Peer Review File

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Reviewer A

Comment 1: My main concern is the lack of novelty in this research as there is nothing new in concept, methodology, results or conclusions. Although the authors argue that there is no evidence on this subject in the available sources, there are many similar studies and even literature reviews which significantly limits the novelty of the present manuscript. Maybe the confusion results from a poorly updated bibliography and an insufficiently thorough literature review?

Reply 1: Thank you for your valuable review of our manuscript and for providing this comment. We have identified the two previously reported literature reviews that you mentioned. Indeed, there have been studies that investigated the microbial profile of asymptomatic bacteriuria and the colonization rate of ureteral stents. However, these studies did not make clear distinctions between bacteriuria and symptomatic UTI. Additionally, only a few studies have assessed the pathophysiology and risk factors for the development of symptomatic UTI in those patients.

Therefore, for clarity, we have added the reports of the literature reviews and modified the existing sentences detailing the previous studies in the Introduction and Discussion as shown in the changes below.

We have also updated the text in the discussion section to account for the added literature reviews: Please see the relevant changes shown below and in the revised manuscript file.

Moreover, we think that our study distinguishes itself from previous literature in that we tried to include patients requiring a retained ureteral stent with more than one replacement in our analysis.

Changes in the text: Pages 5-6, lines 83-95, "Introduction"

Most previous studies have mainly evaluated the pattern of asymptomatic bacteriuria and the colonization rate of ureteral stents in patients with these medical devices (12,14,15). However, the results reported in the literature are extremely varied. Moreover, these studies did not make clear distinctions between bacteriuria and symptomatic UTI. Additionally, only a few studies have assessed the pathophysiology and risk factors for the development of symptomatic UTI in these patients. Recently, two literature reviews have attempted to clarify the epidemiological characteristics and risk factors for UTI in patients with ureteral stents; however, they could not come to clear conclusions due to discrepancies in methodology and a low level of evidence of the included studies (16,17).

Accordingly, this study aimed to identify the microbiological profile of bacteriuria and the incidence of ESBL-producing bacteria in patients who, in particular, required retained ureteral stents and to determine the predisposing factors associated with the bacteriuria and ESBL-producing bacteria in these patients.

Pages 15-16, lines 292-295, "Discussion":

In addition, previous systematic reviews have reported that Staphylococcus, E. coli, Klebsiella species, P. aeruginosa, Enterococcus, and Candida species were the microorganisms most commonly identified in urine or ureteral stents (16,17).

Page 17, lines 328-331, "Discussion":

Additionally, recent literature reviews demonstrated that a longer duration of ureteral stenting was an associated risk factor for the occurrence of bacteriuria, although the included studies presented multiple methodological biases (16,17).

Page 20, lines 407-411, “Discussion”:

In previous literature reviews (16,17), CKD appeared to be associated with an increased colonization rate of ureteral stents without any clear evidence of an increase in the development of UTI, although the methodological quality of the included studies was inadequate to derive any clear conclusions.

References:

16. Bey E, Bouiller K, Pimpie R, et al. Recommendations of the AFU Infectious Diseases Committee on the prevention, diagnosis and treatment of infections of endo-ureteral equipment. Prog Urol 2021;31:557-75.

17. Vallee M, Bey E, Bouiller K, et al. Epidemiology and risk factors for ureteral stent-associated urinary tract infections in non-transplanted renal patients: a systematic review of the literature. World J Urol 2021;39:3845-60.

Comment 2: My second concern is the poor description of the "methodology" section. It is not clear to me if ABU was observed in urine samples collected before or after the procedure? Or maybe both?

Reply 2: We collected urine samples before the procedure. In accordance with your suggestion, we have modified the sentences.

Changes in the text: Page 3 lines 7-10, “Abstract”:

From August 2018 to January 2021, urine samples from 307 consecutive patients who required stent indwelling and had replaced ureteral stents more than once were collected before the replacement procedure and analyzed by microbiological testing.

Page 8, lines 128-129, “Methods”

Urine samples from patients systematically collected before ureteral stent replacement were examined using microbiological testing.

Comment 3: What exactly type of stent was used (double J? Single J?)?

Reply 3: Thank you for your comment. The type of stent used in our study was a double J stent. We have added this information in the text.

Changes in the text: Page 9, lines 137-138, “Methods”:

A silicone ureteral stent, double J stent type (Boston Scientific Corp., Natick, MA, USA), was used in all the patients.

Comment 4: What "conventional method" (line 148) was used and according to what "mandatory microbiological standards" (line 149-150)? Perhaps the authors would like to provide an appropriate quote and explain briefly what it means?

Reply 4: Thank you for your suggestion. In our study, the acquired urine samples were inoculated on

eosin methylene blue (EMB) agar and blood agar for cultural studies. The plates were incubated for 48 hours at 37℃. The resultant microbial growth was evaluated quantitatively (growth of $>10^5$ colony-forming units/mL was considered significant). Additionally, all microbial isolates were tested for antibiotic susceptibility. The evaluation and analysis of microbiological characteristics and antibiotic resistance patterns were conducted according to the definitions and recommendations of the National Committee for Clinical and Laboratory Standards Institute. We have added the sentences and references in the text.

Changes in the text: Pages 8-9, lines 128-136, "Methods"

Urine samples from patients systematically collected before ureteral stent replacement were examined using microbiological testing. The acquired urine samples were inoculated on eosin methylene blue (EMB) agar and blood agar for cultural studies. Plates were incubated for 48 hours at 37℃. The microorganisms that grew on the agar were evaluated quantitatively (growth of >105 colony-forming units [CFU]/mL was considered significant). Additionally, all microbial isolates were tested for antibiotic susceptibility. The evaluation and analysis of microbiological characteristics and resistance patterns were conducted according to the definitions and recommendations of the National Committee for Clinical and Laboratory Standards Institute (19,20).

References:

19. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309-32.

20. CLSI Standards and Guidelines - CLSI Standards Center. 2021.

Comment 5: The text about UTI and its treatment is also unclear. What antibiotics were used? In what doses? For how long? What tests were used?

