

Bacterial Urinary Isolates Associated with Prostate Cancer and Their Relationship to Gleason Score

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ABSTRACT

Introduction: Prostate cancer ranks as the second cancer among men all over the world. Prostate cancer in Iraq is about 4.08% of all male cancers however after age 50 the incidence has been observed to rise significantly. Prostate cancer has been proposed to be caused by bacteria prostatitis and chronic inflammation. The gram-negative bacteria, particularly *Escherichia coli*, are commonly implicated in UTI and prostatitis, and can also facilitate the development of inflammatory microenvironment, associated with cancer development. **Purpose:** This research attempt was designed to isolate and identify bacterial species in urine specimens of patients having prostate cancer and to determine their distribution in terms of Gleason grade, age and other clinical risk factors. **Methods:** The study was a descriptive cross-sectional one, which was carried out between November 2021 and April 2022. Fifty prostate cancer patients aged between 35 and 80 years who were attending Imam Hussain Center of Oncological and Hematological Diseases, Kerbala, Iraq were recruited. Midstream urine of 10 mL was collected in sterile samples and placed under MacConkey and Blood agar culture. Bacterial identification was done through the traditional biochemical tests and then the VITEK-2 Compact System. The statistical analysis has been conducted using SPSS; Chi-square test and Pearson correlation have been implemented, and the level of significance has been set to $p \leq 0.05$. **Findings:** In 42% of the urine samples, there was significant growth of bacteria. The most common isolate was *Escherichia coli* (81 percent of the positive cultures), then *Enterobacter cloacae* (14.3%) and *Klebsiella pneumoniae* (4.7%). Most of the patients were aged between 70 and 79 years (46%). The most common was Gleason score 8-10 (50%). Forty eight percent of patients were found to smoke. Gleason score of 8 -10 showed the highest *E. coli* isolate growth (56%). There was no statistically significant difference between age groups and bacterial isolates ($p > 0.05$), no statistically significant difference in mean age between Gleason score groups ($p = 0.689$). **Conclusion:** The most frequent category among prostate cancer patients was Gleason score 8-10. *Escherichia coli* was the bacterial isolate that was detected to be most common. The risk factors were identified as older age (70-79 years) and smoking. The results were in line with the expectation of the possible role of the long-term presence of bacteria in the inflammatory process involving prostate cancer.

Keywords: Prostate cancer; *Escherichia coli*; Chronic inflammation; Urinary tract infection; Gleason score.

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1. INTRODUCTION

The second most widespread cancer in men all over the globe is prostate cancer and poses a serious public health issue [1]. Globally, prostate cancer is among the commonest cancers in men with its prevalence rising especially after the age of 50 years in Iraq, prostate cancer is reported to cause some 4.08 women out of every 100 men [2]. Histopathological grading systems play a critical role in the diagnosis and prognosis of prostate cancer, the most commonly used system of which is the Gleason score that allows to evaluate the aggressiveness of the tumor and predict the clinical outcome [3]. The Gleason scoring system is used to find the pattern of glandular architecture in prostate cancer and higher scores represent more aggressive disease and poor prognosis [4].

It has been postulated that chronic inflammation and bacterial prostatitis are risk factors that cause prostate cancer [5,6]. Most urinary tract infections and prostatitis often isolate gram-negative bacteria, and *Escherichia coli* in particular, which can help support inflammatory microenvironment associated with cancer progression [7,8].

The urine bacterial infection and its association with the prostate cancer development among Iraqi patients are still not clear. The local data that can identify bacterial species that relate to prostate cancer and the association with clinical parameters, including Gleason score, age, level of prostate-specific antigen, and comorbidities are limited [9,10]. The objective of the study was to isolate and identify bacterial species that exist in the urine samples of prostate cancer patients and assess their distribution with regard to Gleason score, age, as well as clinical risk factors.

2. RESEARCH QUESTIONS

This research answered the following questions:

- (1) What are the most frequent bacterial isolates in urine samples of patients with prostate cancer?
- (2) Is there a correlation between the presence of bacteria and severity of the Gleason scores?
- (3) How do the bacterial isolates spread among various age groups?

As shown in previous researches, prostate cancer develops with chronic inflammation and bacterial prostatitis [11,12]. *Escherichia coli* has been indicated to cause 50-90% of acute bacterial prostatitis [13]. High levels of inflammatory cytokines including interleukin-6 related to infection by bacteria have been identified to facilitate the initiation and progression of tumors [14,15].

