Research Article

Optimizing the Catheter Care and Maintenance Strategy of Short-Term Catheterization among Hospitalized Patients in Microbiological Approach

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Objective. To optimize the allocation of nursing resources, we investigate an alternative strategy for indwelling catheter cleaning.

Methods. The present study involved a total of 117 male patients and 54 female patients, who were catheterized after urinary surgery from Aug 2018 to Feb 2019. The samples of indwelling catheter cleaning solutions were divided by two parts for microbiological culture and microbiome analysis.

Results. No pathogenic bacteria were observed in the microbiological culture of the indwelling catheter cleaning samples from 24 h-uncleaned group and 48 h-uncleaned group. The microbiome analysis also showed no significant difference in bacterial diversity and quantity of the indwelling catheter cleaning solutions between the two groups.

Conclusion. The indwelling catheter cleaning for male after urinary surgery can be prolonged to 48 h. The result of this study provided reliable basis for optimizing the allocation of clinical nursing resources.

1. Introduction

Indwelling catheterization is one of the most common clinical diagnosis and treatment techniques [1]. But the subsequent catheter-associated urinary tract infection (CAUTI) has troubled medical staff for a long time. According to previous studies and reports, urinary tract infections currently account for 40% of nosocomial infections [2], and about 80% of them are caused by indwelling catheterization [3]. The risk factors for CAUTI include the patient’s own factors, the catheter duration time, the operation of catheter implantation, the meatal care, and the unreasonable clinical use of antibacterial drugs. Especially the duration of the catheter is the most important factor for the occurrence of CAUTI [4]. Many research reports on different populations have suggested that the short duration of catheterization is beneficial compared to the longer one [5–7]. According to reports, the risk of urethral bacterial infection each day in people undergoing catheterization is about 5%, reaching 100% within 4 weeks. The longer the catheter stays in place, the higher the risk of infection [8]. Guideline for Prevention of Catheter-Associated Urinary Tract Infections 2009 issued
by the Healthcare Infection Control Practices Advisory Committee (HICPAC) suggested "Do not clean the periurethral area with antisepsics while the catheter is in place. Routine hygiene (e.g., cleansing of the mental surface during daily bathing or showering) is appropriate (Category IB)" and "Further research is needed on optimal cleaning and storage methods for catheters used for clean intermittent catheterization" [9]. In clinical practice, catheter care is usually performed twice a day after defecation (ensure the catheter is also cleaned) or when needed. However, Centers for Disease Control and Prevention Guidelines wrote “Insufficient evidence to recommend daily or twice-daily cleansing with soap and water or povidone-iodine solution” [10]. These existing vague materials caused us to think. The cleaning fluid of urinary catheters has been changed from the use of traditional disinfectants to normal saline [11]. It is inferred from this that the large number of daily urinary catheter care work increases the workload of care, is it also redundant? The duration of indwelling urinary catheter is greater than 3 days, and the incidence of CAUTI will be greater than 20% [12]. From the perspective of the practical significance of reducing the risk of infection and the economics of nursing, is it necessary to clean the patients with indwelling short-term catheters frequently within 72 hours after surgery? This needs to be confirmed based on a precise medical method and the concept of evidence-based medicine.

Based on the above speculations, we tested and analyzed the catheter wipe liquids of 117 male and 54 female patients with indwelling short-term catheterization after urinary system surgery. The microbes around the catheter of patients with short-term indwelling catheters of different lengths of indwelling and different cleansing frequencies were compared, which provided a specific biological basis for the improvement of indwelling catheter cleansing strategies.

2. Methods and Materials

2.1. General Information. We continuously collected data on patients who were catheterized after surgical surgery from Aug 2018 to Feb 2019 in the Department of Urology, Huashan Hospital, Fudan University. Patients who did not meet all three of the following criteria were excluded: (1) indwelling catheter after surgery; (2) clear mind, normal communication; (3) agree to be involved in this study. The samples of precleaning and postcleaning were collected and classified according to the operation, the time of indwelling catheter, and the cleaning frequency (Table 1).