Reply 5: The patients with clinical symptoms suggestive of UTI, including dysuria, fever, or flank pain who showed the presence of $>10^5$ colony-forming units (CFU)/mL of a bacterial species isolated from midstream clean urine were diagnosed with UTI. We modified the sentences regarding definition of UTI. Additionally, white blood cell (WBC) count, erythrocyte sedimentation rate, and C-reactive protein values were checked to clarify the disease status.

The patients with symptomatic UTI were administered antibiotics, and antibiotic selection depended on the microbiological susceptibility results. The doses and duration of each antibiotic followed clinical practice guidelines (23,24). Symptomatic UTI was considered to be successfully treated if urinary clinical symptoms disappeared and when urine culture results were negative.

We modified the sentences regarding the diagnostic criteria for symptomatic UTI and added the following sentences in the Methods section for clearer understanding.

Changes in the text: Page 9, lines 145-157, "Methods"

The patients with clinical symptoms suggestive of UTI, including dysuria, fever, or flank pain who showed the presence of $\geq 10^5$ CFU/mL of a bacterial species isolated from midstream clean urine were diagnosed with UTI (21,22). Additionally, white blood cell (WBC) count, erythrocyte sedimentation rate, and C-reactive protein values were checked to clarify the disease status.

Asymptomatic bacteriuria was defined as the presence of $\geq 10^5$ CFU/mL of one bacterial species

isolated from two consecutive urine culture samples without any clinical symptoms suggestive of UTI (21). The patients with symptomatic UTI during the study were administered appropriate antibiotics based on microbiological susceptibility results, and the dose and duration of each antibiotic followed clinical practice guidelines (23,24). Subsequently, ureteral stents were removed if the patients' conditions were stable. Symptomatic UTI was considered to be successfully treated if urinary clinical symptoms disappeared with negative urine culture results.

References:

23. Eliakim-Raz N, Yahav D, Paul M, et al. Duration of antibiotic treatment for acute pyelonephritis and septic urinary tract infection-- 7 days or less versus longer treatment: systematic review and meta-analysis of randomized controlled trials. J Antimicrob Chemother 2013;68:2183-91.

24. Sobel JD. Duration of antibiotic treatment for urinary tract infection. Curr Infect Dis Rep 2008;10:483-4.

Comment 6: Maybe the authors would like to answer a few additional questions to further support their research and make it more interesting? How does ABU affect the incidence of UTI? Is there any positive correlation? If so, is the ABU microbial profile correlated with the bacteria that cause UTIs?

Reply 6: We tried to investigate the incidence and predisposing factors of symptomatic UTI only among the patients with bacteriuria. Asymptomatic bacteriuria was defined as the presence of $\geq 10^5$ CFU/mL of one bacterial species isolated from two consecutive urine culture samples without any clinical symptoms suggestive of UTI. Therefore, the patients in the two groups did not overlap.

Regarding the results of microbiological characteristics, bacteriuria was found in 187 patients (60.9%) of all patients with a retained ureteral stent. Among patients with bacteriuria, asymptomatic bacteriuria was identified in 165 (88.2%) patients, with *Escherichia coli* being the most isolated microorganism in the latter (55), followed by *Enterococcus* (40). Additionally, *Candida, Staphylococcus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were detected in 21, 17, 13, and 5 patients, respectively.

In comparisons with these results, symptomatic UTI developed in 22 (11.8%) of the patients with bacteriuria. Among patients with symptomatic UTI, the most isolated microorganism was *E. coli* (5), followed by *Enterococcus* (four), *Candida* species (four), *P. aeruginosa* (three), and *Proteus mirabilis* (one). Out of the isolated *E. coli*, four were ESBL-producing.

The results showed that significant differences in bacterial frequency were not identified between patients with symptomatic UTI and asymptomatic bacteriuria. However, the proportion of ESBL-producing bacteria among overall *E. coli* species was higher in patients with symptomatic UTI (80%) than asymptomatic bacteriuria (71%), although it was not significant.

Changes in the text: Page 12, line 204-210, "Results"

When comparing the patients with symptomatic UTI with asymptomatic bacteriuria, asymptomatic bacteriuria was identified in 165 (88.2%), while symptomatic UTI developed in 22 (11.8%) patients with bacteriuria, respectively. No significant differences in bacterial frequency were identified between patients with symptomatic UTI and asymptomatic bacteriuria. However, the proportion of ESBL-producing bacteria among overall E. coli species was higher in patients with symptomatic UTI (80%) than asymptomatic bacteriuria (71%), although it was not significant.

Comment 7: Why there is no control group in this study? (e.g., patients after single ureteral stent

insertion). This comparison could be both useful and interesting.

Reply 7: Thank you for your comment. We tried to investigate bacterial characteristics and factors associated with bacteriuria and symptomatic UTI in patients requiring indwelling ureteral stents with more than one replacement. Therefore, we did not include a control group of patients with single ureteral stent insertion. In future studies, we plan to incorporate your suggestion and include patients with single ureteral stent insertion as a control group.

Comment 8: Is there a difference in ABU microbial profile before and after ureteral stent procedure?
Reply 8: Thank you for your comment. In this study, we collected urine samples only before the procedure of ureteral stent replacement. We modified the sentences in the text.

Changes in the text: Page 3 lines 7-10, "Abstract"

From August 2018 to January 2021, urine samples from 307 consecutive patients who required stent indwelling and had replaced ureteral stents more than once were collected before the replacement procedure and analyzed by microbiological testing.

Page 8, lines 128-129, "Methods"

Urine samples from patients systematically collected before ureteral stent replacement were examined using microbiological testing.

Comment 9: Maybe the authors would like to focus on the treatment of UTIs in patients with permanent ureteral stents? Which antibiotics are most effective? Maybe it would be beneficial to compare a groups with a retained or replaced stent?

Reply 9: Thank you for your suggestion. In the present study, we focused on patient management regarding retained ureteral stents, including periodic replacement of the stents and antibiotic treatment if symptomatic UTI developed.