The urinary tract which was previously thought to be sterile in healthy people has been found to host various communities of microbes which may have significant health and disease implications [16]. There is an increasing body of evidence to indicate that the composition of urinary microbiota can change in prostate cancer patients in comparison to those with benign prostate-related disorders or healthy controls [6,7].

The research is based on the hypothesis of inflammation cancer, which suggests that persistent bacterial infection triggers inflammatory microenvironment, which promotes carcinogenesis and tumor development [17,18]. The theories of chronic inflammatory responses to persistent colonization of bacteria, generation of bacterial metabolites that can alter cellular functions and immune surveillance system regulation have been put forward as possible lines of action through which the presence of bacteria can have an impact on the prostate cancer development [19,20].

The research regarding urinary microbiota in prostate cancer patients has been limited in the region of Iraq. No available data correlating the bacterial isolate with the Gleason scores, as well as demographic variables, was organized using automated identification systems [21,22]. Also, the particular patterns of distribution of bacterial species within each category of Gleason score within this population are not adequately reported.

3. METHODS

The study was a descriptive cross-sectional one aiming to cover a specific timeframe between November 2021 and April 2022 [23]. The study was pre-empted with ethical approval of the Imam Hussain Center of Oncology and blood diseases Directorate.

The target population was the patients who were diagnosed with prostate cancer. A total of 50 patients with prostate cancer aged 35-80 years who visit Imam Hussein Center Oncological and Hematological Diseases, Kerbala, Iraq was included in the sample. The criteria used in the inclusion were adult male patients with reported and confirmed histologically prostate adenocarcinoma and Gleason score. The exclusion criteria were a history of antibiotic use within four weeks before the collection of the sample, active treatment of urinary tract infection, and the presence of urinary catheters.

Sterile containers that were used to collect clean-catch midstream urine samples (10 mL) were filled with the enrolled patients. The patients were asked to wash the glans penis using sterile wipes before the collection. The first segment of urine stream was thrown away and midstream urine was taken. The samples were taken to the laboratory, not later than two hours after collection and processed promptly.

MacConkey agar and Blood agar plates were used to culture the urine samples. Incubation of the inoculated plates was done in an aerobic environment at 37°C in 24-48 hours. The growth of the bacteria was determined by counting the colony-forming units. Bacteriuria was considered significant when the growth of bacteria would be more than 10⁷ colony-forming

units per milliliter.

The bacterial identification was carried out using the traditional biochemical tests and then using VITEK-2 Compact System [24]. A preliminary classification was done through gram staining. Identification was done at the species level using automated VITEK-2 system, which was fast and precise, identifying bacteria using biochemical reactions.

The statistical analysis was done by the SPSS software. Descriptive statistics were computed to get the prevalence of bacterial isolates. The patients were grouped according to their ages and Gleason score groups. The chi-square test was used to determine the relationships between categorical variables. It was analyzed through Pearson correlation to test the relationships between continuous variables. The level of statistical significance was established at p 0.05.

All the participants signed informed consent written before enrollment.

4. ETHICS AND COMPLIANCE

Ethics Approval: It was done with Imam Hussain Center oncological and Hematological Diseases Directorate.

Conflict of Interest: There was no conflict of interest in the article.

Informed Consent: Patients gave informed consent to the collection of urine samples. Questionnaires and institutional records were used to get medical data.

5. RESULTS

The study was conducted on 50 prostate cancer patients. Among the 50 samples of the urine that were tested, 42 percent exhibited notable bacterial growth and 58% exhibited no notable bacterial growth. The *Escherichia coli* was found to be the most common with 81 percent of the positive cultures, followed by *Enterobacter cloacae* (14.3) and *Klebsiella pneumoniae* (4.7). The bacterial species that were isolated were predominantly found to be gram-negative.

Table 1: Demographic and Clinical Characteristics of Patients.

Characteristic	Value
Number of patients	50
Age (mean ± SD)	68.74 ± 6.93 years
Age range	51–80 years
PSA levels (mean ± SD)	12.7 ± 9.3 ng/mL
Smoking (smokers)	24 (48%)
Smoking (non-smokers)	26 (52%)

Most of the patients fell within the 70-79 years age group (46%), and then 60-69 years (34%). The most common category was Gleason score 8-10 with 50 percent and next was Gleason score 7 (34 percent) and Gleason score 6 (16 percent). Forty eight percent of patients were found to smoke.