2.2. The Method of Indwelling Catheter Cleaning and Sampling. The method of indwelling catheter cleaning and sampling was standardized by using sterilized saline water to clean the surrounding area of urinary meatus, surface of catheter, and the area of 10 cm down to urin meatus, and the cleaning time was 30 sec. The method of cleansing is spiral scrubbing from top to bottom. Patients with each disease were divided randomly into the bis in die (BID) group and quaque die (QD) group and started to clean at 24 h and 48 h after surgery. Two q-tips for sampling were used before

| Table 1: The distribution of patients with different diseases and ages. |
|-----------------------------------------------|
| Type of disease | Number | Age (average) | |
|-----------------------------------------------|
| (a) For male |
| Calculus | 24 | 55.3 ± 14.3 |
| Prostate tumor | 23 | 68.1 ± 6.5 |
| Kidney tumor | 19 | 48.2 ± 12.7 |
| Bladder tumor | 22 | 63.7 ± 14.3 |
| Adrenal tumor | 18 | 54.0 ± 13.3 |
| Prostatic hyperplasia | 11 | 68.5 ± 5.2 |
| Total | 117 | 59.6 ± 13.4 |
| (b) For female |
| Calculus | 10 | 56.5 ± 15.5 |
| Kidney tumor | 23 | 55.4 ± 11.2 |
| Bladder tumor | 6 | 70.2 ± 14.3 |
| Adrenal tumor | 15 | 51.3 ± 11.2 |
| Total | 54 | 58.0 ± 13.05 |

2.3. Observation Index

2.3.1. Bacterial Culture. All the samples were used to perform bacterial culture, drug sensitivity test, and 16s rDNA real-time PCR. Some of the samples were performed with 16s rRNA for microbial diversity analysis.

2.3.2. 16s rDNA Real-Time PCR. The DNA copy number of bacteria in each sample was tested by 16s rDNA r-PCR. The probes are 515F'—GGACTACHVGGGTWTCTAAT and 806R,5′—GGACTACHVGGGTWTCTAAT—3′′. The experiments were performed by Slan 96P real-time PCR system (Shanghai Hongshi Medical Technology Co., Ltd) and qPCR MIX kit (RR430A, Takara) according to the manufacturer’s instructions. The amplification program was set to run at 95°C for 3 min followed by 30 cycles of 30 s of denaturation at 94°C, 20 s of annealing at 60°C, and 20 s of extension at 72°C. pMD19T bacterial 16s was used as the reference standard. The conversion formula of plasmid initial copy number: \( \text{copies/μl} = \text{concentration (ng/ml)} \times 10 - 9 \times 6.02 \times 1023 \times / \text{base pair} \times 324.5 \times 2 \).

2.3.3. Microbial Diversity Analysis. Bacterial 16s rRNA gene sequencing of catheter cleaning samples was used for microbial diversity analysis. The probes are 16S V4-V5: 515F-907R, 18S V9: 1380F-1510R, and ITS1: ITS1F-ITS2R. The allocated sequencing region was amplified into specific probe
with barcode. All the PCR products were collected by Gene-JET Gel Extraction Kit (Thermo Scientific) and quantified by QuantiFluor™-ST (Promega). The sequencing library was set by NEB Next®Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) and sequenced by Illumina MiSeq platform to generate 250 bp/300 bp pair-end reads.

Sequencing read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged. A quality filter was applied to the resulting merged reads and those containing above 0.05% expected errors were discarded. Sequences were stepwise clustered into operational taxonomic units (OTUs) to determine taxonomies. The beta-diversity analysis was performed by the `vegdist` function in R package “vegan” with the method of “bray,” to generate Bray-Curtis distance. The hierarchical clustering was performed using complete lineage method to calculate the linkage distances based on the Bray-Curtis distance between samples. Principal Co-ordinates Analysis (PCoA) was performed by the `betadisper` function in R package “vegan.”

2.4. Statistical Analysis. Statistical analysis was done using R 3.2.1. Normally distributed data were expressed in mean ± standard deviations (SD) and compared using Student’s t test; otherwise was indicated as median (Q1-Q3) and compared using the nonparametric Mann-Whitney test. P < 0.05 was considered statistically significant.

3. Result

A total of 117 male postoperative patients and 54 female postoperative patients were included in this study. The number and age of patients in each operation type is shown below (Table 1). There was no statistical difference between the routine urine examination of patients in all groups before and after cleansing (Table 2).

