

Isolation and Identification of Bacteria from Patients with Transitional Cells Carcinoma of Urinary Bladder

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الخلاصة

أجريت هذه الدراسة لغرض عزل وتوصيف البكتيريا المرافقة للخلايا السرطانية للمثانة البولية ، فضلا عن الكشف عن التغيرات النسيجية المرضية للخلايا الطلائية للمثانة البولية. جمعت 130 عينة إدرار وتوزعت على ثلاثة مجاميع (36 عينة لمرضى مصابين بسرطان المثانة البولية ، 64 عينة من مصابين بالتهابات المجاري البولية و30 عينة من أشخاص أصحاء) تجربة ضابطة)). أخذت هذه العينات من كلا الجنسين وفي أعمار مختلفة واستمر جمع العينات مدة تراوحت بين كانون الثاني لغاية حزيران 2010 لمراجعي مستشفى الديوانية التعليمي.

أظهرت النتائج إن 32 عينة من أصل 36 عينة لمرضى سرطان المثانة كانت موجبة للزرع (88.8%) ، إما مرضى التهاب المجاري البولية أظهرت النتائج إن جميع العينات كانت موجبة للزرع (100%). وكانت البكتيريا المعزولة الشائعة والمرافقة للخلايا السرطانية للمثانة البولية والتهابات المجاري البولية متشابهة وشملت: (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). إلا إن نسب عزلها كانت مختلفة، حيث كانت اعلي نسبة عزل لبكتيريا *Escherichia coli* (35.9%) بينما كانت النسبة الأقل لبكتيريا *Klebsiella pneumoniae* (6.2%).

Abstract

This study was conducted to isolate and identify the bacteria associated with tumor cells urinary bladder, in addition to investigate the histopathological changes in the urothelium of the urinary bladder.

A total of 130 urine samples were taken and classified into three groups:(36 samples belong to patients with urinary bladder cancer, 64 samples of urinary tract infections(UTIs), and 30 samples as healthy group (control). These samples were collected from both sexes and different ages and lasted a period from January to June 2010 at Al-Diwaniyah Teaching Hospital.

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The results revealed that 32/36 (88.8%) of urinary bladder cancer samples were positive for bacterial growth, while was 64/64 (100%) for UTIs samples. The common isolated bacteria associated with tumor cells of urinary bladder and UTIs were similar and included six species (*Escherichia coli*, *Staphylococcus aureus*, *Proteus morganii*, *P. mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*). But the percentage of isolation was varied and the high percentage belonged to *Escherichia coli* (34.5%), and the lowest was *Klebsiella pneumonia* (6.2%).

Introduction

It is estimated that over 15% of malignancies worldwide can be attributed to infections or about 1.2 million cases per year. Infections involving viruses, bacteria and schistosomes have been linked to higher risks of malignancy¹. Although viral infections have been strongly associated with cancers², bacterial associations also are significant. Important mechanisms by which bacterial agents may induce carcinogenesis include chronic infection, immune evasion and immune suppression³. It has been shown that several bacteria can cause chronic infections or produce toxins that disturb the cell cycle resulting in altered cell growth⁴. The resulting damage to DNA is similar to that caused by carcinogenic apoptosis. Processes that encourage the loss of cellular control may be tumor initiators (directly causing mutations) or promoters (facilitating mutations)⁵. Tumor genesis is initiated when cells are freed from growth restraints, later promotion results when the immune system is evaded favoring further mutations and increased loss of cell control. Subsequent invasion occurs if the tumor breaks down surrounding tissues. The worst outcome is metastasis which results when cells break away from the tumor and seed tumors at distant sites⁶. The immune system is an important line of defense for tumor formation of malignancies that expresses unique antigens. Certain bacterial infections may evade the immune system or stimulate immune responses that contribute to carcinogenic changes through the stimulatory and mutagenic effects of cytokines released by inflammatory cells. These include reactive oxygen species

(ROS),⁷ , interleukin-8 (IL-8),⁸ , cyclooxygenase-2(COX-2),⁹ , reactive oxygen species (ROS) and nitricoxide (NO)¹⁰. Chronic stimulation of these Substances along with environmental factors such as smoking, or a susceptible host appears to contribute significantly to carcinogenesis. Several researcher found that certain bacteria are associated with human cancers but their role ,however, is still unclear. Convincing evidence links some species to carcinogenesis while others appear promising in the diagnosis, prevention or treatment of cancers¹¹.

