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Isolation of Some Intestinal Bacteria and Study of Their Antibiotic Resistance and Detection of Virulence Genes

Sundus Khalid Ahmed[1] , Rana Jalal Shaker [2]
[1]*Master student ,Department of Biology , College of Education for Women. , Tikrit University, Iraq.*
Email [1]. Sundus.Ahmed437@st.tu.edu.iq
Email: [2] ranajalal@tu.edu.iq

ABSTRACT

This study aimed to isolate and identify intestinal bacteria from various clinical sources. A total of 76 bacterial isolates were collected, encompassing six types. The most prevalent was Escherichia coli (48.68%, 37 isolates), followed by Klebsiella pneumoniae (34.21%, 26 isolates), Proteus mirabilis (13.15%, 10 isolates), and one isolate each of Enterococcus faecalis, Enterobacter cloacae, and Raoultella ornithinolytica (1.31% each). Antimicrobial susceptibility testing revealed that E. coli showed the highest resistance to tetracycline (64%) and the lowest resistance to meropenem (5.4%). K. pneumoniae exhibited the highest resistance to ceftriaxone (73%) and the lowest to meropenem (11.53%). P. mirabilis showed high resistance to tetracycline (90%) but was fully sensitive to meropenem (0% resistance). The R. ornithinolytica isolate was fully resistant to ceftriaxone and cefixime (100%) and fully sensitive to meropenem. The E. cloacae isolate was fully resistant to cefixime (100%) and fully sensitive to amikacin, while E. faecalis showed full sensitivity to meropenem and full resistance to cefixime. Molecular analysis was conducted on 12 isolates (6 E. coli and 6 K. pneumoniae) to detect virulence genes. E. coli isolates showed a high detection rate for the ymdB and fimH genes (83.33% each), whereas K. pneumoniae isolates showed detection rates of 66.6% for KPHS and 83.33% for KFim genes. These findings highlight the diversity in antimicrobial resistance among intestinal bacteria and the presence of virulence genes that may contribute to their pathogenicity, emphasizing the need for ongoing antimicrobial stewardship and infection control.

Keywords : UTIs , TmdB , fimH, *K.pneumoneiae , E.coli, PCR*

عزل بعض البكتيريا المعوية ودراسة مقاومتها للمضادات الحيوية والكشف عن جينات الضراوة

سندس خالد أحمد ١، رنا جلال شاكر ٢
طالبة ماجستير، قسم علوم الحياة، كلية التربية للبنات، جامعة تكريت، العراق.
البريد الإلكتروني: Sundus.Ahmed437@st.tu.edu.iq1 .
البريد الإلكتروني: ranajalal@tu.edu.iq2 .

الملخص

هدفت هذه الدراسة إلى عزل وتحديد البكتيريا المعوية من مصادر سريرية مختلفة. جُمعت 76 عينة بكتيرية، شملت ستة أنواع. كانت الإشريكية القولونية الأكثر انتشارًا (48.68%، 37 عزلة)، تليها الكلبسيلة الرئوية (34.21%، 26 عزلة)، ثم بروتيوس ميرابيليس (13.15%، 10 عزلات)، وعزلة واحدة لكل من المعوية البرازية، والمعوية المجاري، وراؤولتيلا أورنيثينوليتيكا (1.31% لكل منها). كشف اختبار حساسية المضادات الحيوية أن الإشريكية القولونية أظهرت أعلى مقاومة للتتراسيكلين (64%) وأقل مقاومة للميروبينيم (5.4%). أما الكلبسيلة الرئوية، فقد أظهرت أعلى مقاومة للسيفترياكسون (73%) وأقل مقاومة للميروبينيم (11.53%). أما البروتيوس ميرابيليس، فقد أظهرت مقاومة عالية للتتراسيكلين (90%)، لكنها كانت حساسة تمامًا للميروبينيم (0%). أظهرت عزلة R. ornithinolytica مقاومة تامة للسيفترياكسون والسيفكسيم (100%) وحساسية تامة للميروبينيم. أما عزلة E. cloacae فكانت مقاومة تامة للسيفكسيم (100%) وحساسة تامة للأميكاسين، بينما أظهرت E. faecalis حساسية تامة للميروبينيم ومقاومة تامة للسيفكسيم. أُجري تحليل جزيئي على 12 عزلة (6 عزلات من الإشريكية القولونية و6 عزلات من K. pneumoniae) للكشف عن جينات الضراوة. أظهرت عزلات الإشريكية القولونية معدل كشف مرتفع لجينات ymdB و fimH (83.33% لكل منهما)، بينما أظهرت عزلات K. pneumoniae معدلات كشف بلغت 66.6% لجينات KPHS و83.33% لجينات KFim. تُسلّط هذه النتائج الضوء على تنوع مقاومة مضادات الميكروبات بين البكتيريا المعوية، ووجود جينات الضراوة التي قد تُسهم في قدرتها على التسبب بالأمراض، مما يُؤكد على ضرورة الاستمرار في إدارة مضادات الميكروبات ومكافحة العدوى.

