacteria in the double J stent and urine grew significantly ($P < 0.05$). See [Table 7](#).

Analysis of the Influencing Factors BF Formation in Malignant Tumor Patients with Double J Stent Indwelling

Patients with an age of ≥ 60 years, female, diabetes, hormonal therapy, chemotherapy, anemia, long duration of tube indwelling, and urinary tract infection had significantly increased rates of ureteral BF formation ($P < 0.05$). See [Table 8](#).

Logistic Regression Analysis for Multiple Factors of Urinary Catheter BF Formation in Malignant Tumor Patients with Double J Stent Indwelling

Advanced age, chemotherapy, anemia, indwelling time ≥ 90 d, and urinary tract infection were risk factors for BF formation in patients with malignancy undergoing double J stent indwelling ($P < 0.05$). See [Table 9](#).

Discussion

Indwelling double J stent is a significant step to prevent upper urinary tract obstruction after malignant tumor surgery to achieve better quality of life.⁹ Relevant evidence suggests that the rate of catheter-associated urinary tract infections remains at a high level in recent years, which requires sufficient attention.¹⁰ The application of double J stent mainly plays a role in fixedly supporting the ureter, relieving the obstruction caused by ureteral edema and inflammation, and preventing postoperative anastomotic leakage and ureteral stenosis,¹¹ which ensures a shorter bed rest and a better rehabilitation effect with no limitation and discomfort from the external drainage stent.¹² However, it has been found that the presence of double J stent predisposes to urinary reflux in the ureter, resulting in a rise in the retrograde infection rate of the kidney.¹³ Zhou Honghui et al¹⁴ reported that the rate of urinary tract infection in patients with double J stent indwelling for early radical cervical cancer was about 22.95%. The rate of BF formation in this study (34.73%) was slightly higher than that reported by others¹⁴ and was related to the inclusion of gastrointestinal and urinary malignancies in this study. The high molecular materials such as polyurethane used in the double J stent facilitate the adherence of bacteria to its surface and the subsequent formation of BF. Prior research demonstrated that the formation of bacterial BF can increase its resistance by more than 1000 times¹⁵ and inhibit its antigenic expression to generate immune escape.¹⁶ In addition, under appropriate conditions, the bacteria in BF can diffuse and free from the membrane, causing re-infection.¹⁷

In this study, urine and double J stent specimens were collected from patients, respectively, and SEM observation revealed that BF formation was found in 39 of 45 positive urine culture specimens, which was consistent with the results of previous studies, and BF formation was found in 13 other negative urine culture specimens, which may be related to the fact that urine bacteria are mostly planktonic, thereby leading to false-negative results; however, the influence of native bacteria on the results was not excluded, which requires further analysis.¹⁸ Moreover, BF formation was found in 58 of 78 positive double J stent culture specimens, and no BF formation was found in negative double J stent culture specimens. These results suggest that the rate of BF formation is higher in patients with malignancy undergoing double J stent indwelling, and double J stent culture more

Table 8 Analysis of Urinary Catheter BF Formation in Malignant Tumor Patients with Double J Stent Indwelling

Item	Number of Cases	BF Forming Group (n=58)	Non BF Forming Group (n=109)	χ^2	P
Age				18.184	<0.001
< 60 years old	78	14	64		
≥60 years old	89	44	45		
Gender				6.340	0.012
Male	71	17	54		
Female	96	41	55		
BMI				5.154	0.076
18.5~22.9kgm ²	57	16	41		
23.0~27.4kgm ²	86	29	57		
≥27.5kgm ²	24	13	11		
Highest level of education				0.372	0.830
Junior secondary and below	44	16	28		
Senior high school and technical secondary school	69	25	44		
College and above	54	17	37		
High blood pressure				0.297	0.586
Yes	96	35	61		
No	71	23	48		
Diabetes				21.120	<0.001
Yes	65	38	27		
No	102	21	81		
Hormone therapy				40.240	<0.001
Yes	43	32	11		
No	124	26	98		
Chemotherapy				11.488	0.001
Yes	113	49	64		
No	54	9	45		
Treat by radiation therapy				0.036	0.850
Yes	56	20	36		
No	111	38	73		
Clinical stages				0.629	0.428
I-II	79	25	54		
III-IV	88	33	55		
Malignant tumor type				1.207	0.751
Digestive system	44	16	28		
Urinary system	62	21	41		
Gynecology	38	15	23		
Others	23	6	17		
Anemia				65.413	<0.001
Yes	63	46	17		
No	104	12	92		
Stent setting time				51.817	<0.001
30~60d	21	4	17		