The patients with symptomatic UTI were administered with antibiotics, whose selection depended on the microbiological susceptibility results.

We could not conduct a study comparing retained and replaced ureteral stents due to ethical constraints.

However, among the patients with symptomatic UTI (22), 15 had their ureteral stent replaced with new stents; subsequently, the UTI symptoms resolved, and bacteriuria was not detected in the urine samples. Meanwhile, in the remaining seven patients with symptomatic UTI, ureteral stents were left in place and although they were treated with appropriate antibiotics, UTI symptoms recurred after a few days. Therefore, the ureteral stents were replaced with new stents after the patients' condition recovered. After replacement of ureteral stents, the remaining UTI symptoms were relieved.

Considering these results, it is possible to deduce that a retained ureteral stent might decrease the effects of antibiotics in patients with symptomatic UTI.

Minor comments
Comment 10: I would advise to shorten the title

Reply 10: Thank you for your suggestion. I have modified the title for increased specificity as follows:

'Determination of microbiological characteristics and risk factors associated with bacteriuria, and symptomatic urinary tract infection in patients with retained ureteral stents: An observational study'.

Abstract
Background:
Comment 11: It looks like the first sentence is just a repeated title whereas should also include a brief rationale for carrying out this study.

Reply 11: Thank you for your suggestion. I have added a sentence to include a brief rationale for this study, as follows:

Changes in the text: Page 3, lines 3-5, "Abstract"

'The maintenance of ureteral stents is vital in patients with severe ureteral stricture or ureteral obstruction. This study aimed to identify microbiological characteristics and factors associated with bacteriuria and symptomatic urinary tract infection in these patients.'

Methods:
Comment 12: There is no explanation of the key elements of the study design. Authors should clarify whether the study is prospective or observational? Is it a cohort or case-control study? When was this study conducted?

Reply 12: Thank you for your comment. I have added the study design in the revised manuscript's text.

This study is an observational cohort study. From August 2018 to January 2021, 307 consecutive patients who required indwelling and replaced ureteral stents more than once at our institution were evaluated. Additionally, this study was conducted during August 2021. We have added these sentences to the text, as follows.

Changes in the text: Page 3, lines 7-10, "Abstract"

This study is an observational cohort study. From August 2018 to January 2021, urine samples from 307 consecutive patients who required stent indwelling and had replaced ureteral stents more than once were collected before the replacement procedure and analyzed by microbiological testing.

Page 7, line 101, "Methods"

This study is an observational cohort study. From August 2018 to January 2021, 307 consecutive patients who required indwelling and replaced ureteral stents more than once at our institution were evaluated.

Comment 13: There is no information on the type of ureteral stent.

Reply 13: Thank you for your comment. The type of ureteral stent used in our study was a double J stent. We have mentioned this information in the text.

Changes in the text: Page 9, lines 137-138, "Methods"

A silicone ureteral stent, double J stent type (Boston Scientific Corp., Natick, MA, USA), was used in all the patients.

Comment 14: I think that lines 44-48 are too detailed while there is no information on the primary and secondary endpoints of the study. My advice would be to clarify the endpoints instead.

Reply 14: Thank you for your suggestion. We modified the sentence briefly and clarified the endpoints in the text as follows 'Patient demographics, laboratory test results, and data of patient conditional status (dependent functional capacity and indwelling urethral catheter use) were collected for all patients. The primary endpoint was the incidence rate of bacteriuria and extended-spectrum beta-lactamase-producing bacteria. The secondary endpoint was the factors predisposing patients with ureteral stents to bacteriuria, extended-spectrum beta-lactamase-producing bacteria, and development of symptomatic urinary tract infections.'

Changes in the text: Page 3, lines 11-17, "Abstract"

Patient demographics, laboratory test results, and data on dependent functional capacity and indwelling urethral catheter use were collected from all patients. Additionally, ureteral stenting duration and number of previous ureteral stent replacements were reviewed. The primary endpoint was the incidence rate of bacteriuria and extended-spectrum beta-lactamase-producing bacteria. The secondary endpoint was the factors predisposing patients with ureteral stents to bacteriuria, ESBL-producing bacteria, and the development of symptomatic urinary tract infections.

Conclusions:
Comment 15: It should be shortened and concise. In this section, it would be useful to answer the question of what is the main result of the study and what it adds to the existing knowledge. I would also omit the last sentence because the authors did not study antibiotic therapy (or did not show these data and analyzes) and did not address it earlier in the abstract.

Reply 15: Thank you for your comment. We have shortened the section as per your suggestion and modified the sentences for conciseness.

Changes in the text: Page 22, lines 438-447, "Conclusions"

Infections related to ureteral stents showed a specific microorganism profile and resistance pattern compared to community-acquired UTIs. Additionally, we identified the factors associated with the occurrence of bacteriuria, ESBL-producing bacteria, and symptomatic UTI in patients with retained ureteral stents.

In our study, the possible short duration of ureteral stent placement and periodic replacement in patients with prolonged use of ureteral stents prevented the occurrence of bacteriuria, ESBL-producing bacteria, and symptomatic UTI. Considering the microbiological profile and factors associated with bacteriuria and symptomatic UTIs may be associated with better outcomes in patients with retained ureteral stents.

Main body
Introduction:
Comment 16: In general, it should be more focused on the problem of ABU and UTI. It is not necessary to list all side effects of stent placement, etc.

Reply 16: Thank you for your suggestion. We have shortened the sentences regarding complications of ureteral stent, and have mainly focused on bacteriuria and symptomatic UTI. Please see the revised manuscript text submitted along with the present re-submission.

Comment 17: Line 120: It is not true that there are no other studies on the microbial profile of ABU and UTI in patients with ureteral stents.

Reply 17: Thank you for your comment. We found that there were previous studies which investigated the microbial profile of asymptomatic bacteriuria and colonization rate of ureteral stents in patient with the said medical device. However, those studies did not make clear distinctions between bacteriuria and symptomatic UTI. Additionally, only a few studies have assessed the pathophysiology and risk factors for development of symptomatic UTI in those patients.