الكلمات المفتاحية: التهابات المسالك البولية، TmdB، fimH، K.pneumoneiae، E.coli، تفاعل البوليميراز المتسلسل.(PCR)

INTRODUCTION

Urinary tract infections (UTIs) are among the most common infections in humans and can lead to serious complications. The majority of these infections are caused by members of the Enterobacteriaceae family. Estimates indicate that UTIs are the most prevalent bacterial infections. Under normal conditions, the urinary tract can eliminate these bacteria through effective defense mechanisms. However, if these bacteria overcome these defenses, they colonize the lower urinary tract. The progression of the infection then depends on the host's susceptibility and the strength of the bacterial virulence factors. Symptoms of the infection vary, ranging from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis, and even bloodstream infections. [1]. The *Enterobacteriaceae* family includes a large number of closely related bacterial species that colonize the large intestines of humans and animals, in addition to being found in soil, water, and decaying organic matter. Due to their natural habitat in humans, they are also known as "enteric bacteria." These organisms are among the leading causes of most hospital-acquired infections, including UTIs, wound infections, pneumonia, meningitis, and sepsis. Virulence, derived from the Latin word meaning "poisonous," is defined as the ability of an organism to cause disease in a specific host. This ability depends on the cumulative effect of one or more distinguishing characteristics, known as virulence factors (VFs), which differentiate potentially pathogenic strains from

harmless enteric strains. [2]. Antibiotics are a broad range of substances produced either naturally by other microorganisms, semi-synthetically derived from natural antibiotics, or entirely synthetic. Antimicrobial agents are used therapeutically as either bacteriostatic (inhibiting bacterial growth) or bactericidal (killing bacteria). They can be classified into easily understandable groups based on their site of action, chemical structure, and nature. [3]. An antibiotic affects microbial growth by targeting vital processes that contribute to their reproduction. A microbial population grows through the replication of individual cells via the duplication of cellular material and cell division into two. For antibiotics to exert their effect, they must first enter the microbial cell, then effectively bind to a cellular structure that plays a key role in the cell's survival or growth, and finally inhibit the biological process in which this structure is fully involved. In this way, antibiotics either halt growth or completely eliminate microbial cells. [4] Urinary tract infections (UTIs) are inflammatory diseases caused by the excessive proliferation of various pathogens in the urinary system, leading to impaired function of the urinary tract and kidneys. [5] Bacteria possess virulence genes, and among the studied virulence factors are

1. FimH Gene in *E. coli*

The fimH gene is part of the fim gene cluster, which encodes a surface organelle known as type 1 fimbriae, found in most Escherichia coli strains. The FimH protein is located at the tip of the fimbrial structure and acts as a specific adhesion factor for D-mannose, contributing to bacterial attachment to biological and non-biological surfaces. Research has shown minor variations in the fim gene sequences.[6]

