



بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**  
**College of Graduate Studies**



**Genotypic detection of the Virulence Factors of Uropathogenic  
*Escherichia coli* Isolated from Diarrheic and Urinary Tract Infected  
Patients in Khartoum State**

الكشف الجزيئي عن عوامل الضراوة في بكتريا الاشريكية القولونية المسببة لعدوى المسالك  
البولية و الاسهالات في مرضى من ولاية الخرطوم

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medical laboratory science (Microbiology)

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى:

آمَنَ الرَّسُولُ بِمَا أُنزِلَ إِلَيْهِ مِنْ رَبِّهِ وَالْمُؤْمِنُونَ كُلٌّ آمَنَ بِاللَّهِ وَمَلَائِكَتِهِ وَكُتُبِهِ وَرُسُلِهِ  
لَا نُفَرِّقُ بَيْنَ أَحَدٍ مِنْ رُسُلِهِ وَقَالُوا سَمِعْنَا وَأَطَعْنَا غُفْرَانَكَ رَبَّنَا وَإِلَيْكَ الْمَصِيرُ

صدق الله العظيم

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## **Dedication**

### **I dedicate this work:**

To whom breastfed me the love and compassion to the symbol of love, and healing balms to pure whiteness heart.

(My mother)

To who spend His life working to give me a drop of love and happiness to that who pave my way to science to the big heart?

(My father)

To the pure hearts and innocent souls to my life basil's.

(My brothers and sister)

Now open the sails and raise the anchor for the ship to start running in the dark sea, the sea of life there is no light in this darkness except candles of distant memory, to those I loved them and loved me.

(My friends)

For all world nation that fight for life, for all Islamic nations and our lovely home.

Best wishes...

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## ABSTRACT

The aim of this study was to determine the virulence factor of Uropathogenic *E. coli* isolated from diarrheic and urinary tract infected patients in Khartoum State by multiplex PCR assay. A total of 100 clinical specimens (50 urine, 50 diarrhea) were collected in this study. Urine samples were culture on CLED agar, while diarrhea samples were culture on MacConky agar, identification scheme was done by conventional method. Modified Kirby-bauer method was performed using the following antibiotic discs; Gentamicin, Amikacin, Ciprofloxacin and Co-trimoxazole. Fifty five percent of samples were found sensitive to Gentamicin, 96% were sensitive to Amikacin, 57% were sensitive to Ciprofloxacin and 63% were sensitive to Co-trimoxazole. Boiling method was adopted for DNA extraction. Finally Multiplex PCR was done for the detection of *E. coli* virulent genes (*pap*, *fim*, *sfa*, *aer* and *hly*).

Most study population were females 57(57%); 42 of them suffering from UTIs and 15 suffering from diarrhea, while males were 43(43%); 8 of them were suffering from UTIs and 35 of them were suffering from diarrhea. Among enrolled subjects, 82 were positive for one or more Uropathogenic *E. coli* virulent genes. While 18 isolates were negative for all genes. The results of multiplex PCR searching for different virulent factors revealed the following: Thirty two (n=32) diarrheal samples appear as *aer* gene positive while the remaining fourteen (n=14) urine samples appear as *aer* gene positive. Thirty three (n=33) urine samples appear as *fim* gene positive while the remaining eight (n=8) diarrheal samples appear as *fim* gene positive. Twenty four (n=24) urine samples appear as *pap* gene positive while the remaining nine (n=9) diarrheal samples appear as

*pap* gene positive. Fourteen (n=14) urine samples appear as *hly* gene positive while the remaining three (n=3) diarrheal samples appear as *hly* gene positive. Fifteen (n=15) urine samples appear as *sfa* gene positive while in diarrheal samples was not detected. The study concluded that *fim* gene was highly prevalent among UTIs patients, *aer* gene was high prevalent among diarrhea patients and Amikacin is the most effective antibiotic

