DOI: 10.29409/ijcmg.v17i1.360

Cancer Research

Iraqi Journal of Cancer and Medical Genetics

Molecular characterization of some resistance genes of E.coli isolated from patients with prostate cancer

Hussein Ali Dnana[1], and Azhar Noory Hussein[2]

1 General Directorate of Missan Education, Ministry of Education, Misssan, Iraq
2 Department of Biology, College of Education, University of Al- Qadisiyah, Al- Qadisiyah, Iraq

Abstract

The current study was conducted to identify pathogenic E.coli that coexist with prostate cancer and characterize these bacteria based on some virulence genes using molecular techniques such as RAPD-PCR. The study was carried out on 200 samples isolated from prostate cancer patients in different hospitals in Maysan Province, Iraq. These samples were cultivated on traditional and specific media agars for the identification of certain pathogenic bacteria. These bacterial organisms were subjected to PCR targeting the 16S rRNA gene for the identification of these bacteria and the genes aac2, blaoxA-1, blaTEM, oxA48 for the detection of virulence factors of isolated bacteria. The results revealed the presence of 200 bacterial isolates, which included 65 (59.09%) E. coli, 20 (18.18%) Proteus mirabilis, 12 (10.9%) P. penneri, 10 (9.09%) Pseudomonas aeruginosa, and 3 (2.72%) Klebsiella pneumonia, 16 (32%) Enterococcus faecalis, 15 (30%) Staphylococcus hemolyticus, 9 (18%) S. lentus, 3 (6%) Aerococcus viridans, , and 7 (14%) Streptococcus uberis. The findings of 16S rRNA-PCR confirmed the results of conventional tools. The virulence genes were detected in the identified bacterial species. The study findings reveal a diverse community of bacterial species present in samples isolated from patients with prostate cancer with high virulence, detected by the presence of virulence genes.

Keywords: *Antibiotic resistance, pathogenic bacteria, prostate cancer*

Introduction

Prostate cancer constitutes one of the most important clinical challenges of urology with a strong impact on the quality of life of patients around the world. Among them, benign conditions, mainly represented by bacterial infections, are of great importance due to the number of affected patients and the complexity of pathophysiology (1). Acute bacterial prostatitis is the less frequent presentation of bacterial infection, with a serious clinical impact, while chronic bacterial prostatitis, a much more frequent disease, also appears to be less severe, although related to a prolonged healing time, mainly due to the recurrence of symptoms. The epidemiological dimensions of the bacterial forms of prostatitis are still undetermined, mainly due to diagnostic ambiguity and the variety of clinical presentations. Since bacterial prostatitis is estimated to affect only about 5–10% of the total cases of prostatitis, the prevalence is around 1-2 per 1000 men in the general population, occurring more frequently in adult males younger than 35 or older than 60 years old, with a clearly bimodal distribution correlated with the sexual activity and prostate gland growth, respectively(2,3).

E. coli, Proteus mirabilis, K. pneumoniae, and P. aeruginosa are the most common Gram-negative bacilli of the gastrointestinal tract that trigger bacterial prostatitis. In fact, E coli is the most common cause of bacterial prostatitis worldwide. In selected cases, sexually transmitted pathogens such as Chlamydia trachomatis and Neisseria gonorrhoeae also play a role. The pathogenesis of bacterial prostatitis reflects the rise of pathogens from the urethra to the prostate under the impetus of infected urine refluxing into the prostatic ducts, especially if there are predisposing factors such as urinary tract infections, obstruction of the bladder outlet and urinary tract instrumentation that breach normal prostatic defenses (4,5).).

Acute bacterial prostatitis is characterized by an abrupt onset, with acute and intense lower UTI (urgency, frequency, dysuria) and painful ejaculation, typically accompanied by systemic symptoms such as fever, chills, and malaise. Chronic bacterial prostatitis can present more insidiously, with recurrent UTIs and pelvic pain that interfere with pa-

Corresponding Address:
Hussein Ali Dnana
General Directorate of Missan Education, Ministry of Education, Misssan, Iraq.
Email: edu.bio.posta14@qu.edu.iq

tient quality of life (6–8).