Therefore, for clarity, we have modified sentences regarding the previous studies in the Introduction and Discussion as follows, 'Most previous studies have mainly evaluated the pattern of asymptomatic bacteriuria and colonization rate of ureteral stents in patients with this medical device (12,14,15). However, the results in the literature are extremely varied. Moreover, these studies did not make clear distinctions between bacteriuria, and symptomatic UTI. Additionally, only a few studies have assessed the pathophysiology and risk factors for development of symptomatic UTI in these patients.'

Changes in the text: Pages 5-6, lines 83-95, "Introduction":

Most previous studies have mainly evaluated the pattern of asymptomatic bacteriuria and the colonization rate of ureteral stents in patients with these medical devices (12,14,15). However, the results reported in the literature are extremely varied. Moreover, these studies did not make clear distinctions between bacteriuria and symptomatic UTI. Additionally, only a few studies have assessed the pathophysiology and risk factors for the development of symptomatic UTI in these patients.

Methods:
Most of my concerns are outlined above.
Comment 18: Additionally, I do not understand why the laboratory tests listed in lines 142-143 were investigated. What method was used to describe GFR? Or maybe eGFR? The relevant citation should be mentioned.

Reply 18: Thank you for your advice. We tried to investigate whether the laboratory test results affected the occurrence of bacteriuria and symptomatic UTI; however, the laboratory results were not associated with the outcomes. We used the Modification of Diet in Renal Disease equation to determine the eGFR according to serum creatinine levels to quantify renal function.

We have added the sentences and associated references in the text.

Changes in the text: Page 8, lines 115-119, "Methods"

Additionally, laboratory test results, such as estimated glomerular filtration rate (eGFR), glucose level, HbA1c level, lipid profile, and data of patient conditional status, including urethral catheter use and dependent functional capacity, were also collected. We used the Modification of Diet in Renal Disease equation to determine the eGFR according to serum creatinine levels (18).

Comment 19: Lines 159-163: I wonder if the UTI was only recognized if every of the listed criteria were met? Are the flank pain and elevated erythrocyte sedimentation rate necessary for the diagnosis of a UTI episode, even if the patient has dysuria and ≥105 CFU/mL?

Reply 19: Thank you for your comment. The patients with clinical symptoms suggestive of UTI, including dysuria, fever, or flank pain who showed the presence of $>10^5$ colony-forming units

(CFU)/mL of a bacterial species isolated from midstream clean urine, were diagnosed with UTI, did not have to qualify all the listed criteria. We have modified the sentences regarding the definition of UTI.

Changes in the text: Page 9, lines 145-147, "Methods"

The patients with clinical symptoms suggestive of UTI, including dysuria, fever, or flank pain who showed the presence of ≥ 105 CFU/mL of a bacterial species isolated from midstream clean urine were diagnosed with UTI (21,22).

Comment 20: I also have some concerns about the statistics Are all data normally distributed? If so, what test was used? What is the goodness of fit of the model? Why were all the data contained in univariable analyzes used in multivariable analysis? I advise to follow the APA 6th Edition Guidelines in reporting statistics.

Reply 20: Thank you for your suggestion. All data were not normally distributed and the normal distribution of variables was checked using the Kolmogorov-Smirnov test. Goodness of fit of logistic regression models was assessed using the Hosmer and Lemeshow test. We have mentioned this in the text.

We modified the tables using the only variables, which have possibility of significance in univariate analyzes, in multivariate analysis, and followed the APA 6th Edition Guidelines in reporting statistics as you advised.

Changes in the text: Page 10, lines 163-165, "Methods"

The normal distribution of variables was checked using the Kolmogorov-Smirnov test. Additionally, goodness of fit of logistic regression models was assessed using the Hosmer and Lemeshow test.

Results:
Comment 21: Any repetition should be avoided in this section. It is not necessary to list all the irrelevant results presented in the tables. Authors should only report the most important results of their research.

Reply 21: In accordance with your suggestion, we have deleted the sentences about prevalence of the underlying disease and cause of ureteral stenting in the Results to only include relevant information.

Changes in the text: we deleted the irrelevant sentences in text.

Discussion:
Comment 22: The first paragraph should contain the most important results of the study. In addition, the entire discussion should be shortened and updated with the current state of knowledge. Also applies to bibliography.

Reply 22: Thank you for your suggestion. We have added sentences which contain only the important results of our study in the Discussion. Additionally, we shortened and updated the text with the current state of knowledge.

Changes in the text: Pages 13, lines 230-234, "Discussion"

In our study, infections related to ureteral stents showed a specific microorganism profile and resistance pattern, in which microorganisms other than E. coli, such as Enterococcus, Staphylococcus species,

Klebsiella, and Candida species, were found more frequently when compared to community-acquired UTI. Additionally, we identified the factors associated with bacteriuria and development of symptomatic UTI in patients with retained ureteral stents.

Comment 23: The figure is of poor quality, there is no description of x and y axes or units.

Reply 23: Thank you for your comment. We have improved the quality of Figure 1 and inserted the axes and units. Please see the revised figure submitted herewith.

Reviewer B

The authors performed a prospective study evaluating the urine and characteristics of patients undergoing exchange of chronic ureteral stents in an attempt to identify factors influencing the development of bacteriuria and symptomatic UTIs.

The authors should address the following:

Comment 1: Based on your definition of significance as $p<0.05$, there does not appear to be a significant association between stent duration and the development of ESBL-producers on multivariate analysis

Reply 1: Thank you for this keen observation. We have made an error in reporting the statistical significance regarding the association between ureteral stent duration and incidence of ESBL-producing bacteriuria. We have corrected the statistical significance (P=0.048) in the text.

Changes in the text: Page 11, lines 196-199

The incidence of ESBL-producing bacteria in urine cultures was associated with old age (OR 1.04; 95% CI 1.02-1.06; P<0.001) and longer duration of ureteral stenting (OR 1.02; 95% CI 1.00-1.03; P=0.048) using multivariate logistic analysis (Table 3).