## المستخلص

هدفت هذه الدراسة الى تحديد عوامل الضراوة في البكتيريا الاشكريكية القولونية المسببة لعدوى المسالك البوية المعزولة من مرضى الإسهال ومرضى المسالك البولية في ولاية الخرطوم, حيث تم الكشف عنها عن طريق فحص تفاعل البلمرة المتعدد المحتوي على عدة بادئات. تم جمع 100 عينة (50 عينة بول، 50 عينة إسهال) في هذه الدراسة. ثم تم تزييع عينات البول علي وسط CLED وعينات الاسهال على وسط MacConkey و تم التعرف علي البكتريا بالطرق التقليدية. تم استخدام طريقة Kirby-bauer المطورة لاجراء إختبار الحساسية باستخدام اقراص المضادات الحيوية التالية:جنتاميسين، أميكاسين، سيبروفلوكساسين والكوتريموكسازول وجدنا أن 55% من العينات حساسة للجنتاميسين، 96% حساسة للأميكاسين، 57% حساسة للسيبروفلوكساسين و 63% حساسة للكوتريموكسازول. تم استخدام طريقة الغليان لاستخراج الحمض النووي.اخيرا تم إجراء إختبار تفاعل البلمرة المتعدد المحتوي علي عدة بادئات للكشف عن عوامل الضراوة (*pap*, *fim*, *sfa*, *aer* and *andhly*) في بكتريا الاشكريكية القولونية.

وكان معظم المشاركين في الدراسة من الإناث (57%)؛ 42 منهم يعانون من عدوى المسالك البولية و 15 منهم يعانون من الإسهال، في حين بلغ عدد الذكور 43 (43%)؛ 8 منهم يعانون من التهاب المسالك البولية و 35 منهم يعانون من الإسهال. من عينات الدراسة، كانت 82(82%) عينة إيجابية لواحدة أو أكثر منجينات الضراوة . بينما كانت 18(18%) عينة سلبية لجميع الجينات. نتيجة اختبار تفاعل البلمرة المتعدد المحتوي علي عدة بادئات للكشف عن عوامل

الضراوة كانت كالتالي: اثنين و ثلاثون (ن=32) عينة إسهال ظهرت موجبة للجين *aer* و المتبقي اربعة عشر (ن=14) عينة بول ظهرت موجبة للجين *aer*. ثلاثة و ثلاثون (ن=33) عينة بول ظهرت موجبة للجين *fim* و المتبقي ثمانية (ن=8) عينات إسهال ظهرت موجبة للجين *fim*. اربعة و عشرون (ن=24) عينة بول ظهرت موجبة للجين *pap* و المتبقي تسعة (ن=9) عينات إسهال ظهرت موجبة للجين *pap*. اربعة عشر (ن=14) عينة بول ظهرت موجبة للجين *hly* و المتبقي ثلاثة (ن=3) عينات إسهال ظهرت موجبة للجين *hly*. خمسة عشر (ن=15) عينة بول ظهرت موجبة للجين *sfa* و لم يظهر نفس الجين في اي عينة إسهال. وخلصت الدراسة إلى أن الجين *fim* كان منتشرا بكثرة بين مرضى عدوى المسالك البولية، وكان الجين *aer* عالي الانتشار بين مرضى الإسهال ووجدنا المضاد الحيوي الاميكاسين هو المضاد الحيوي الأكثر فعالية.



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## List of Abbreviations

<b>Abbreviation</b>	<b>Complete word</b>
DNA	Deoxyribonucleic Acid
UTIs	Urinary Tract Infections
UPEC	Uropathogenic <i>Escherichia coli</i>
<i>fim</i>	fimbriae type 1 gene
<i>pap</i>	pyelonephritis associated pili gene
<i>sfa</i>	S-family adhesions gene
<i>aer</i>	aerobactin gene
<i>hly</i>	hemolysin gene
PCR	Polymerase chain reaction
ELISA	Enzyme Linked Immunosorbant Assay
GUD	Beta-D-glucuronidase
UV	Ultraviolet light