In bacterial prostatitis, the diagnosis is usually made clinically, based on urine / prostate secretion microbiology and occasionally on imaging; standard approach to diagnosis includes the following. Four-glass test, which remains the gold standard for distinguishing between types of prostatitis. In case of acute presentation, an imaging study, such as transrectal ultrasound or magnetic resonance imaging, may be necessary to rule out abscess formation or other complications(9).

Antibiotics are needed to treat acute bacterial prostatitis, and patients need to receive treatment immediately to avoid the development of complications such as abscesses in the prostate. Prolonged courses of antibiotics, often lasting many weeks or months. In chronic bacterial prostatitis, where causative agents tend to hide in the prostatic ducts, treatment can be difficult, and the course of the drug is usually prolonged (10).

The current study was conducted to identify pathogenic bacterial species that coexist with prostate cancer and characterize these bacteria based on some virulence genes using molecular techniques.

Methods

Samples and Bacterial Culture

During the study, 200 clinical samples were collected from patients suffering from prostate infections at Al-Sadr Teaching Hospital, private laboratories, and the Oncology Center in Maysan Governorate for the period from 4/1/2023 to 11/1/2023. Clinical samples included urine samples, blood samples, and biopsy samples. All of these samples were cultured on culture medium represented by MacConkey agar medium, basic blood agar medium, and solid nutrient medium. Positive laboratory culture results were distributed over 90 urine samples (81.81%), 40 blood samples (80%), and 30 biopsies (75%) of the total samples belonging to the same type. Gram-negative bacterial isolates were diagnosed using morphological, cultural, biochemical and Vitech tests, while Gram-positive isolates were diagnosed using morphological, cultural, and Vitech tests as a final diagnosis. Since E.coli bacteria are more numerous than isolated bacterial isolates, it was the focus of the study. The results of the statistical analysis showed that there were no significant differences between the samples in terms of growth at the 0.05 level.

PCR

DNA was extracted from bacterial broth using a bacterial extraction kit (Anatolia, Turkey). The protocol of the kit was followed for the isolation of bacterial DNA. A nanodrop was utilized for the estimation of the quality and quantity of DNA. DNA was stored at -20 ° C for later work procedures.

The bacterial organisms were subjected to PCR targeting the 16S rRNA gene for the identification of these bacteria and the genes: aac2, blaoxA-1, blaTEM, and oxA48 for the detection of the virulence factors of the isolated bacteria. The primers (ordered from Macrogen and IDT, Korea) are shown in Table 1.

Table 1: Primers used in the study for the detection of *E.coli* and virulence genes from samples of prostate cancer patients

Primers	Sequence 5----------3	Product size	References
16srRNA	F: AGAGTTTGATCCTGGCTCAG	1500 bp	Clementino *et al.,* 2001(11) [11]
	R: GGTTACCTTACGACTT		
aacC2	F: TAGAGGAGATATCGCGATGC	896 bp	Aleisa *et al.,* 2013 (12)
	R: ATTATCATTGTCGACGGCCT		
blaoxA-1	F: TCAACTTTCAAGATCGCA	609 bp	Speldooren *et al*., 1998(13)
	R: GTGTGTTTAGAATGGGTGA		
bla-TEM	F: TTTCGTGTCGCCCTTATTCC	516 bp	Sharif *et al.*, 2014(14)
	R: CCGGCTCCAGATTTATCAGC		
bla-SHV	F: ATCGTTGTCAGAAGTAAGTTGG R: TTTATGGCGTTACCTTTGACC	390 bp	Yagi *et al*., 2000 (15)
OXA-48	F: GCTTGATCGCCCTCGATT	285 bp	Gurung *et al*., 2020 (16)
	R: GATTTGCTCCGTGGCCGAAA		

The PCR reaction and contents are mentioned in Table 2.

Table 2: PCR master mix and components of the study for the detection of bacterial species and virulence genes from urine samples from prostate cancer patients.