(Continued)

Table 8 (Continued).

Item	Number of Cases	BF Forming Group (n=58)	Non BF Forming Group (n=109)	χ^2	P
61~90d	48	5	43		
91~120d	56	16	40		
121~150d	28	21	7		
≥151d	14	12	2		
Urinary tract infection				38.498	<0.001
Yes	45	11	34		
No	122	5	117		
Type of urinary tract infection				22.474	0.215
Symptomatic	21	14	7		
Asymptomatic	24	13	11		

accurately responds to bacterial colonization and BF formation in the urinary catheter than urine culture. The results of bacterial identification showed that *Staphylococcus* was the dominant type of bacteria in the double J stent and urine culture samples, followed by *Enterococcus*, *Pseudomonas*, *Enterobacter*, and *Acinetobacter*, which was basically in line with the previous research results.^{8,14} This suggested that, compared with other types of bacteria, *Staphylococcus* might be more prone to produce biofilm bacteria, which provides a reference for the selection of clinical antibacterial drugs.

Moreover, this study found that the viable count of bacteria in both double J stent and urine rose sharply with the increase of stenting duration, indicating that the risk of BF formation grew with the increase of stenting duration, which was confirmed by the results of electron microscope scan. In clinical practice, the application of indwelling double J stent requires strict management to reduce unnecessary use. To reduce the formation of BF, Double J stent requires regular replacement or the periodical evaluation of double J stent removal in a timely manner. Analysis of factors influencing the formation of BF in

the urinary catheter in patients with malignancy undergoing double J stent indwelling found that patients with an age of ≥60 years, female, diabetes, hormonal therapy, chemotherapy, anemia, long duration of tube indwelling, and urinary tract infection had significantly increased rates of ureteral BF formation, and advanced age, chemotherapy, anemia, indwelling time ≥90 d, and urinary tract infection were risk factors for BF formation in patients with malignancy undergoing double J stent indwelling. It indicated that enhanced nutritional management for elderly patients, urethral care for female patients, and strict control of blood glucose levels in diabetic patients are warranted to reduce the rate of urinary catheter-related infections.

In conclusion, there is a high rate of BF formation in patients with malignancy undergoing double J stent indwelling, with *Staphylococcus* as the dominant species. Treatment requires enhanced urinary catheter management and nutritional status to inhibit BF formation and lower the rate of urinary catheter-related infections. The shortcoming of this study lies in the absence of characterization analysis of the isolates,¹⁹ which limits the determination of treatment protocols and will be explored in further studies.

Table 9 Multi-Factor Logistic Regression Analysis of Urinary Catheter BF Formation in Malignant Tumor Patients with Double J Stent Indwelling

Factor	β	SE	Wald	P	OR (95% CI)
Age ≥60 years old	1.123	0.487	5.364	0.012	3.236 (1.198~7.456)
Chemotherapy	1.012	0.326	4.264	<0.001	3.697 (2.342~6.397)
Anaemia	1.214	0.418	6.978	<0.001	3.523 (2.971~7.448)
Catheter time 90 d or higher	1.435	0.523	11.751	<0.001	5.044 (1.893~11.784)
Urinary tract infection	1.237	0.272	8.233	<0.001	3.912 (1.292~8.396)

Disclosure

The authors report no conflicts of interest in this work.

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