3.1. Detection of the Absolute Number of Bacteria

(1) The overall situation of the sample: all patients of various diseases have no significant difference before and after cleansing at 24 hours postoperatively; there is no significant difference between the samples before and after cleansing at 48 hours postoperatively (Table 3). There was no significant difference between the 24-hour and 48-hour postoperatively samples before cleansing (P = 0.776 for male; 0.174 for female).

(2) The patients’ samples after cleansing according to BID are divided into two groups: cleaned before noon (am) and after noon (pm). The results showed that no matter am or pm, there was no significant difference in the total amount of bacteria in the samples before and after cleansing at 24 hours or 48 hours postoperatively (Table 4).

(3) Compare within groups according to different disease groups: there is no difference in the samples before and after 24-hour or 48-hour cleansing within each disease group. There was no difference between the samples within the disease group (except for the male calculus group) at 24 hours or 48 hours postoperatively before cleansing. In the male calculus group, the number of bacteria in the samples before cleansing at 24 hours postoperatively was significantly higher than that before cleansing at 48 hours (P < 0.001; Table 5).

3.2. Bacterial Species Detection

(1) The results of bacterial culture were partially consistent with that of 16s rDNA fluorescence quantitative PCR detection distribution (Figures 1-3).

(2) Before and after QD cleansing for male at 24 hours postoperatively, the proportion of pathogenic bacteria consistent with the culture results decreased (Figure 1; left 1-2: Staphylococcus), and the proportion of other major types of bacteria did not change significantly.

(3) For the male group, there was no significant change in the proportion of bacteria and other major types of bacteria consistent with the culture results before and after QD cleansing at 48 hours postoperatively. And there was no significant change in the proportion of major bacterial species before and after BID cleansing at 24 hours no matter cleansing before or after noon (Figures 1, 3(a), and 3(c)).

(4) For the female group, the large variation in bacterial species before and after cleansing was mainly due to genital-associated flora rather than urinary tract infections. Therefore, the reference value of the change of bacterial species sampled in the female group remains to be discussed (Figures 2, 3(b), and 3(d)).

4. Discussion

According to the guidelines of various countries, it is recommended to clean the inlet and outlet of the urinary catheter 1-2 times a day. Therefore, we cleaned the indwelling catheters at 24 hours and 48 hours postoperatively once a day (QD) and twice a day (BID). The results suggest that although bacterial culture and microbial diversity analysis have a certain consistency in the detection of bacterial species, uncleanliness within 48 hours will not cause significant urinary tract bacterial increase, urinary tract infection, and bacteriuria. In addition, there was no significant difference in the samples before and after cleansing for all patients of each disease at 24 hours and 48 hours postoperatively and no significant difference between the samples before cleansing at 24 hours and 48 hours postoperatively, which indicated that no cleansing within 48 hours will not cause significant urinary tract infections. In order to further confirm this conclusion, we conducted a study on the time frequency. Two cleansings (forenoon and afternoon) were performed at 24 or 48 hours postoperatively, and samples were taken before and after the cleansings. There was no significant difference in the total...
Table 2: The routine urine tests of patients referred to hospital, precleaning and postcleaning catheterized after urinary surgery.

