

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى:

(واتقوا الله ويعلمكم الله والله بكل شيء عليم)

صدق الله العظيم

سورة البقرة الآية "282"

Dedication

I dedicate this work to

My parents

My wife

My son

MOHAMED

&

daughter

YOMENA

My brothers & sisters

To all who has ever taught me
anything

Acknowledgment

First of all my thanks are due to ALMIGHTY ALLAH for granting me the will and confidence to perform this study.

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Abstract

Mycolasmagenitalium (*M. genitalium*) is a gram negative bacterium that does not have a cell wall. It is classified as one of the sexually transmitted diseases and its infection may lead to sterility, if it is not treated in early stages.

With regards to the diagnosis of *M. genitalium*, the nucleic acid amplification test (NAAT), polymerase chain reaction (PCR) technique is recommended to be used rather than traditional methods for instance cultural and serological techniques, as they are not accurate and precise in investigating the *M. genitalium*. Thus molecular technique constitutes the theme of this thesis to detect mutations of the *M. genitalium* among Sudanese.

Two types of specimens, vaginal swab and first void urine were collected randomly from one hundred women with genitourinary symptoms, two hundred specimens in total. These specimens were taken from two hospitals of Khartoum area, Khartoum north teaching hospital and Alhya health centre during the period 2011-2012.

Prior samples collection, interviews and questionnaires were designed to collect demographic: age, sex, family history and clinical data. The urine specimens were collected in one ml phosphate buffer saline (PBS) container, whereas high vaginal swabs were collected in five ml tris-HCL to preserve DNA from damage. DNA was then extracted from all specimens and stored at -80C until time of PCR analysis.

The specimens were firstly tested by real-time PCR and secondly the positive ones were confirmed, using conventional PCR for targeting domain V 23SRNA and *mgpB* genes. Finally, DNA sequencing was done for both genes, V 23SRNA and *mgpB*. This was to detect macrolides antibiotics resistance for the former and to know genotyping of

M.genitalium for the latter. Therefore, mutation was recognized by comparison between isolated sequences versus control strains of *M.genitalium* G-37.

Of studied samples, 4% of Sudanese women were detected with *M.genitalium*. These strains were studied to recognize their single nucleotide polymorphisms (SNPs) of *mgpB* gene, using MgPa-13. Accordingly, six mutations were detected, four in SDN19 and two in SDN51 or SDN151, when aligned with control strain sequence of *M.genitalium* G-37. After that, the sequences of *mgpB* were submitted to GenBank under accession numbers (KF612736-38) to be as a reference for further studies in the future.

In conclusion, the first void urine specimen is recommended to be used for detecting *M.genitalium* rather than high vaginal swab. All detected polymorphisms of *M.genitalium* among Sudanese were sensitive to Macrolide antibiotic. The primary screening leucocyte esterase test was not sensitive in detecting *M.genitalium*. Therefore, PCR method is accurate and precise to diagnose patients with *M.genitalium*.

الخلاصة

المفطورة التناسلية عبارة عن بكتريا سالبة الجراملا تحتوي على جدار خلوي, وتعتبر إحدى أنواع البكتريا المنقولة جنسيا, لأنها تسبب الكثير من الأمراض التناسلية لدى النساء, منها التهابات عنق الرحم والمبايض. وإذا أهمل علاجها في مراحلها الأولى قد تؤدي إلى عقم.

تقنية سلسلة تفاعلات البلمرة تعتبر هي الطريقة المثلى لتشخيص المفطورة التناسلية حاليا بدلا من استخدام الطرق التقليدية القديمة مثل التزريع والتفاعلات السيرولوجية. عليه فإن هذه الدراسة استخدمت الطريقة الأولى لتحديد الإصابة بهذه البكتريا لمعرفة التركيبة الجينية لها والطفرات المختلفة وسط المرضى السودانيين ومن ثم تحديد العلاج المناسب لها, مستخدما أحدث أنواع تقنية الاحياء الجزيئية.