2. YmdB Gene in *E. coli*

YmdB is an evolutionarily conserved protein in *E. coli*. Researchers have found that YmdB regulates RNase III cleavage processes, meaning it influences how this enzyme functions. YmdB interacts with the catalytic region of RNase III, affecting its activity. The expression of YmdB is activated when bacteria are exposed to various stresses, such as cold shock, or when they enter a dormant state where growth ceases.[7]

3. Fim Gene in *K. pneumoniae*

The fimK gene in *Klebsiella pneumoniae* encodes a protein that is part of the gene cluster responsible for forming fimbriae (hair-like structures on the bacterial surface that play a crucial role in adhesion to host tissues). This is a critical factor in *K. pneumoniae's* ability to cause disease. In *K. pneumoniae,* fimbriae help the

bacteria adhere to epithelial cells in the urinary tract, lungs, and other tissues, facilitating colonization and infection. The genes responsible for fimbrial formation, including fimK, are involved in the synthesis, assembly, and regulation of fimbriae. The FimK protein itself is part of the fimbrial gene cluster and plays a role in the expression and regulation of fimbriae. This gene can influence the bacteria's ability to adhere to surfaces, thereby affecting its pathogenicity. Changes in this gene may also affect bacterial resistance to host immune defenses, contributing to its virulence.[8]

4. KPHS Gene in *K. pneumoniae*

The KPHS gene in *Klebsiella* species is associated with the synthesis of capsular polysaccharides. *K. pneumoniae* is known for its large polysaccharide capsules, which play a vital role in virulence by helping the bacteria evade host immune responses. The KPHS gene contributes to the production of these capsular structures, which may vary among different strains, leading to diverse *Klebsiella* serotypes. *Klebsiella* species, such as *K. pneumoniae*, are opportunistic pathogens that can cause a range of infections, including pneumonia, UTIs, and bloodstream infections. Variations in capsule types due to differences in genes like KPHS are significant in terms of virulence, as the capsular polysaccharide can affect the bacteria's ability to survive in the body and evade immune defenses. [9]

The Study aimed To isolate and identify selected intestinal bacterial species responsible for urinary tract infections (UTIs) from clinical and environmental sources , evaluate the antibiotic resistance profiles of the isolated bacterial strains using the Kirby-Bauer disk diffusion method, detect virulence-associated genes (fimH and YmdB in *E. coli*; Kfim and KPHS in *K. pneumoniae*) using Polymerase Chain Reaction (PCR) techniques and analyze the correlation between the presence of virulence genes and antibiotic resistance patterns, in order to better understand pathogenic mechanisms and clinical implications.

MATERIALS AND METHODS

1- Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed on all clinical and environmental isolates using the disk diffusion method (modified Kirby-Bauer method, 1996) on Mueller-Hinton agar, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2024). [10]

Procedure:

1. Bacterial suspensions were prepared by transferring fresh colonies from solid culture media to 5 mL of sterile physiological saline (0.85% NaCl).

2. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard (NO).

3. Using a sterile cotton swab, the suspension was evenly spread on Mueller-Hinton agar plates.

4. Plates were allowed to dry for 2-3 minutes, then antibiotic disks were placed using sterile forceps (1 disk per plate).

5. Plates were incubated at 37°C for 24 hours.

6. Inhibition zones around antibiotic disks were measured in millimeters and interpreted according to CLSI standard tables (2024).

2- Detection of Virulence Genes

2.1 DNA Extraction

Protocol:

1. 1-1.5 mL of actively growing bacterial culture was centrifuged at 13,000 rpm for 3 minutes, and the supernatant was discarded.

2. 20 µL of proteinase K solution was added.

3. 500 µL of preheated Lysis Buffer was added and mixed using a vortex mixer for 10-15 seconds.

4. The sample was incubated at 60°C for 15 minutes, with inversion every 5 minutes.

5. Cooled at room temperature for 2 minutes.

6. 500 µL of binding buffer was added and mixed thoroughly using a vortex mixer for 10 seconds.