The aim of study is concerned with the tumor cells associated with urinary bladder and aimed to isolation and identification of bacteria accompanied with tumor cells

Materials and Methods

-Samples Collection : The collection samples was divided into three groups:

1-The patients group of UTIs with bladder cancer contained 36 samples (27 males and 9 females).

2- The patients group with UTIs only(without bladder cancer)contained 64 samples(22 males and 42 females).

3- Healthy group: 30 urine samples of healthy individuals as control (did not have cancer and UTI). Ten ml of urine samples were taken and placed in test tubes, labeled and transported to the laboratory. The samples were collected from individuals who admitted to Al-Diwaniya teaching hospital after they were diagnosed by surgeon during the period from January to June 2010.

- Samples processing and culturing: The urine was mixed thoroughly and centrifuged before inoculation and the top of the container was removed. The calibrated loop was inserted vertically into the urine in a cup. The following prepared culture media (Blood agar, Nutrient agar and MacConkey agar) were inoculated and streaked to obtain isolated colonies. Urine culture was incubated over-night at 37C° for 18-24 hours to detect uropathogens¹². The isolated colonies were studies using the standard cultural characteristics to describe and identify the bacterial isolates.

Statistical Analysis

The results were analyzed statistically by Chi-square (X^2) test at the level of significant when P-value < 0.01.¹³.z

Results and Discussion

The results showed that 32 of 36 urine samples of patients with cancer were positive for culture (88.8%), while only 4(11.2%) urine samples were negative. While in the case of patients with UTI, the results showed that 64 of 64(100)% urine samples were positive for culture (Table:1)

Table (1): Number and percentage of bacterial growth of urine samples

Infection type	Samples No.	Positive	%	X^2 value
UTIs with bladder cancer	36	32	88.8	Cal X^2 =7.4 Tab X^2 =5.34 df= 1 significant (P<0.01)
UTIs without bladder cancer	64	64	100	
Control group	30	0	0	

A total of six bacterial species were isolated and identified which included the G-ve bacteria (*E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Proteus morgani*) and the G+ve bacteria included *Staphylococcus aureus* (Table:2)

Table (2): Bacterial species isolated from urinary bladder cancer and UTIs based on biochemical tests for Gram –ve and Gram +ve bacteria

Bacteria	Oxidase	MR	VP	Urean	H ₂ S production	Citrate	Nitrate reductase	Gas production	Triple-Sugar Iron	Indole	Coagulase
<i>E.coli</i>	-		-	-	-	-			A/A		-
<i>Proteus mirabilis</i>	-		-						ALK/A	-	-
<i>Klebsiella Pneumonia</i>	-		V	V	-				ALK	-	-
<i>Proteus morgani</i>	-	-	-		-	-			ALK		-
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	+	+	-	ATK/A	-	-
<i>S. aureus</i>	-	-	-	-	-			-	ALK		

The conformational diagnosis of Gram negative enteric bacteria was performed by Mini API 20 E system and the results were

obtained depending on interpretation kit chart and result entry to Mini API 20 E system(Table:3)

Table (3): Confirmative Biochemical Tests Using Mini API20E System:

Bio chemical tests	<i>E.coli</i>	<i>P. aeruginosa</i>	<i>Proteus. mirabilis</i>	<i>P.Morgan ii</i>	<i>K. pneumonia</i>
ADH	-	+	-	-	-
AMY	-	-	-	-	+
ARA	+	+	-	-	+
CIT	-	+	V	V	+
GEL	-	+	V	-	-
GLU	+	-	+	+	+
H2S	-	-	+	V	-
IND	+	-	-	+	-
INO	-	-	-	-	+
LDC	+	-	-	V	+
MAN	+	-	-	-	+
MEL	+	-	-	-	+
OMP	+	-	-	V	+
ODC	+	-	+	+	-
Oxidase	-	+	-	-	-
RHA	+	-	-	-	+
SAC	-	-	V	V	+
SOR	+	-	-	-	+
RHA	-	-	+	+	-

Table(4) shows the majority of bacterial species isolated from patients with bladder cancer were *E.coli* (34.5)%, followed by *Pseudomonas aeruginosa* (28.2)%, then *Proteus mirabilis*(15.5)% followed by *Proteus morganii* (9.4%),*Staphylococcus aureus* and *Klebsiella pneumonia* (6.2)%.

Bacteria are considered the major microorganisms isolated from urine¹⁴. The weakness of immune system of patient with bladder cancer is one major factor that predisposes danger of infection by bacteria. Most cases of cancer are associated with immune deficiency due to the engagement of body to get rid of tumor cells, which leads to secondary bacterial infection to the patients. The

source of secondary infection with bacteria is not determined yet¹⁵. The possibility of getting infection increases in immune compromised patients as patients with bladder cancer, especially when they take anti cancer drugs. Also the source of *S. aureus* bacteria may be from skin or air¹⁶.