(a) For male

| Term                          | In-hospital | Statistical value | P value | Precleaning | Statistical value | P value | Postcleaning |
|-------------------------------|-------------|-------------------|---------|-------------|-------------------|---------|--------------|
| Age                           | 59.60 ± 13.40 |                  |         | 59.60 ± 13.40 |                  |         | 59.60 ± 13.40 |
| Bacterial count (µL)          | 18.8 (9.1~49.8) | 0.225            | 0.822   | 20.8 (8.65~40.25) | -0.852 | 0.396 | 20.75 (6.73~45.6) |
| Occult blood (positive %)     | 56.9        | 38.231            | ***     | 96.1        | 0                 | 0.122  | 100          |
| Tube count (µL)               | 0.38 (0.128~1.24) | -0.610           | 0.542   | 0.43 (0.12~1.26) | -1.692 | 0.092 | 0.27 (0.123~0.985) |
| Red blood cell count (µL)     | 12.3 (5.2~70.9) | -4.873           | ***     | 262.95 (44.88~972.1) | -0.441 | 0.658 | 204.15 (50~834.85) |
| White blood cell count (µL)   | 9.3 (3.7~43.2) | -3.121           | ***     | 92.55 (35.43~199.38) | -0.477 | 0.631 | 63.6 (36.15~165.5) |
| Epithelial cell count         | 3.9 (1.28~7.78) | -0.774           | 0.44    | 5.4 (1.9~11.2) | -1.373 | 0.172 | 3.7 (2.0~9.43) |
| Glucose (positive %)          | 13.89       | 11.732            | ***     | 38.83       | 0                 | 1      | 38.89        |
| Ketosone (positive %)         | 2.78        | 14.291            | ***     | 25.24       | 0.020             | 0.887  | 23.33        |
| Leukocyte lipase (positive %) | 29.17       | 50.39             | ***     | 83.50       | 3.015             | 1      | 83.33        |
| Nitrite (positive %)          | 2.78        | 1.442             | 1       | 1.94        | 0                 | 0.501  | 0            |
| pH                            | 6.03 ± 0.57 | -5.361            | ***     | 6.50 ± 0.57 | 0.622             | 0.536  | 6.56 ± 0.56 |
| Protein (positive %)          | 61.11       | 1.101             | 0.294   | 69.90       | 0.113             | 0.739  | 66.67        |
| Urine specific gravity        | 1.02 ± 0.009 | 3.368            | ***     | 1.01 ± 0.008 | -0.58             | 0.56   | 1.01 ± 0.008 |
| Turbidity (turbid %)          | 86.11       | 10.137            | **      | 64.08       | 1.050             | 0.311  | 71.11        |
| Urobilinogen (negative %)     | 0           | 0                 | 0.511   | 1.94        | 0.572             | 1      | 1.11         |
| Small round epithelial cell (negative %) | 0 | 0 | 1 | 0 | 0.004 | 0.946 | 1.10 |

Statistical values are t value for Student’s t test, χ² for chi-square test, and OR value for Fisher test; P value: **, <0.01; *** <0.001.

Data were shown by mean ± SD or M(p25 – p75).

(b) For female

| Term                          | In-hospital | Statistical value | P value | Precleaning | Statistical value | P value | Postcleaning |
|-------------------------------|-------------|-------------------|---------|-------------|-------------------|---------|--------------|
| Age                           | 58.00 ± 13.05 |                  |         | 58.00 ± 13.05 |                  |         | 58.00 ± 13.05 |
| Bacterial count (µL)          | 75.9        | (27.5~542.2)      | 1.94    | 0.058       | 31.6 (7.875~70.45) | -1.38 | 0.17 | 40.9 (9.4~90.7) |
| Occult blood (positive %)     | 56.41       | 7.54              | **      | 84.62       | 3.86              | 0.10   | 95.56        |
| Tube count (µL)               | 0.38 (0.13~1.20) | -0.19            | 0.85    | 0.395 (0.09~1.0125) | -0.76 | 0.45 | 0.5 (0.00~0.83) |
| Red blood cell count (µL)     | 15 (6.2~38.25) | -2.84            | **      | 115.10 (23.05~247.4) | -0.441 | 0.95 | 153.75 (27.85~349.2) |
| White blood cell count (µL)   | 31.4 (9.3~161.6) | -0.34            | 0.74    | 51.65 (16.825~172.975) | 0.11 | 0.91 | 75.4 (20.8~152.8) |
| Epithelial cell count         | 7.0 (3.0~26.0) | 2.39             | *       | 4.6 (2.825~7.9) | -0.57 | 0.52 | 4.6 (2.8~7.9) |
| Glucose (positive %)          | 5.13        | 0.30              | 0.18    | 15.38       | 0.36              | 0.55   | 22.22        |
| Ketosone (positive %)         | 10.26       | 0.42              | 0.52    | 17.31       | 0.38              | 0.54   | 24.44        |
| Leukocyte lipase (positive %) | 58.97       | 0.001             | 0.98    | 61.54       | 0.0071            | 0.93   | 64.44        |
| Nitrite (positive %)          | 7.69        | 19.7              | ***     | 0          | 0                 | 1      | 0            |
| pH                            | 6.12 ± 0.54 | -2.63             | *       | 6.41 ± 0.52 | 0.63              | 0.53   | 6.48 ± 0.49 |
| Protein (positive %)          | 38.46       | 0.49              | 0.48    | 48.08       | 0.098             | 0.75   | 53.33        |
| Urine specific gravity        | 1.02 ± 0.007 | 2.74             | **      | 1.01 ± 0.008 | -0.29 | 0.77 | 1.01 ± 0.008 |
| Turbidity (turbid %)          | 23.08       | 0.17              | 0.68    | 17.31       | 0.12              | 0.73   | 22.22        |
| Urobilinogen (negative %)     | 0           | 0                 | 1       | 0           | 0                 | 1      | 0            |
| Small round epithelial cell (negative %) | 0 | 0 | 1 | 0 | 0 | 1 | 0 |

Statistical values are t value for Student’s t test, χ² for chi-square test, and OR value for Fisher test; P value: **, <0.01; *** <0.001.