7. The mixture was transferred to a collection tube, then loaded into a spin column and centrifuged at 11,000 rpm for 1 minute at room temperature.

8. The flow-through was discarded, and the column was placed back in the same collection tube.

9. 500 µL of Wash Buffer-1 was added, followed by centrifugation at 11,000 rpm for 1 minute.

10. The flow-through was discarded, and the column was returned to the collection tube.

11. 500 µL of Wash Buffer-2 was added, followed by centrifugation at 13,000 rpm for 1 minute.

12. The flow-through was discarded, and the column was placed back in the collection tube.

13. A final centrifugation at 13,000 rpm for 3 minutes was performed to remove residual ethanol.

14. The column was transferred to a new 1.5 mL microcentrifuge tube.

15. 150 µL of preheated (60°C) elution buffer was added and left at room temperature for 5 minutes.

16. Centrifugation was performed at 11,000 rpm for 2 minutes at room temperature.

17. The DNA was either used immediately or stored at 4°C (short-term) or -20°C (long-term).

2.2 Quality Control

The quality of genomic DNA was assessed using a NanoDrop spectrophotometer.

2.3 Primer Preparation

- Primers were provided by Macrogen in lyophilized form.

- Lyophilized primers were reconstituted in nuclease-free water to prepare a 100 pmol/µL stock solution.

- A working solution (10 pmol/µL) was prepared by mixing 10 µL of stock primer with 90 µL of nuclease-free water and stored at -20°C.

2.4 Primer sequences

The primer sequences used in the study were designed using the NCBI website

Table 1 : Sequences of primers used in the study

The resulting base pair	direction	prefix sequence	Gen	ت
349	F	TTCATGTTGTGCAGGGTGAT	*E.coli* YmdB_	1
	R	ATTGCAGGAAAAGCCACTGA		
460	F	TATTTGACGCCTGTGAGCAG	*E.col* FimH_	2
	R	CTCCGGTACGTGCGTAATTT		
186	F	GGACATCAGCACCGTTGATA	*Klebsiella* KPHS_	3
	R	TCCTGGTATTCGCCAGATTC		
345	F	CGGACGGTACGCTGTATTTT	*Klebsiella* Kfim_	4
	R	CGTTGGGATCGAAAGGTTTA		

2.5 Polymerase Chain Reaction (PCR)

The following genes were detected:

- For *E. coli:* fimH and YmdB genes

- For *Klebsiella*: fimK and KPHS genes

The PCR reaction mixture was prepared using the AccuPower PCR PreMix kit (Bioneer, Korea) according to the manufacturer's instructions:

1. The mixture was prepared in pre-sterilized PCR tubes containing the basic reaction components. Additional components were added as per the manufacturer's protocol.

2. After preparation, tubes were tightly sealed and mixed carefully using a vortex mixer for 10 seconds.

3. Finally, tubes were transferred to a PCR thermocycler to complete the required thermal cycles.

Reaction setup details:

1. 12 µL of Master Mix

2. 2 µL of primer (forward + reverse)

3. 3 µL of DNA template

4. 8 µL of nuclease-free water

5. The mixture was vortexed briefly and centrifuged to collect the contents.

2.6 PCR Thermocycler Conditions

The thermocycler was programmed according to the parameters specified in the following table:

Table 2 PCR setup : Detailed Thermocycler Conditions

Step	Temperature	Duration	Cycles
Initialdenaturation	C95°	min5	1
Denaturation	C95	S30	30

Annealing	C57	S30	30
Extension	C72	S30	30
Final Extension	C72	min7	1
Hold	C4	forever	Hold

2.7Agarose Gel Electrophoresis

Solutions:

- TAE Buffer

- DNA ladder marker

- Ethidium bromide stain

Agarose Gel Preparation:

1. Added 10% sodium bromide solution (10 mL sodium bromide + 90 mL distilled water).

2. Added 1.2 g of agarose powder.

3. Mixed thoroughly and heated on a hot plate for 10 minutes until boiling

4. Allowed to cool, then added 2-3 µL of ethidium bromide per 100 mL of agarose solution

Horizontal Agarose Gel Preparation:

1. Poured agarose solution into a gel tray with sealed edges using sealing tape.

2. Allowed agarose to solidify at room temperature for 30 minutes.

3. Carefully removed the comb and placed the gel in the electrophoresis tank.

4. Filled the tank with sodium bromide solution until 3-5 mm above the gel surface.

2.8 DNA Electrophoresis

- PCR products were directly loaded (5 µL per well).