PCR Master Mix	16srRNA µl	PCR (*aacC2-blaoxA-bla-TEM-bla-SHV-Oxa-48*) µl	Concentration
Master Mix	25	12.5	1X
Forward primer	2.5	1.5	pmol 10
Reveres primer	2.5	1.5	
Free nuclease water	16	6.5	1X
DNA template	4	3	1-150
Total volume	50	25	-

The agarose gel at 2% was used to run the PCR products using electrophoresis at 100 volts and 80 Amp for one hour. The gel was then visualized under a UV imager.

DNA Sequencing

The PCR products were sent to a sequencing in Macrogen, Korea. The sequences received were then processed and analyzed using NCBI-related websites and MEGA X software to generate a phylogenetic tree.

Results

The results revealed that the presence of 200 bacterial isolates, which included positive laboratory culture results, were distributed among 90 urine samples (81.81%), 40 blood samples (80%), and 30 biopsies (75%) of the total samples belonging to the same type.Table3. The findings of 16S rRNA-PCR confirmed the results of conventional tools. The bands were amplified as revealed in Fig. 1. The virulence genes were detected in the identified bacterial species. These genes were amplified using the conventional PCR techniques as displayed in Fig. 2.

Table3. The findings of 16S rRNA-PCR confirmed the results of conventional tools.

Sample type	Total	Positive growth%	Negative growth%
urine	110	90(81.81)	20
Blood	50	40(80)	10(20)
biopsy	40	30(75)	10(25)
Total number	200	160	40
Chi-square value X2	-	0.852	
Calculated P-value		0.653 (not significant)	

Table 4: Numbers and percentages of Gram-negative bacteria isolated from the samples under study.

P value	X2	Total	*K.pneuomonee* (٪) number	*p. aerugi-nosa* number (٪)	*pro. penneri* (٪) number	*Pro. mira-bilis* number(٪)	*E. coli* number (٪)	Sample type
<0.0001 (Significant)	118.4	66	1(1.51)	5(7.57)	4(6.06)	12(18.18)	44(66.66)	urine
<0.0001 (Significant)	26.16	28	2(7.14)	2(7.14)	5(17.85)	4(14.28)	15(53.57)	Blood
0.119 (No signifi-cant)	7.34	16	0(0)	3(18.75)	3(18.75)	4(25)	6(37.5)	Biobsy
<0.0001 (Significant)	139.6	110	3(2.72)	10(9.09)	12(10.9)	20(18.18)	65(59.09)	Total

Table 5: Numbers and percentages of Gram-positive bacteria isolated from the samples under study.

P-Value	X2	Total	*Streptococcus uberis* Number (٪)	*Aerococcus viridans* Number (٪)	*Staph. lentus* Number (٪)	*Staph.haemolytic* Number (٪)	*Enterococcus faecalis* (٪) Number	Sample Type
0.137 (No significant)	6.97	24	4(16.66)	1(4.16)	6(25)	5(20.83)	8(33.33)	Urine
0.309 (No significant)	4.79	12	3(25)	2(16.66)	0(0)	4(33.33)	3(25)	Blood
0.008 (Significant)	13.75	14	0(0)	0(0)	3(21.42)	6(42.85)	5(35.71)	Biopsy
0.005 (significant)	15	50	7(14)	3(6)	9(18)	15(30)	16(32)	Total

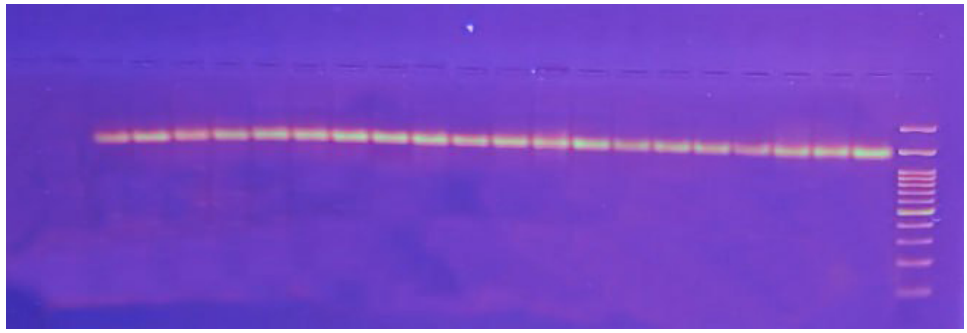

Fig. 1: Agarose gel electrophoresis of PCR for the detection of the 16S rRNA gene of bacterial species from samples from patients with prostate cancer. 1500 bp, Horizontal bands: Positive. Vertical bands: DNA ladder.