Table 2: Gleason Score Categories Distribution.

Gleason Score Category	Number of Patients	Percentage
Low-grade (Gleason 6)	8	16%
Intermediate grade (Gleason 7)	17	34%
High-grade (Gleason 8–10)	25	50%
Total	50	100%

Figure 1: The Gleason Score Categories Distribution among the participants of the study.

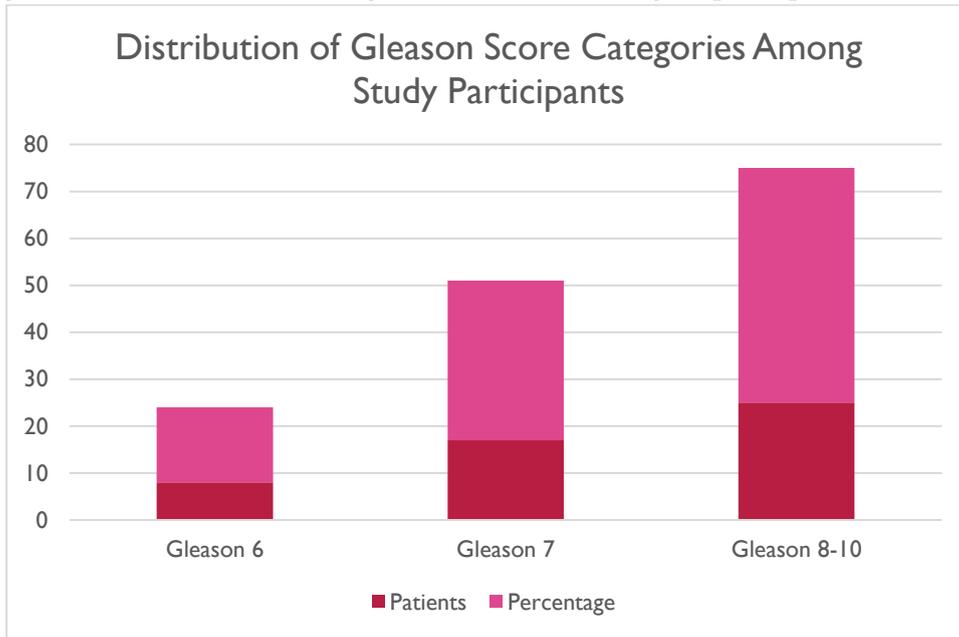
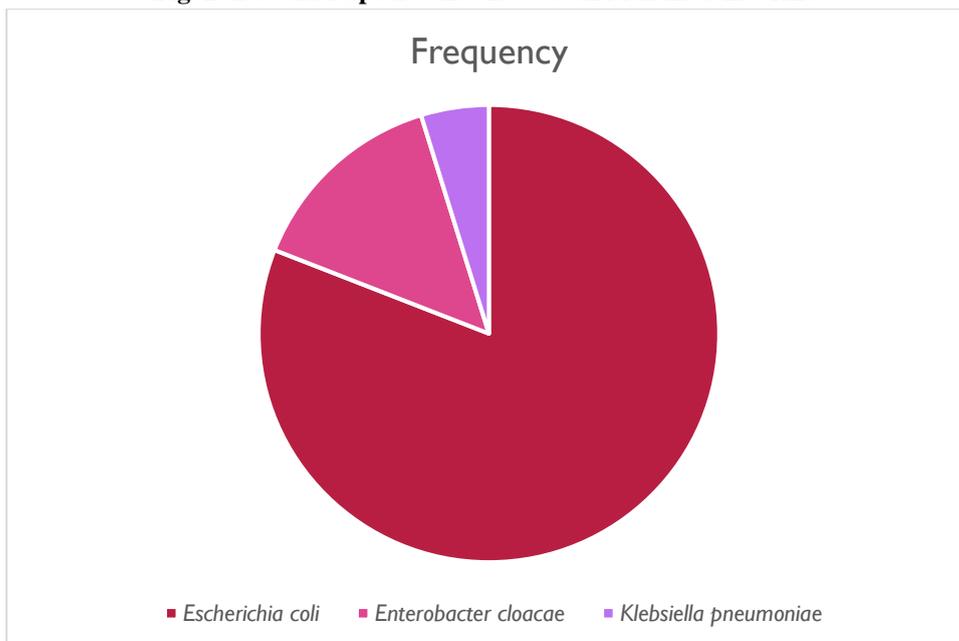


Table 3: Bacterial Species Isolated Urine Samples.