Data were shown by mean ± SD or M(p25 – p75).
amount of bacteria in the samples before and after cleansing, no matter forenoon and afternoon. In terms of disease types, there was no difference among the groups, which supported this conclusion. However, the calculus group was an exception, the number of bacteria in the sample of patients in this group before the 24 hours cleansing was significantly higher than that before the 48 hours cleansing. The above results are applicable to both male and female patients.

We have found in clinical practice that due to the increase in postoperative urethral secretions and the application of catheter coatings, stains on patients’ catheters will form biofilms. Some studies believe that the biofilm formed by bacterial plaque on the catheter is one of the causes of repeated or

Table 3: Comparison of the absolute number of bacteria between precleaning and postcleaning. b: before cleaning; a: after cleaning.

| Postsurgery | b vs. a |
|-------------|---------|
| 24 h        | 0.840   |
| 48 h        | 0.551   |

(a) For male

| Postsurgery | b vs. a |
|-------------|---------|
| 24 h        | 0.128   |
| 48 h        | 0.284   |

(b) For female

Table 4: Comparison of the absolute number of bacteria between precleaning and postcleaning after cleansing according to BID. 24 h: 24 h postsurgery cleaning; 48 h: 48 h postsurgery cleaning; b: before cleaning; a: after cleaning.

| Postsurgery | b vs. a-am | b vs. a-pm |
|-------------|------------|------------|
| 24 h        | 0.82       | 0.659      |
| 48 h        | 0.285      | 0.786      |

(a) For male

| Postsurgery | b vs. a-am | b vs. a-pm |
|-------------|------------|------------|
| 24 h        | 0.533      | 0.138      |
| 48 h        | 0.652      | 0.349      |

(b) For female

Table 5: Comparison of the absolute number of bacteria between precleaning and postcleaning based on different type of diseases. 24 h: 24 h postsurgery cleaning; 48 h: 48 h postsurgery cleaning; b: before cleaning; a: after cleaning; NA: no data.

| Type of disease | 24 h b vs. a | 48 h b vs. a | 24 h b vs. 48 h b |
|-----------------|--------------|--------------|------------------|
| Calculus        | 0.745        | 0.695        | 0.001*           |
| Prostate tumor  | 0.708        | 0.297        | 0.309            |
| Kidney tumor    | 0.786        | 0.314        | 0.314            |
| Bladder tumor   | 0.613        | 0.505        | 0.882            |
| Adrenal tumor   | 0.539        | 0.935        | 0.653            |
| Prostatic hyperplasia | 0.739 | 0.902 | 0.133 |

(a) For male

| Type of disease | 24 h b vs. a | 48 h b vs. a | 24 h b vs. 48 h b |
|-----------------|--------------|--------------|------------------|
| Calculus        | 0.842        | 0.549        | 1                |
| Kidney tumor    | 0.935        | 0.4          | 0.159            |
| Bladder tumor   | 0.905        | NA           | NA               |
| Adrenal tumor   | 0.014*       | 0.31         | 0.298            |