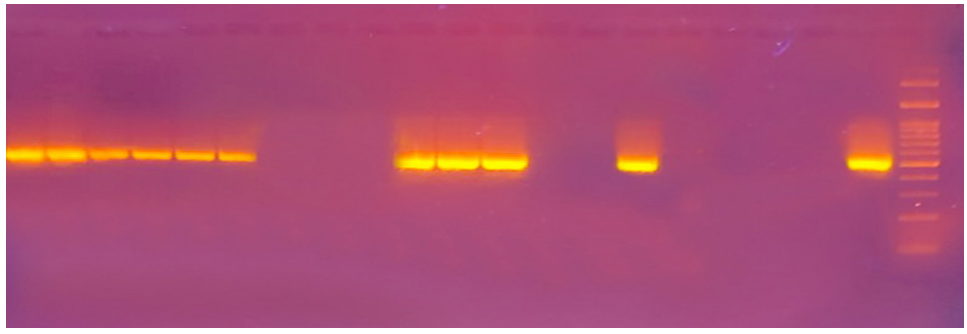

Fig. 2: Electrophoresis of PCR products of the blaTEM gene from E.coli isolates in 2% agarose and 100 V, where lane M represents the size index 100-16000 base pairs. 516 bp, Lanes 1-20 represent E.coli isolates that possess the blaTEM gene.

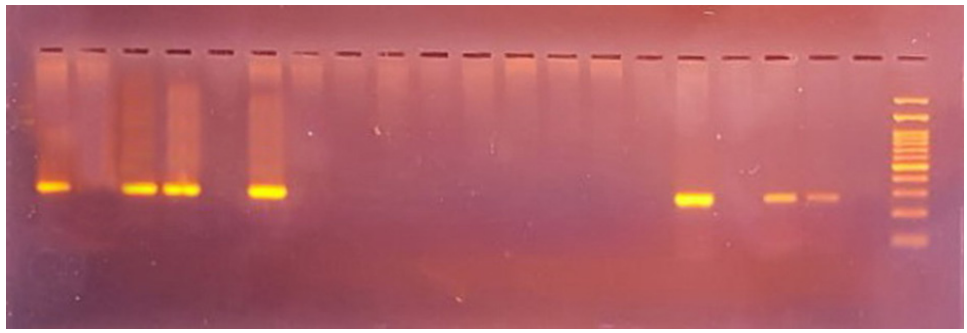

Fig. 3: Electrophoresis of 48-oxA PCR products of E.coli isolates in 2% agarose and 100 V, where lane M represents the size index 100-16000 base pairs. 285 bp, Lanes 1-20 represent E.coli isolates that possess the 48-oxA gene.

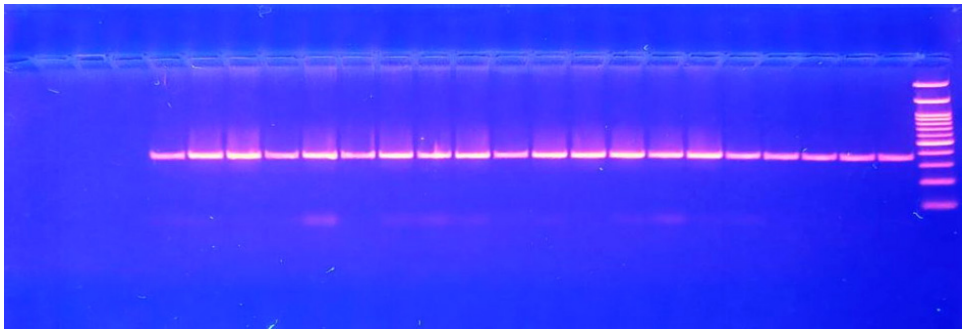

Fig. 4: Electrophoresis of PCR products of the blaSHV gene of E.coli isolates in 2% agarose and 100 V, where lane M represents the size index 100-16000 base pairs. 390 bp, Lanes 1-20 represent E.coli isolates that possess the blaSHV gene.