Bacterial Species	Frequency	Percentage of Positive Cultures
<i>Escherichia coli</i>	17	81.0%
<i>Enterobacter cloacae</i>	3	14.3%
<i>Klebsiella pneumoniae</i>	1	4.7%
Total positive cultures	21	100%
Negative cultures	29	–
Total samples	50	–

Fig. 2: Bacterial Species that have been identified in Urine.



The maximum growth of *E. coli* isolates was observed in the Gleason score of 8-10 range (56%), which proves that the given range can be regarded as one of the risk factors of *E. coli* proliferation in prostate cancer patients.

Table 4: Classification of Bacterial Isolates based on Gleason Score.

Bacterial Species	Gleason 6 (n=8)	Gleason 7 (n=17)	Gleason 8–10 (n=25)	Total
<i>E. coli</i>	3 (37.5%)	4 (23.5%)	14 (56.0%)	17
<i>Enterobacter cloacae</i>	0 (0%)	1 (5.9%)	2 (8.0%)	3
<i>K. pneumoniae</i>	0 (0%)	1 (5.9%)	0 (0%)	1
Total bacteriuria	3 (37.5%)	6 (35.3%)	16 (64.0%)	25*

Note: There are patients who gave more than a single isolate.

Figure 3: Distribution of Bacterial Isolates According to Gleason Score Categories.

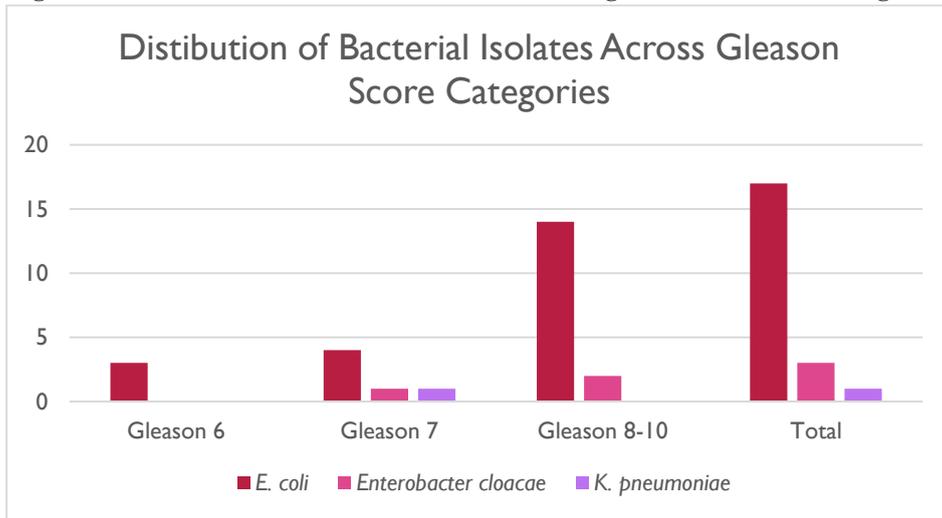
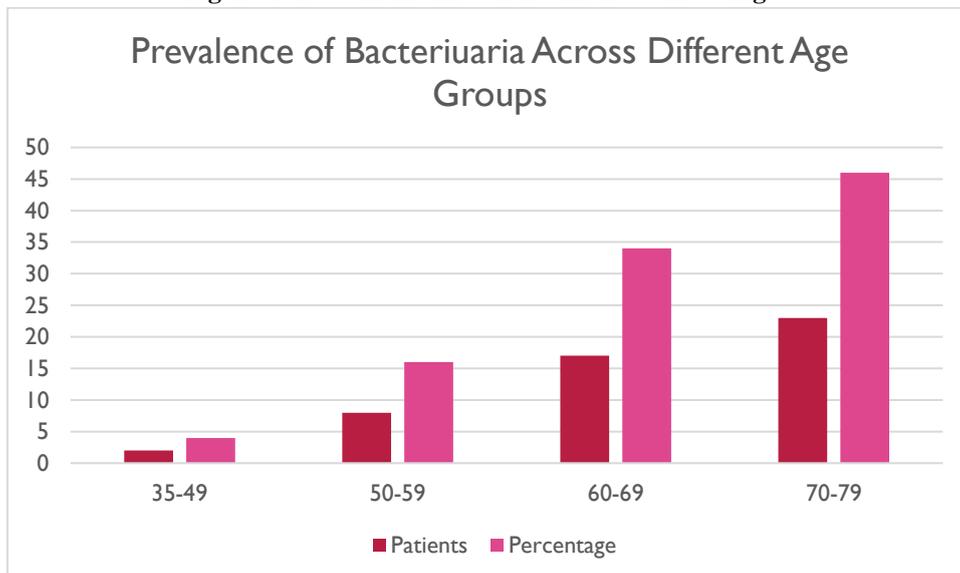


Table 5: Age Distribution of the participants of the study.