Comment 2: Line 483: you recommend broad-spectrum antibiotics for patients at risk of UTI. Can you clarify this statement? As written, it appears that you are recommending this preemptively for all patients with this risk. If not, please rephrase. If so, how do you justify this in light of the concerns for the development of resistance, particularly in a population who will presumably require this repeatedly if not persistently given their ongoing stent use?

Reply 2: Thank you for your comment. The broad-spectrum antibiotics may be helpful in preventing symptomatic UTI in patients with ureteral stents, provided they are at risk for the disease. However, repeated use of broad-spectrum antibiotics might increase the resistance in patients requiring periodic replacement of ureteral stents as you have pointed out. Considering this, we have deleted the sentences about 'broad-spectrum antibiotics for patients at risk of UTI' in the text.

Comment 3: Line 483: How many of these specific patients actually developed UTIs?

Reply 3: Thank you for your comment. In our study, symptomatic UTI developed in 22 patients (7.2%)

among all patients with a retained ureteral stent.

Changes in the text: Page 11, line 200, "Results"

Additionally, symptomatic UTI developed in 22 patients (7.2%) among all patients.

Comment 4: Line 517-519- where dis you demonstrate this? Please discuss these findings. Otherwise please remove this statement

Reply 4: Thank you for your comment. In our study, a shorter duration of a ureteral stent significantly decreased the occurrence of bacteriuria, ESBL-producing bacteria, and symptomatic UTI in patients with a retained ureteral stent.

The sentences regarding these results is described in "Results" as follows,

'Using the multivariate logistic analysis, the occurrence of bacteriuria was significantly associated with old age, female sex, presence of DM, and CKD. Additionally, a lower GFR and longer duration of ureteral stenting significantly increased the incidence of bacteriuria.

The incidence of ESBL-producing bacteria in urine cultures was associated with old age (OR 1.04; 95% CI 1.02-1.06; P<0.001) and longer duration of ureteral stenting (OR 1.02; 95% CI 1.00-1.03; P=0.048) using multivariate logistic analysis (Table 3).

Additionally, a longer ureteral stenting duration correlated significantly with the development of symptomatic UTI (Table 4)'.

Therefore, the periodic replacement of ureteral stents is thought to prevent bacteriuria and development of symptomatic UTI in these patients.

Reviewer C

Title

Comment 1: The title is somewhat redundant and could be more concise.

Reply 1: Thank you for your suggestion. I modified the title for conciseness and specificity and it now is as follows:

'Determination of microbiological characteristics and risk factors associated with bacteriuria, and symptomatic urinary tract infection in patients with retained ureteral stents: An observational study'.

Changes in the text: Title

Introduction

Comment 2: Line 103 – the authors report several challenges of ESBL-producing bacteria, including ecological consequences. Perhaps "economic" or "financial" consequences is what is meant here? If ecological consequences is the intended point, please explain further. The term is used again in line 386

of the Discussion.

Reply 2: Thank you for your comment. After considering your comment, I feel 'economic' is more appropriate in this context and have modified the text accordingly.

Changes in the text: Pages 5-6, lines 66-68, "Introduction"

ESBL-producing bacteria represent a major concern, especially in the hospitalized patient population, and a costly therapeutic challenge requiring broad-spectrum antibiotics with an increased length of hospital stay with economic consequences (10).

Pages 17-18, lines 337-340

[…] the increased incidence of ESBL-producing bacteria that present resistance to most antibiotics, except the carbapenem group, is a major concern, with an increased length of stay and economic consequences, as proper antibiotic treatment is challenging (9).

Materials and Methods

Comment 3: Credit to the authors for their exclusion criteria, however, why were patients with bilateral stents excluded from the study? Bilateral obstruction is not uncommon in patients requiring long-term indwelling stents, and therefore represent a meaningful number of patients in this group. Previous data has revealed that bilateral stenting is associated with UTI development, and could further support the authors' main hypothesis. They typically do not undergo any additional operative interventions compared to patients with unilateral stents, and therefore bilateral stents should not be considered a confounder, but rather, an important factor predicting UTI development.

Reply 3: Thank you for your comment. In our study, we focused on the occurrence of bacteriuria and symptomatic UTI in patients with a retained ureteral stent. Therefore, we excluded the patients with bilateral stents from the study because those patients might have a higher probability of bacterial colonization on the ureteral stent and symptomatic UTI when compared to patients with unilateral stents, thus possibly affecting the results of the study.

However, I agree that bilateral stents could be an important factor predicting UTI development, and therefore, include patients with bilateral stents in future studies.

Comment 4: A major concern is the inclusion of patients with indwelling urethral catheters, which account for 7.5% of the cohort. Indwelling catheters are a significant independent risk factor for bacteriuria, UTI, and UTI-related sepsis. It is difficult to ascertain whether microbiologic data in these patients is related to the stents, or more likely, the urethral catheter.

Reply 4: Thank you for your comment. Indwelling urethral catheters are a significant independent risk factor for bacteriuria and UTI as you have pointed out.

However, we found that indwelling urethral catheter was not significantly associated with bacteriuria, ESBL-producing bacteria, and development of symptomatic UTI in our study.

Therefore, it could be thought that the factors, including old age, female sex, presence of DM, CKD, and longer duration of ureteral stenting, were more likely associated with bacteriuria than indwelling urethral catheters.

Additionally, the patients with indwelling urethral catheters account for 7.5% among population, while most of subjects were without indwelling urethral catheters.

However, we added the possibility of indwelling urethral catheters acting as a confounding factor in the study's limitations.

Changes in the text: Pages 21-22, lines 431-434, "Discussion"

Additionally, it is known that indwelling catheters and female sex are risk factors for bacteriuria and UTI. Therefore, these factors may be potential confounding factors, although analysis of this study was adjusted for variables, including these factors. We have also focused on other factors affecting bacteriuria and symptomatic UTIs, such as longer duration of ureteral stenting.