- Electrophoresis was performed at 100 V/100 mA for 60 minutes.

- DNA migrated from cathode (-) to anode. (+)

- Ethidium bromide-stained bands were visualized using a gel documentation system

RESULTS AND DISCUSSION

The table 3 presents the antibiotic susceptibility test results for the studied isolates, showing wide variations in resistance patterns:

For *E. coli*

Highest resistance: Tetracycline (TE) - 64% , Other resistances: Cefixime (CFM) - 59%, Ceftriaxone (CRO) - 59.45%, Levofloxacin (LEV) - 56.75%, Trimethoprim (TMP) , 56%, Ciprofloxacin (CIP) - 48.64% , Lowest resistance: Amikacin (AK) - 27%, Meropenem (MEM) - 5.4%, Ciprofloxacin (CIP) - 8.10%

For *K. pneumoniae:*

Highest resistance: Ceftriaxone (CRO) - 73% , Other resistances: Cefixime (CFM) - 65.38%, Tetracycline (TE) - 57%, Ciprofloxacin (CIP) - 42.30%, Amikacin (AK) - 38%, Trimethoprim (TMP) - 34.61%, Levofloxacin (LEV) - 30% , Lowest resistance: Imipenem (IPM) - 15%, Meropenem (MEM) - 11.53%

For *Proteus mirabilis:*

Tetracycline (TE) showed highest resistance at 90% , Other resistances: Trimethoprim (TMP) and Ceftriaxone (CRO) - 40%, Levofloxacin (LEV) - 30%, Amikacin (AK) and Ciprofloxacin (CIP) - 20% , Lowest resistance: Imipenem (IPM) and Cefixime (CFM) - 10%, Meropenem (MEM) - 0%

For *R. ornithinolytica*

100% sensitive to Meropenem (MEM) and Levofloxacin (LEV) , 100% intermediate to Ciprofloxacin (CIP) and Imipenem (IPM) , 100% resistant to Ceftriaxone (CRO), Cefixime (CFM), Amikacin (AK), Trimethoprim (TMP), and Tetracycline (TE).

For *E. cloacae*

100% sensitive to Imipenem (IPM), Levofloxacin (LEV), Amikacin (AK), Meropenem (MEM), and Tetracycline (TE), 100% intermediate to Ciprofloxacin (CIP) , 100% resistant to Trimethoprim (TMP), Ceftriaxone (CRO), and Cefixime (CFM)

For *E. faecalis*

100% sensitive to Meropenem (MEM) and Amikacin (AK),100% intermediate to Ciprofloxacin (CIP), Imipenem (IPM), Levofloxacin (LEV), and Tetracycline (TE) , 100% resistant to Trimethoprim (TMP), Cefixime (CFM), and Ceftriaxone (CRO)