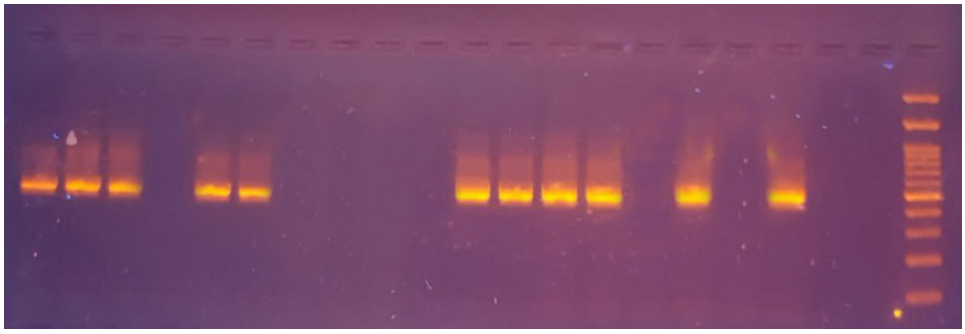

Fig. 5: Electrophoresis of PCR products of blaoxA-1 gene from E.coli isolates in 2% agarose and 100 V, where lane M represents the size index of 100-16000 base pairs. 609 bp, Lanes 1-20 represent E.coli isolates that possess the blaoxA-1 gene.

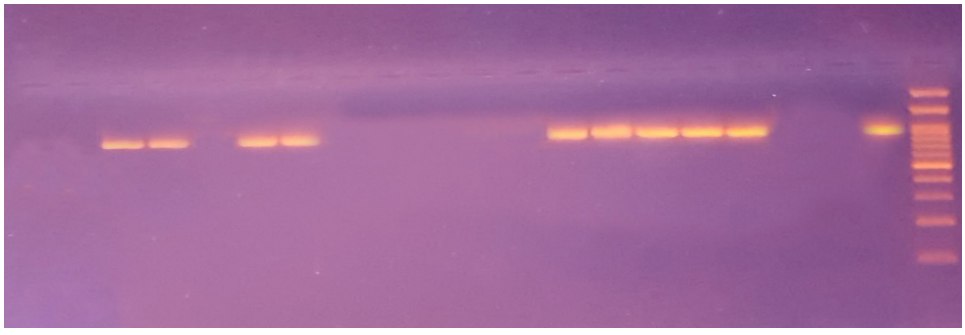

Fig. 6: Electrophoresis of the PCR product of aacC2 gene of E.coli isolates in 2% agarose and 100 V, where lane M represents the size index 100-16000 base pairs. 806 bp, Lanes 1-20 represent E.coli isolates that possess the aacC2 gene.

Discussion

We noticed a large community of bacterial isolates that was gram-negative in nature, compared with gram-positive (60 per cent of the isolates versus 40%, respectively). This evolved disparity is predictable and supported by epidemiological knowledge that Gram-negative pathogens are the most common etiological agents of UTIs (17). Of these, 46.7% were acquired Gram-negative isolates, mostly composed of Escherichia coli, as expected, it being the most frequent pathogen responsible for uncomplicated UTIs (up to 50%), according to the literature (18). The relevant high proportion of other Gram-negative pathogens, such as P. aeruginosa (9.09%) and K. pneumonia (2.72%) also mirrors recent reports on the increasingly recognized roles of these pathogens in complicated UTI (19).

The representativity of our samples by Proteus species including P. mirabilis (18.18%) and P. penneri (10.9%) is considerably higher than what is normally represented in UTI, with P. mirabilis mostly representing a minority fraction of infections 19. It is either a regional phenomenon in terms of the predominance of bacteria or a specific vulnerability of our study population. Among Gram-positive bacteria, E. faecalis stood out with 32% vs a consensus of other studies that indicated that it makes up a smaller proportion of UTI pathogens (2-10%) (20). The finding of S. aureus and especially of S. lentus, which does not usually predominate in UTI, might reflect a unique microbial picture in our cohort or might need confirmation using advanced molecular methods (21).