Table (4): No. and percentage of bacteria associated with bladder cancer (n=36), than UTIs isolates(n=64)

Urinary bladder cancer			Urinary tract infection		
Bacteria species	No.	%	Bacteria species	No.	%
<i>Escherichia coli</i>	11	34.5	<i>Escherichia coli</i>	23	35.9
<i>Pseudomonas aeruginosa</i>	9	28.2	<i>Pseudomonas aeruginosa</i>	19	29.6
<i>Staphylococcus aureus</i>	2	6.2	<i>Staphylococcus aureus</i>	8	12.6
<i>Proteus mirabilis</i>	5	15.5	<i>Proteus mirabilis</i>	6	9.5
<i>Proteus morganii</i>	3	9.4	<i>Proteus morganii</i>	3	4.6
<i>Klebsiella pneumonia</i>	2	6.2	<i>Klebsiella pneumonia</i>	5	7.8
Total	32	100%	Total	64	100%

E coli was isolated from patients with bladder cancer in percentage (34.5%). This ratio agrees with¹⁷ and¹⁸ who isolated *E coli* rates (33.8 %) and (31.4%) respectively. While *Pseudomonas aeruginosa* was isolated in a percentage (28.2%). *Klebsiella pneumoniae* is considered the micro flora of intestine; they pose important virulence factors as capsule helping in increasing the opportunity to infect urinary system. The capsule protects the bacteria from harsh conditions and increases their resistance to immune system as phagocytosis process¹⁹. They also pose pili which help in adhesion to epithelia and increase the chance to cause infection.

The majority of *E coli* in urine samples agreed with a number of studies: ²⁰ isolated *E. coli* in a percentage (28.2%), ²¹ (30%) and ²² (32%). While other studies showed that the isolation of *E. coli* was in a ratio higher than the previous one; ²³ isolated the bacteria in rating (82%) and ²⁴ in a ratio (85%). Also, ²⁵ confirmed that this species occupies the pole position of list of bacteria isolated from urine, followed by *Pseudomonas*, *Proteus*, *Klebsiella* in different ratios. This agrees with the results of this study. The cause of high incidence infection with *E coli* belongs to the fact that these

bacteria leave their natural place (micro flora of intestine) to urinary pathways causing inflammation of urinary tract because they pose numerous virulence factors as pili helping in adhesion to epithelia of urinary system ²⁶.

The incidence of *Proteus sp* infection is considered less than the infection with *E. coli*; *Proteus mirabilis* were isolated in ratio (15.5)% and this ratio was almost similar to a number of studies: ²⁷ isolated *Proteus sp* from UTI patients in ratio (12.2)% ,while ²⁸ isolated this bacteria in ratio (13.7)%. ²⁹ showed that the infection with *Proteus* bacteria in men is higher four times than women.

The results of this study have shown that *Pseudomonas aeruginosa* bacteria was isolated from patients with UTI in ratio 29.6%, this ratio is higher than ratio of ³⁰ who found that the ratio was(17.1%) and ³¹ who reported the ratio (11%).

Previous studies have showed that the bacteria are one of the causative agents of nosocomial infections.

In this study, *Streptococcus. spp* did not isolated which is considered rare bacteria presented in urinary pathways ³².The incidence of mixed growth of two types of bacteria is considered a chronic inflammation of UTIs ³³. One of the stimulating factors that enhance the incidence of UTI is suffering some diseases as diabetes and prostate enlargement in men which may lead to block the stream of urine in urinary bladder that leads to the chance to bacterial growth and initiation of infection ³⁴.

Conclusions

- 1- There are a little variations in the number and percentage of bacteria isolated from urine samples of bladder cancer in comparison with urinary tract infection and the *E. coli* was the most common bacteria in urine samples.
- 2- Ciprofloxacin and Norfloxacin were the more effective antibiotics against G-ve isolates while *Staphylococcus aureus* was resistant to Trimethoprem and Amoxicillin.

Recommendations

- 1- Early diagnosis and treatment of UTIs in order to avoid the development of bladder cancer as a result of chronic irritation.
- 2- Determination of the genotype of isolated bacteria and detection are these bacteria play a role in cancer or not using PCR technique.
- 3- The need to follow up the resistance to antibiotics of isolated bacteria and determine the MICs to these antibiotics before using by patients.

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