(b) For Female
| Time  | Cleaning | Bacteria                           |
|-------|----------|-----------------------------------|
| 24 h  | QD       | Acinetobacter_unclassified        |
| 24 h  | BID      | Anaerococcus_uncultured bacterium |
| 24 h  | BID      | Campylobacter_unclassified        |
| 24 h  | BID      | Corynebacterium_1_unclassified    |
| 24 h  | BID      | Corynebacterium_1_uncultured      |
| 24 h  | QD       | Enterococcus faecium              |
| 24 h  | QD       | Enterococcus_unclassified         |
| 24 h  | BID      | Escherichia-Shigella_unclassified |
| 24 h  | BID      | Ezakiella_uncultured bacterium    |
| 24 h  | BID      | Faecalibacterium_1_uncultured     |
| 24 h  | BID      | Finegoldia_uncultured bacterium   |
| 24 h  | BID      | Gardnerella_1_unclassified        |
| 24 h  | BID      | Lactobacillus_1_unclassified      |
| 24 h  | BID      | Lactobacillus_1_uncultured        |
| 24 h  | QD       | Mollicutes_RF39_1_uncultured      |
| 24 h  | BID      | Mycoplasma_unclassified           |
| 24 h  | BID      | PMMR1_1_unclassified              |
| 24 h  | BID      | Peptostreptococcus_1_uncultured   |
| 24 h  | BID      | Prevotella_1_unclassified         |
| 24 h  | BID      | Pseudomonas_1_unclassified        |
| 24 h  | BID      | Ralstonia_1_uncultured bacterium  |
| 24 h  | BID      | Ruminococcaceae_UGG-014_uncultured|
| 24 h  | BID      | Sphingomonas_1_unclassified       |
| 24 h  | BID      | Staphylococcus_1_uncultured       |
| 24 h  | BID      | Streptococcus_1_unclassified      |
| 24 h  | BID      | Ureaplasma_1_unclassified         |
| 24 h  | BID      | [Ruminococcus]_torques_group_1_unclassified|
|       |          | Other                             |

Figure 2: 16s RNA analysis of different approaches to the cleansing of indwelling catheterization for female. QD: quaquedie; B: bisindie; b: before cleaning; a: after cleaning.
Figure 3: Continued.
serious urethral infections [13–15]. If these biofilms are removed in time, it can play a positive role in the prevention of urethral infections caused by catheters. Our results suggest that the biofilm bacterial culture and microbial diversity analysis have a certain consistency in the detection of bacterial species. But results confirm that even if there are secretions in the urethra or biofilms in the catheter within 48 hours postoperatively, the culture results will not show significant infection. Therefore, as such a hidden danger, we are considering whether it is enough to wipe off the plaque on the surface of the catheter.

The evidence basis of the previous clinical nursing cleansing program mainly comes from urine examination and bacterial culture. With the development of molecular biology technology, the evidence-based medical evidence in the past catheter care guidelines has shown certain limitations. We used the microbiological diversity detection technology to obtain accurate biological evidence of catheter samples, so as to further explore and study the cleansing strategy of catheter. We used this technology to test the catheter cleaning fluid and found that whether the catheter was cleaned within 24 to 48 hours postoperatively did not affect the distribution of bacterial species and changes in the number of bacteria on the catheter, nor would it cause CAUTI. The change of bacterial species in the samples of female patients before and after cleansing was limited to the killing of long-term colonized bacteria of the reproductive tract and was not associated with urinary infection. Therefore, cleansing the catheter after 48 hours is a crucial period for short-term indwelling catheter care. In addition, our study also found that after the catheter was not cleaned 72 hours postoperatively, the total copy number of bacteria did not increase significantly. However, the proportion of positive bacteria in bacterial culture increased significantly, and the expansion of the bacterial plaque and changes in color could be observed on the appearance of the catheter, and the number of white blood cells in the urine of patients was also increased. It corroborates the conclusions of previous researchers that the incidence of urinary tract infections has increased significantly since the third day after surgery [16].

5. Conclusions

This study conducted an in-depth study on the routine nursing work of indwelling catheterization, suggesting that the cleansing care of indwelling short-term catheterization for
patients within 48 hours after urological surgery can be adjusted according to the needs, that is, to reduce the number of mental care or only wipe the secretion or biofilm. It provides a reliable reference basis for promoting the rational allocation and effective use of medical and nursing resources. From the perspective of nursing economy, the research results have greatly saved manpower costs, changed traditional concepts, or nursing strategies, and allowed nurses more time to return to serving patients. But the sample size of this study is not large enough; we will continue to collect samples in the future, expand to more patients, and use more data to verify our conclusions.

Data Availability

The original data presented in this study can be inquired from the corresponding authors.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors’ Contributions

All the authors were involved in this work. HJ and CQ conceived the idea of the study. SJ and GX designed the study. PX, QW, and WZ collected the samples. ZJ and SL analyzed the data and prepared the manuscript. SJ provided the financially supporting for this work. Xiaoqiong Peng, Wei Qian, and Jingming Zhuang contributed equally to this work.

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