Comment 5: Did the authors make an effort to exclude patients with other independent risk factors for UTI development, such as other urologic conditions that contribute to urinary stasis (eg. voiding dysfunction, BPH, neurogenic or hypotonic bladder)? Given that these conditions predispose patients (even those without stents) to UTIs, there should at minimum be discussion of the rationale for inclusion.

Reply 5: Thank you for your advice. We regularly checked the voiding pattern and residual urine amount in patients with retained ureteral stents. Additionally, we excluded the patients with urinary stasis due to voiding dysfunction, BPH, neurogenic or hypotonic bladder from our study because this feature might act as confounding factor. We have added this information in the Methods.

Changes in the text: Page 7, lines 108-111, "Methods"

We excluded patients with urinary stasis developed due to various etiologies, including bladder outlet obstruction and impaired bladder function, because this condition predisposes patients to UTI. We evaluated the voiding pattern and residual urine amount regularly to identify patients with urinary stasis.

Comment 6: The authors state that urine samples were collected before and after ureteral stent exchange. The authors should be more specific. Were these collected in preoperative testing days/weeks prior or In the OR immediately prior to stent exchange? For postoperative testing was this done immediately following stent exchange or at routine follow up weeks later?

Reply 6: Thank you for your comment. Urine samples from patients were collected immediately prior to the replacement procedure of ureteral stent. We have modified the sentences in the text for more clarity.

Changes in the text: Page 3 lines 7-10, "Abstract":

From August 2018 to January 2021, urine samples from 307 consecutive patients who required stent indwelling and had replaced ureteral stents more than once were collected before the replacement procedure and analyzed by microbiological testing.

Page 8, lines 128-129, "Methods"

Urine samples from patients systematically collected before ureteral stent replacement were examined using microbiological testing.

Comment 7: For the urine culture UTI cutoff, the authors state ≥105 CFU/ml. Do they mean ≥105,000 CFU/ml? If so, this should be corrected.

Reply 7: Thank you for your comment. We considered the presence of $\geq 10^5$ CFU/ml of a bacterial species in the urine as the cutoff for bacteriuria. We have modified the sentences in the text for a clearer

understanding.

Changes in the text: Page 9, lines 145-149, "Methods"

The patients with clinical symptoms suggestive of UTI, including dysuria, fever, or flank pain who showed the presence of $\geq 10^5$ CFU/mL of a bacterial species isolated from midstream clean urine were diagnosed with UTI (21,22). Additionally, white blood cell (WBC) count, erythrocyte sedimentation rate, and C-reactive protein values were checked to clarify the disease status.

Comment 8: The authors state on line 175 that bivariate and multivariate regression analysis was performed. The abstract and tables mention univariate analysis rather than bivariate. This should be addressed.

Reply 8: Thank you for pointing this out. We performed univariate and multivariate regression analysis to identify the factors predisposing patients with ureteral stents to bacteriuria and development of symptomatic UTI. We have rectified this error in the text.

Changes in the text: Page 10, lines 165, "Methods"

Univariate and multivariate logistic regression analyses were performed to identify the factors predisposing patients with ureteral stents to bacteriuria, ESBL-producing bacteria, and the development of symptomatic UTI.

Comment 9: The authors state twice in the final paragraph of the Methods that $P < 0.05$ was considered statistically significant. One of these statements should be removed.

Reply 9: We have deleted the sentences repeated in the text.

Results

Comment 10: Is it known if any of the 22 patients with symptomatic UTI developed sepsis as a result of UTI?

Reply 10: Thank you for your comment. In our study, none of the 22 patients with symptomatic UTI developed sepsis as a result of UTI. This may be due to antibiotic treatment and replacement of ureteral stent.

Comment 11: The authors report that there were 7 patients with symptomatic UTI who did not undergo stent exchange. The authors state that "symptoms related to UTI relapsed after a few days (mean, 10 days)." Did UTI symptoms recur in all seven patients? Was urine culture obtained to support this diagnosis? The authors should be as specific as possible here. Would also caution against using colloquial terms like "a few". The authors should state "mean time to symptom recurrence was 10 days," or something similar.

Reply 11: Thank you for your suggestion. Symptoms related to UTI recurred in all seven patients who did not undergo stent replacement. Urine culture obtained in those patients showed positive results. The mean time for symptom recurrence was 10 days. We have clarified the above information in the text.

Changes in the text: Page 12, lines 215-217, "Results"

Symptoms related to UTI recurred in these patients, and the mean time to symptom recurrence was 10 days. Urine culture obtained in these patients showed positive results.

Page 20, lines 386-388, "Discussion"

However, in seven patients in whom the same ureteral stent was retained, UTI recurred, and the mean time to symptom recurrence was 10 days.

Discussion

Comment 12: The authors claim that the present study demonstrates that infections related to ureteral stents show a "specific" microorganism profile. There should be more commentary in the discussion on specifically how, if at all, the demonstrated microbiome differs from a cohort of non-stented patients with UTIs in the literature.

Reply 12: Thank you for your comment. In our study, *E. coli* was the most commonly isolated microorganism in urine (60 patients), followed by *Enterococcus* (44). Additionally, *Candida, Staphylococcus, K. pneumoniae*, and *P. aeruginosa* were detected in 25, 17, 13, and 8 patients, respectively. Our results demonstrate that bacteria other than *E. coli*, such as *Enterococcus*, *Staphylococcus* species, *Klebsiella*, and *Candida* species, were found more frequently in patients with ureteral stents when compared to those in patients with community-acquired UTI, which is consistent with previous several reports (9,19,31). The sentences have been included in the Discussion previously. Additionally, we modified the sentences as follows 'infections related to ureteral stents showed a specific microorganism profile and resistance pattern when compared to community-acquired UTIs.'

Changes in the text: Page 13, lines 230-234, "Discussion"

In our study, infections related to ureteral stents showed a specific microorganism profile and resistance pattern, in which microorganisms other than E. coli, such as Enterococcus, Staphylococcus species, Klebsiella, and Candida species, were found more frequently when compared to community-acquired UTI. Additionally, we identified the factors associated with bacteriuria and development of symptomatic UTI in patients with retained ureteral stents.