Our study identified virulence in numerous antibiotic-resistant bacteria isolated from the urine of prostate cancer patients containing 896 bp-aaC2, 619 bp blaOXA_1, 609 bp blaOX_1, 516 bp blaTEM, 390 bp blaSHV, and 285 bp OXA48 where other resistance genes are involved in a complicating threat for the treatment of UTIs related to prostate cancer disease.

It is noteworthy that it is the same aaC2 gene that is found in other clinical settings, where its presence was recently reported and associated with multidrug resistance in E. coli isolates from various sources of infections by Ramírez-Castillo and coworkers (22). The presence of the aacC2 gene in E. coli is of great concern because it could contribute to the development and spread of antibiotic resistance. Aminoglycoside antibiotics are commonly used to treat many bacterial infections, and the presence of the aac2 gene could impair the effectiveness of these drugs. The high prevalence of the aacC2 gene observed in this study highlights the need for continued surveillance and implementation of strategies to reduce the spread of antibiotic resistance(23).

Of the OXA-type β-lactamases, blaOXA_1 and blaOXA48 have been the subject of particular attention: for example, Potron et al. (24) reviewed the epidemiology of blaOXA-48-like genes in Enterobacteriaceae, a group that includes E. coli and K. pneumoniae (23): Numerous genotypes of blaOXA-48-like genes have been described and are particularly distributed in Asia, where strains with the NDM-1 gene and OXA-48-like carbapenemases are spreading together. These enzymes impair the efficacy of treatment. The blaOXA_1 gene has already been identified in isolates from prostate cancer patients and is capable of incorporating the blaOXA48 gene. The spread of this gene in bacteria is attributed to its ability to be easily transmitted between bacteria through genetic elements that move between different types of bacteria in medical environments, since cases of the appearance of this gene in bacterial isolates from different geographical areas have been recorded. It was named oxA-B-lactamase as a result of its ability to degrade oxacillin, as these enzymes are characterized by their ability to degrade both the antibiotics Cloxacillin and Oxacillin by 50%. (24).

Moreover, blaTEM and blaSHV, the most commonly encountered genes associated with broad-spectrum β-lactamase activity, have been widely reported. The occurrence of blaTEM in urinary isolates described by Bevan et al. (25) concurs with our results and re-emphasizes the continuing role both genes play in thwarting β-lactam therapy (26). The high prevalence of the bla SHV gene in E. coli isolates is of great concern, as it contributes to the development of antibiotic resistance and limits the effectiveness of commonly used antibiotics. The bla SHV gene is often associated with mobile genetic elements, such as plasmids and transposons, that facilitate its horizontal transfer between bacterial species. This horizontal gene transfer can lead to rapid spread of antibiotic resistance, which poses a major threat to public health (27).

Conclusions

The study findings reveal a diverse community of bacterial species present in urine samples isolated from prostate cancer patients with high virulence, detected by the presence of virulence genes.

The results of the study showed that E.coli isolates possessed antibiotic resistance genes, namely the aacC gene, which confers resistance to aminoglycosides, the blaox-1 gene, which is associated with beta-lactam resistance, the blaTEM gene, which encodes beta-lactamase enzymes that degrade penicillin and cephalosporins, and the blaSHV gene, which is resistant to beta-lactam antibiotics.

Acknowledgments

I would like to thank my supervisor who helped me prepare this research. Furthermore, I would like to extend my thanks to the Maysan Health Department for providing the facilities to conduct this research.

Authors' Contribution

All authors have made equal contributions to this work.

Conflict of Interest

There is no conflict of interest.