Age Group	Number of Patients	Percentage
35–49 years	2	4%
50–59 years	8	16%
60–69 years	17	34%
70–79 years	23	46%
Total	50	100%

Figure 4: Bacteriuria Prevalence in the Various Ages.

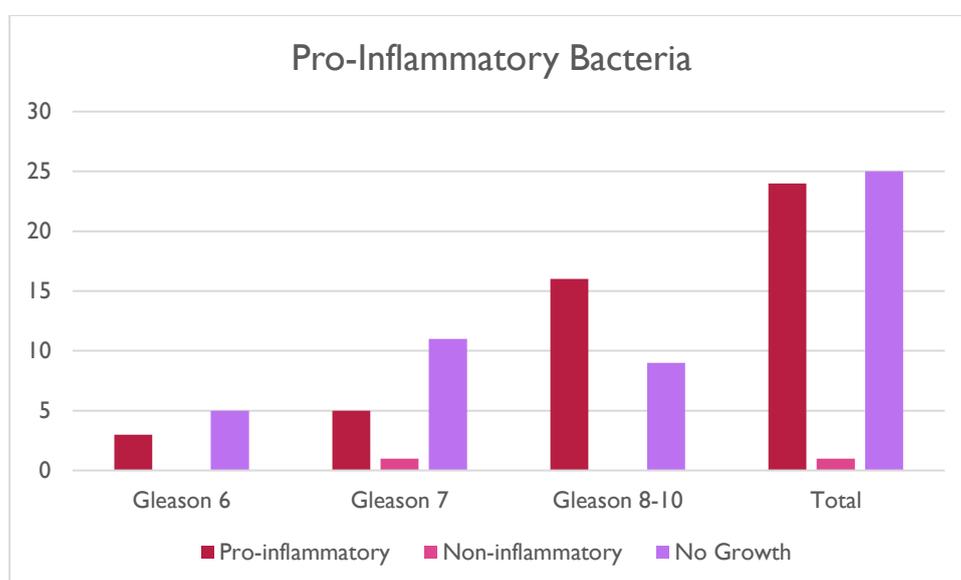


There was no statistically significant relationship between age groups and bacterial isolates ($p > 0.05$). There were no significant differences in the mean age between different groups of Gleason scores ($p = 0.689$). Even though a tendency towards the increase in the rate of bacteriuria was noted in patients with high Gleason scores, especially in the Gleason 8-10 group (64%), they were not statistically significant because of the sample size of the study.

Table 6: Bacterial Profile of inflammatory by Gleason score.

Bacterial Category	Gleason 6 (n=8)	Gleason 7 (n=17)	Gleason 8-10 (n=25)	Total
Pro-inflammatory bacteria*	3 (37.5%)	5 (29.4%)	16 (64.0%)	24
Non-inflammatory bacteria	0 (0%)	1 (5.9%)	0 (0%)	1
No growth	5 (62.5%)	11 (64.7%)	9 (36.0%)	25
Total	8	17	25	50

Among the pro-inflammatory bacteria, there are *E. coli*, *Enterobacter cloacae*, and *K. pneumoniae*.



6. DISCUSSION

The growth of bacteria in 42 percent of the urine samples of patients with prostate cancer suggests that bacteriuria is very high in these patients [25]. The clinical implications of the present observation are that a significant percentage of patients with prostate cancer carries urinary bacteria that could be involved in local inflammation and eventually determine the progression of the disease [26,27].

The *Escherichia coli* predominating with 81% of the positive cultures is in agreement with its known status as the most prevalent uropathogen [28]. *E. coli* has been identified as the major pathogen of UTI and bacterial prostatitis, and high concentration of this pathogen in the urine of patients with prostate cancer can be considered an indication of a high rate of chronic inflammation [29,30]. It was observed that the greatest *E. coli* isolate growth occurred at the Gleason score 8-10 range (56) which indicates that the Gleason score group of 8-10 might be linked to high bacterial growth.