Comment 13: Bacteriuria and ESBL-producing bacteriuria were both associated with old age on multivariate analysis. We know from extensive literature that old age is associated with voiding dysfunction (bladder hypotonicity and bladder outlet obstruction (eg. BPH)), which is an independent risk factor for bacteriuria. Given that the authors did not report on the incidence of voiding dysfunction in this cohort, they should comment specifically on this as a confounding factor and limitation.

Reply 13: Thank you for your suggestion. We routinely checked the voiding pattern and residual urine amount of patients with retained ureteral stents. Additionally, we excluded the patients with urinary stasis due to voiding dysfunction, BPH, neurogenic or hypotonic bladder from our study because this feature might act as confounding factor. We have added these sentences in the Methods.

Changes in the text: Page 7, lines 108-111, "Methods"

We excluded patients with urinary stasis developed due to various etiologies, including bladder outlet obstruction and impaired bladder function, because this condition predisposes patients to UTI. We evaluated the voiding pattern and residual urine amount regularly to identify patients with urinary stasis.

Comment 14: The association between female sex and bacteriuria in the present study is another

observation that is likely subject to confounding bias. It is well known that women are at significantly increased risk of UTIs compared to men in the general population. Therefore, the significance of this finding is questionable.

Reply 14: Thank you for your comment. As you mentioned, it was known that women are at a significantly increased risk for UTIs when compared to men in the general population. In our analysis, bacteriuria was significantly increased in female population compared to male, even after the analysis was adjusted for variables. Moreover, female sex was not associated with ESBL-producing bacteria and symptomatic UTI in the multivariate analysis. We have added the possibility of female sex acting as a confounding factor as a study limitation.

Changes in the text: Pages 21-22, lines 431-434, "Discussion"

Additionally, it is known that indwelling catheters and female sex are risk factors for bacteriuria and UTI. Therefore, these factors may be potential confounding factors, although analysis of this study was adjusted for variables, including these factors. We have also focused on other factors affecting bacteriuria and symptomatic UTIs, such as longer duration of ureteral stenting.

Comment 15: In line 436, "the incidence of symptomatic UTI was 22 (7.2% n = 22)" is redundant, and "n = 22" can likely be omitted.

Reply 15: Thank you for your comment. We have deleted 'n = 22' in the text as you have mentioned.

Changes in the text: Page 19, line 381, "Discussion"

In our study, the incidence of symptomatic UTI was 22 (7.2%) among all patients.

Comment 16: The authors state that patient poor patient conditional status was more associated with the development of UTI than baseline clinical characteristics (age, sex, DM), which were significantly associated with bacteriuria. The authors should again note the limitations of the retrospective analysis and confounders here, given that logically, there is likely a relationship between bacteriuria and symptomatic UTIs.

Reply 16: As you mentioned, the retrospective design of study has led to many limitations. We think that poor patient conditional status, such as dependent functional capacity and longer duration of ureteral stent, may be more associated with symptomatic UTI when compared to other patient characteristics, such as age and sex, although those patient characteristics might also affect the development of UTI to some degree. We have modified the text accordingly in the main manuscript and added the limitation of the observational design of our study.

Changes in the text: Page 21, line 423, "Discussion"

Our study was not without limitations and had a potentially limited observational design and small sample size.

Comment 17: The authors discuss the association of CKD with symptomatic UTI development, while postulating that this difference may be in part due to more "concentrated bacteria". The authors do not stratify patients with CKD by stage. Most patients with mild-to-moderate CKD excrete a normal amount of urine. Therefore, the argument that this association may be due to less flow, or bacterial concentration, is likely flawed, unless most of the patients with CKD in this study have CKD-5/ESRD and truly

produce little-to-no urine.

Reply 17: Thank you for your comment. As you have pointed out, we stratified the patients with CKD stage by using calculated eGFR. The participants were categorized into the following 5 stages according to their baseline eGFR: stage 1, eGFR >90 mL/min/1.73 m^2; stage 2, eGFR of 60–89 mL/min/1.73 m^2; stage 3, eGFR of 30–59 mL/min/1.73 m^2; stage 4, eGFR 15-29 mL/min/1.73 m^2; and stage 5, eGFR <15 mL/min/1.73 m^2. We divided the patients stratified by eGFR into 2 groups, which consisted of group A (preserved urine amount) including CKD stage 1, 2, and 3, and group B (decreased urine amount) including CKD stage 4, and 5, due to the small number of patients with CKD stage 5. We have added the sentences in Methods and Table 1.

It is known that patients with CKD stage 4 or 5 have severely impaired renal function, which could be associated with decreased urine amount.

In the multivariate analysis, group B showed significant increased occurrence of bacteriuria and symptomatic UTI compared to group A, which supported the hypothesis that severely impaired renal function (in particular CKD state 4, and 5) may result in both concentrated bacteria and subsequent development of symptomatic UTI. We have added the sentences in the Result, Table 2, 3, 4, and Discussion.

Changes in the text: Page 8, line 118-126, "Methods"

We used the Modification of Diet in Renal Disease equation to determine the eGFR according to serum creatinine levels (18). Then, we stratified the patients with CKD stage by using calculated eGFR. The participants were categorized into the following five stages according to their baseline eGFR: stage 1, eGFR >90 mL/min/1.73 m2; stage 2, eGFR of 60–89 mL/min/1.73 m2; stage 3, eGFR of 30–59 mL/min/1.73 m2; stage 4, eGFR 15–29 mL/min/1.73 m2; and stage 5, eGFR <15 mL/min/1.73 m2. We divided the patients stratified by eGFR into 2 groups: group A (preserved urine amount group) including CKD stage 1, 2, and 3, and group B (decreased urine amount group) including CKD stage 4, and 5, due to a small number of patients with CKD stage 5.

Page 11, lines 190-191, "Results"

Moreover, the patients in group B with CKD stage 4 and 5 showed significantly increased occurrence of bacteriuria compared to those in group A.