Table 3 : Results of antibiotic sensitivity and resistance on bacterial isolates

R.Ornithinolytic			*E.faecalis*			*E.cloaca*			*P.mirabilis*			*K.pneumonia*			*E.coli*			Antibiotics
R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	
0%	100%	0%	0%	0%	100%	0%	100%	0%	30%	40%	30%	30.7%	46.1%	23%	56.7%	%32.4	%10.81	Levofloxacin
100%	0%	0%	100%	0%	0%	100%	0%	0%	10%	60%	30%	65.3%	30.7%	3.8%	59.4%	81.91%	%21.5	Cefixime
0%	0%	100%	0%	0%	100%	0%	0%	100%	40%	40%	20%	34.6%	53.8%	11.5%	56.7%	24.32%	%81.91	Trimethoprim
100%	0%	0%	100%	0%	0%	100%	0%	0%	40%	20%	40%	73%	23%	8.3%	3.8%	%24.3	%16.21	Ceftriaxone
0%	100%	0%	100%	0%	0%	0%	100%	0%	0%	90%	10%	11.5%	80.7%	7.6%	5.4%	%91.8	%2.7	Meropenem
0%	0%	100%	0%	100%	0%	0%	100%	0%	10%	80%	10%	15.3%	65.3%	19.2%	8.1%	%81	%10	Impenem
10	0%	0%	0%	10	0%	0%	10	0%	20	20	60	38.	19.	42.	27	%27	%44.9	Amikacin

0 %				0 %			0 %		%	%	%	4 %	2 %	3 %	%			
100 %	0 %	0 %	0 %	0 %	100 %	0 %	100 %	0 %	90 %	10 %	0 %	57.6 %	30.7 %	11.5 %	64.8 %	%21.6	%13.51	Tetracycline

PCR Results for Virulence Genes

For *E. coli:*

fimH gene (460 bp): 83% positive (6 isolates tested) , YmdB gene (349 bp): 83% positive.As show in figure 1

For *K. pneumoniae:*

KPHS gene (186 bp): 66.6% positive ,Kfim gene (345 bp): 83% positive.as show in figure 2 .

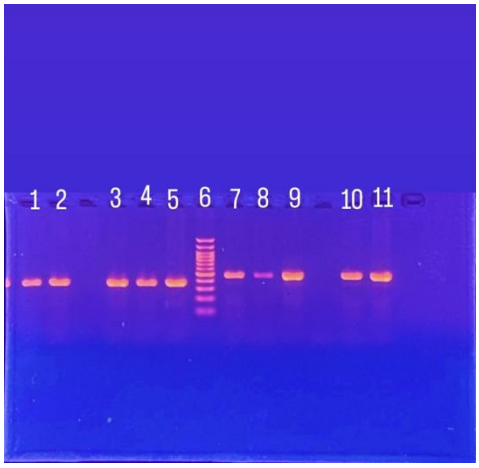

Figure 1 : electrophoresis results for *E.coli* bacteria

1, 2, 3, 4, 5 YmdB gene

6 Leader gene

7, 8, 9, 10, 11 FimH gene

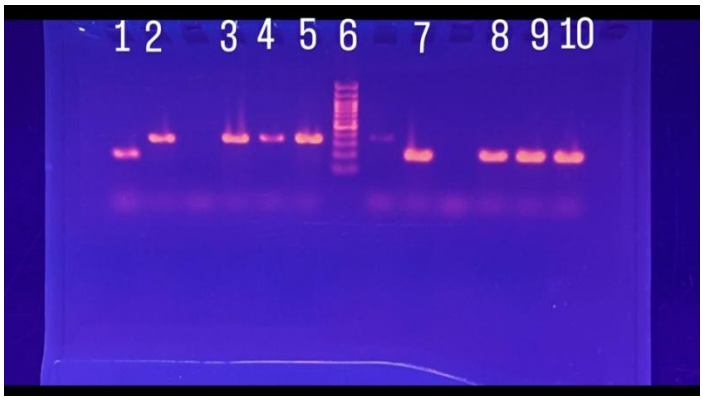

Figure 2 : electrophoresis results for *K. pneumoniae*

1, 2, 3, 4, 5 K fim gene

6 Leader

7, 8, 9, 10 KPHS gene

CONCLUSIONS

Enteric bacteria responsible for UTIs exhibit high resistance to commonly used antibiotics, Carbapenems remain the most effective therapeutic agents. The fimH and Kfim genes are significant indicators of adhesion and virulence in E. coli and K. pneumoniae, respectively and Molecular techniques such as PCR enhance understanding of infection mechanisms and support targeted therapeutic decisions.

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