الجدير بالذكر أنه لا توجد دراسات سابقة في السودان على حد علمي - تحدد نسبة وجود هذا النوع من البكتريا ولذلك الدراسة الحالية حذبت على معرفت سلالتها المختلفة وصولا إلى العلاج المناسب للقضاء عليها مبكرا.

جمعت مائتا عينة عشوائياً في الفترة ما بين (2011م-2012م), منها مائة عينة بول ومائة مسحة من المهبل, وأخذت هذه العينات من مائة امرأة, راجعن مستشفى الخرطوم بحري التعليمي بقسم النساء والتوليد و مركز صحيا الحياة الخيري بأمدردمان بغرض العلاج والمتابعة, وبعد ذلك أخذت موافقتهم, ومن ثم تمت تعبئة استمارة الاستبيان, ووضعت عينات البول في حاوية معقمة تحتوي على (1مل) من (PBS) . ووضعت مسحة المهبل في أنبوب يحتوي على (5 مل) من محلول (Tri-HCl) لحفظ (DNA) من التلف, وأرسلت العينات إلى المختبر, وتم استخلاص الحمض النووي من العينات وحفظها في درجه (- 80 مئوية) إلى حين تحليلها. وقد تم تحليل المائتي عينة باستخدام تقنية البلمرة الجزيئية السريعة, ثم تم

تأكيد صحة العينات الموجبة باستخدام تقنية البلمرة الجزيئية التقليدية التي تستهدف الجين (23S rRNA)، ومن ثم تم التوصيف الجزيئي للجين (mgpB)، وتحديد الشفرات الجينية ومقارنتها بالسلالة الأصل G-37 لمعرفة الطفرات الجينية في السلالات المعزولة.

أظهرت النتائج أن نسبة الإصابة ببكتريا المفطورة التناسلية 4%. كما تم توصيف الجين mgpB بوضع الشفرات في بنك الجينات العالمي بالأرقام: (KF612736-38). وبمقارنة الشفرات المعزولة مع السلالة الأصل تبين وجود ست طفرات وراثية.

خُلصت الدراسة إلى وجود بكتريا المفطورة التناسلية في السودان، ووجود طفرات في السلالات المعزولة التي ليست لها مقاومة لعقار الماكرولايت. عينات البول كانت أفضل من مسحات المهبل في الكشف عن المفطورة التناسلية. وكانت كل السلالات المعزولة أكثر حساسية للعقار الماكرولايت المستخدم لعلاج هذه البكتريا. الاختبار المسحي الأولي المسمى (Leucocyte esterase test) للكشف عن المفطورة التناسلية في البول، ليس له فائدة كبيرة في التشخيص. عليه فإن الدراسة الحالية توصي باستخدام تقنية سلسلة تفاعلات البلمرة في مراكز الخدمات الصحية لدقة نتائجها وصولاً للتشخيص السليم.

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List of Abbreviations

Abbreviation	Meaning
PID	Pelvic inflammatory disease
TFI	Tubal factor infertility
NAATS	Nucleic acid amplification test
TMA	Transcription –mediated amplification
RFLP	Restriction fragment length polymorphism
SPSS	Statistical package for social science
IRS	Inhibitor removal solution
WS	Wash solution
AC	Absorption column
EB	Elution buffer
HVS	High vaginal swab
<i>M.genitalium</i>	<i>Mycoplasma genitalium</i>
NGU	Non gonococcal urethritis
PBS	Phosphate buffer saline
UK	United Kingdom
USA	United States of America
STD	Sexually transmitted diseases
<i>C.trachomatis</i>	<i>Chlamydia trachomatis</i>
GAPDH	glyceraldehyde-3-phosphate
SP	Sucrose phosphate
LE	leucocytes esterase
MgPa	<i>Mycoplasma genitalium</i> protein
PCR	Polymerase chain reaction
NTPs	Nucleotriphosphates
BV	Bacterial vaginosis
TV	<i>Trichomonasvaginalis</i>
PEACH	PID <i>Evaluation</i> And Clinical Health
Ct	Threshold cycle