Page 12, lines 220-223, "Results"

Dependent functional capacity and impaired renal function were significantly associated with the development of symptomatic UTI. In particular, group B patients with CKD stage 4 and 5 showed significantly increased development of symptomatic UTI compared to those in group A.

Page 20, lines 404-411, "Discussion"

Additionally, renal function may be considered as the capacity to produce and excrete urine, in which microorganisms flow into the outer environment. Therefore, severely impaired renal function (in particular CKD state 4 and 5) may result in both concentrated bacteria and subsequent development of symptomatic UTI which is consistent with our results. In previous literature reviews (16,17), CKD appeared to be associated with an increased colonization rate of ureteral stents without any clear evidence of an increase in the development of UTI, although the methodological quality of the included studies was inadequate to derive any clear conclusions.

Comment 18: What is meant by "adequate management of the functional status of patients" on line

474? In a patient population where this is typically not feasible, the authors should describe how this would be achieved.

Reply 18: After considering your comment, I have deleted the sentence due to its confusing nature.

Comment 19: On line 483, the authors recommend that patients at risk for symptomatic UTI or bacteremia be "covered" by broad spectrum antibiotics. The authors should clarify whether they are referring to one-time surgical prophylaxis or a course of antibiotics.

Reply 19: Thank you for your comment. The broad-spectrum antibiotics may be helpful in preventing symptomatic UTI in patients with ureteral stents, provided they are at risk for the disease. However, repeated use of broad-spectrum antibiotics might increase the resistance in patients requiring periodic replacement of ureteral stents as you have pointed out. Considering this, we have deleted the sentences about 'broad-spectrum antibiotics for patients at risk of UTI' in the text.

Comment 20: As stated previously, the authors should discuss the retrospective design and confounding bias as significant limitations of this study.

Reply 20: We have added the observational design of this study and other confounding factors as limitations.

Changes in the text: Page 21, line 423, "Discussion"

Our study was not without limitations and had a potentially limited observational design and small sample size.

Conclusion

Comment 21: The conclusion is slightly lengthy and could be more concise.

Reply 21: As per your suggestion, we have modified the Conclusions more concisely.

Changes in the text: Page 22, lines 438-447, "Conclusions"

Infections related to ureteral stents showed a specific microorganism profile and resistance pattern compared to community-acquired UTIs. Additionally, we identified the factors associated with the occurrence of bacteriuria, ESBL-producing bacteria, and symptomatic UTI in patients with retained ureteral stents.

In our study, the possible short duration of ureteral stent placement and periodic replacement in patients with prolonged use of ureteral stents prevented the occurrence of bacteriuria, ESBL-producing bacteria, and symptomatic UTI. Considering the microbiological profile and factors associated with bacteriuria and symptomatic UTIs may be associated with better outcomes in patients with retained ureteral stents.

Comment 22: In the discussion, the authors advise, based on the study findings, that patients with symptomatic UTI should undergo stent exchange after appropriate antibiotic treatment due to high rate of recurrence. This is one of the more important points of the study that should likely be mentioned in the Conclusion.

Reply 22: Thank you for your comment. As you have suggested, we have included the sentences in the

text:

Changes in the text: Page 22, lines 443-445, "Conclusions"

In our study, the possible short duration of ureteral stent placement and periodic replacement in patients with prolonged use of ureteral stents prevented the occurrence of bacteriuria, ESBL-producing bacteria, and symptomatic UTI.

Tables

Comment 23: Values should be presented as N (%) and mean ± SD, rather than N (%) and mean (± SD).

Reply 23: I have modified the tables accordingly.

Comment 24: The authors should consider stratifying patients by CKD stage, given that they discuss the association between CKD and symptomatic UTI. This is an important stratification given the vast difference between CKD-1 and CKD-5 or ESRD.

Reply 24: Thank you for your comment. As you have suggested, we have stratified the patients with CKD stage by using calculated eGFR. The participants were categorized into the following 5 stage according to their baseline eGFR: stage 1, eGFR >90 mL/min/1.73 m^2; stage 2, eGFR of 60–89 mL/min/1.73 m^2; stage 3, eGFR of 30–59 mL/min/1.73 m^2; stage 4, eGFR 15-29 mL/min/1.73 m^2; and stage 5, eGFR <15 mL/min/1.73 m^2. Then, we divided the patients stratified by eGFR into 2 groups, which consisted of group A (preserved urine amount) with CKD stage 1, 2, and 3, and group B (decreased urine amount) with CKD stage 4, and 5, due to the small number patients with CKD stage 5. It is known that patients with CKD stage 4 or 5 have severely impaired renal function, which could be associated with decreased urine amount.

In the multivariate analysis, group B showed significant increased occurrence of bacteriuria and symptomatic UTI compared to group A, which supported the hypothesis that severely impaired renal function (in particular CKD state 4, and 5) may result in both concentrated bacteria and subsequent development of symptomatic UTI.

We have added the sentences in the text and tables.

Changes in the text: Page 8, line 118-126, "Methods"

We used the Modification of Diet in Renal Disease equation to determine the eGFR according to serum creatinine levels (18). Then, we stratified the patients with CKD stage by using calculated eGFR. The participants were categorized into the following five stages according to their baseline eGFR: stage 1, eGFR >90 mL/min/1.73 m2; stage 2, eGFR of 60–89 mL/min/1.73 m2; stage 3, eGFR of 30–59 mL/min/1.73 m2; stage 4, eGFR 15–29 mL/min/1.73 m2; and stage 5, eGFR <15 mL/min/1.73 m2. We divided the patients stratified by eGFR into 2 groups: group A (preserved urine amount group) including CKD stage 1, 2, and 3, and group B (decreased urine amount group) including CKD stage 4, and 5, due to a small number of patients with CKD stage 5.

Comment 25: Are serum triglycerides and cholesterol necessary for Table 1? Consider excluding these.

Reply 25: As you have suggested, we have excluded serum triglycerides and cholesterol from Table 1.