References:

1. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016;14(8):e1002533.
2. Quigley EMM. Gut bacteria in health and disease. Gastroenterol Hepatol (N Y). 2013;9(9):560.
3. Nicolaro M, Portal DE, Shinder B, Patel H V, Singer EA. The human microbiome and genitourinary malignancies. Ann Transl Med. 2020;8(19).
4. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007;449(7164):804–10.
5. Chen YB, Hochstedler B, Pham TT, Alvarez MA, Mueller ER, Wolfe AJ. The urethral microbiota: a missing link in the female urinary microbiota. J Urol. 2020;204(2):303–9.
6. Devaraj S, Hemarajata P, Versalovic J. The human gut microbiome and body metabolism: implications for obesity and diabetes. Clin Chem. 2013;59(4):617–28.
7. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci. 2012;13(10):701–12.
8. Purcell R V, Visnovska M, Biggs PJ, Schmeier S, Frizelle FA. Distinct gut microbiome patterns associate with consensus molecular subtypes of colorectal cancer. Sci Rep. 2017;7(1):11590.
9. Miyake M, Ohnishi K, Hori S, Nakano A, Nakano R, Yano H, et al. Mycoplasma genitalium infection and chronic inflammation in human prostate cancer: detection using prostatectomy and needle biopsy specimens. Cells. 2019;8(3):212.
10. Collins MM, Stafford RS, O'LEARY MP, Barry MJ. How common is prostatitis? A national survey of physician visits. J Urol. 1998;159(4):1224–8.
11. Clementino MM, de Filippis I, Nascimento CR, Branquinho R, Rocha CL, Martins OB. PCR analyses of tRNA intergenic spacer, 16S-23S internal transcribed spacer, and randomly amplified polymorphic DNA reveal inter-and intraspecific relationships of Enterobacter cloacae strains. J Clin Microbiol. 2001;39(11):3865–70.
12. Aleisa K, Al-Dwairi ZN, Alwazzan K, Al-Moither M, Al-Shammari M, Lynch E. Convergence angles of clinical tooth preparations achieved by dental students at King Saud University, Saudi Arabia. J Dent Educ. 2013;77(9):1154–8.
13. Speldooren V, Heym B, Labia R, Nicolas-Chanoine MH. Discriminatory detection of inhibitor-resistant β-lactamases in Escherichia coli by single-strand conformation polymorphism-

PCR. Antimicrob Agents Chemother. 1998;42(4):879–84.
14. Sharif MK, Butt MS, Anjum FM, Khan SH. Rice bran: a novel functional ingredient. Crit Rev Food Sci Nutr. 2014;54(6):807–16.
15. Yagi T, Kurokawa H, Shibata N, Shibayama K, Arakawa Y. A preliminary survey of extended-spectrum β-lactamases (ESBLs) in clinical isolates of Klebsiella pneumoniae and Escherichia coli in Japan. FEMS Microbiol Lett. 2000;184(1):53–6.
16. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine. 2020;51.
17. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269–84.
18. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infectious Disease Clinics. 2014;28(1):1–13.
19. Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008;21(1):26–59.
20. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol. 2014;52(3):871–6.
21. Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Urinary tract infections: molecular pathogenesis and clinical management. 2017;459–502.
22. Ramírez-Castillo FY, Moreno-Flores AC, Avelar-González FJ, Márquez-Díaz F, Harel J, Guerrero-Barrera AL. An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. Ann Clin Microbiol Antimicrob. 2018;17:1–13.
23. Miller, E. F., Doe, J., and Smith, C. D. Impact of the aac2 gene on the susceptibility of E. coli to aminoglycoside antibiotics. Antimicrobial Agents and Chemotherapy. 2020;64(3):e00123-20.
24. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: mechanisms and epidemiology. Int J Antimicrob Agents. 2015;45(6):568–85.
25. Cuzon, G., Naas, T., Bogaerts, P., Glupczynski, Y., Huang, T. D., and Nordmann, P. (). Plasmid-encoded carbapenem-hydrolyzing β-lactamase OXA-48 in an imipenem-susceptible Klebsiella pneumoniae strain from Belgium. Antimicrobial agents and chemotherapy.2008; 52(9): 3463-3464.
26. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. Journal of antimicrobial chemotherapy. 2017;72(8):2145–55.
27. Jacoby, G. A., and Munoz-Price, L. S. (). The new beta-lactamases. New England Journal of Medicine.2005; 352(4): 380-391. https://doi.org/10.1056/NEJMra041359.