Enterobacter cloacae and *Klebsiella pneumoniae* were also found to be present albeit in lesser numbers compared to *E. coli*. These gram-negative organisms are reported to have the ability to cause inflammatory response and have been involved in a number of urinary tract and prostate infections [31,32].

The results of this research are in accordance with other researchers who reported *E. coli* to be the most prevalent pathogen of prostatitis and urinary tract infections [33,34]. As shown by the results, the findings can be associated with the wider body of literature on the relationship between chronic inflammation and the progression of prostate cancer that formed the basis of the inflammation-cancer hypothesis employed in this study [35,36]. It has been established by past research that

persistent inflammatory conditions are encouraged by chronic colonization with bacteria, release of pro-inflammatory cytokines including interleukin-6, tumor necrosis factor-alpha, and interleukin-1- that may mediate cancer growth and progression [37,38].

The pro-inflammatory bacteria of such a high prevalence in the study especially in the high-grade Gleason score group is similar to that of other studies conducted to investigate urinary microbiota in prostate cancer patients [39,40]. Identification of bacteria by the VITEK-2 Compact System could be considered a strength of the methodology, and this automated system could help identify bacteria with high accuracy and in a short amount of time, based on the reactions of the biochemical [41].

Early identification of complications caused by inflammation and the severity of the disease may be the subject of interest of bacterial screening in prostate cancer patients [42]. Provided that regular links of bacteria and disease severity are determined in large studies, microbiological evaluation may be used to add to risk stratification and customized treatment methods. The use of infection control beliefs into the management practices of prostate cancer can be helpful in addition [43].

The sample size is too small (n=50 patients) to have a statistical power to find associations and especially comparing subgroups. Single-center study design is also not able to reflect the entire breadth of the population and restricts external validity. There is no healthy control group, which prevents a comparative study of the prevalence of the bacteria in patients diagnosed with prostate cancer and healthy people. The cross-sectional design does not provide sufficient capability to determine causal links or temporal order among presence of bacteria and cancer development or progression [44].

These restrictions restrict the extrapolativeness of the results and are the reasons that do not allow establishing a direct causal relationship between bacteria and the development of prostate cancer. Considering the dependence upon culture-based approaches is both reasonable and validated, this approach might not reflect the complete diversity that is present in urinary microbiota. Molecular techniques that are culture-independent like the 16S rRNA gene sequencing have been proved to be able to identify bacteria that could be hard or impossible to obtain in the classic methods [45]. The sample size is relatively small and therefore can be one of the factors that led to the lack of statistical significance in some of the associations studied.

7. FUTURE RESEARCH DIRECTIONS

It is suggested that larger multicenter studies with larger sample sizes are needed to increase statistical power and generalizability. The use of control groups, including healthy people or patients with benign prostatic hyperplasia, would allow making stronger comparisons. Next-generation sequencing analysis of microbiota as a form of molecular analysis would give more insights into microbial diversity and community composition [46]. The cause and time-based association between the presence of bacteria and cancer progression should be conducted using longitudinal study design. Future studies should include antimicrobial susceptibility testing which will further guide clinical management decisions [47].

8. CONCLUSION

It was a descriptive cross-sectional study that investigated bacterial urine isolates in 50 prostate cancer patients at Imam Hussain center of Oncological and Hematological Diseases at Kerbala, Iraq, between November 2021 and April 2022. The most significant category was the Gleason score 8-10, which indicated 50 percent of cases. The bacterial isolate that was the most common isolate was *Escherichia coli* which occupied 81 percent of the positive cultures. Notable risk factors were identified as older age (70-79 years) and smoking. *E. coli* isolate growth was highest at the Gleason score 8-10 range (56%), and *E. coli* and *Enterobacter cloacae* were observed to be prolific in the urine of the prostate patients who had cancer and this indicates a high level of inflammation.

9. RECOMMENDATIONS

Microbiological screening of prostate cancer patients is suggested as a routine procedure to be performed at an early stage to determine the complications of inflammation and the severity of the disease. More studies on bacteria-related

inflammation and its role in cancer development should be conducted. The issue of infection control should be included in prostate cancer management interventions. To understand the mechanistic links between urinary bacteria and prostate cancer progression, larger multicenter studies that combine molecular microbiome analysis with longitudinal, study designs are required.

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