

***Mycoplasma genitalium*, passenger or pathogen?**

BY

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DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy in Microbiology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Signature of candidate

_____ day of _____ 20 ____

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SUMMARY

Mycoplasma genitalium is the smallest existing self-replicating prokaryote, lacks a cell wall and has a genome consisting of only 580 kilo base pairs. It has characteristic pear/flask-like morphology with a terminal tip organelle used for attachment. Many researchers, mainly in developed countries, have investigated the role the organism plays in the aetiology of male urethritis and the majority of studies show an association between *M. genitalium* and male urethritis. In this study, the modified Koch's postulates were applied to answer the question whether *M. genitalium* is a true pathogen, or merely a passenger, invading already inflamed or damaged cells.

A total of 300 urine specimens were collected from adult males with symptoms and/or signs of urethritis and 75 from asymptomatic men. In the first study, three molecular assays; viz, a commercial conventional PCR test, a real-time PCR (q-PCR) test and a transcription mediated amplification (TMA) assay were evaluated for the detection of *M. genitalium*. The comparison between the assays was based on the extended gold standard concept, where a specimen was deemed positive when any two nucleic acid amplification tests were positive. In the second study, the specimens were tested for four common urethral pathogens (*N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis* and *M. genitalium*) using TMA assays. Finally, the bacterial loads for *M. genitalium* were determined using the q-PCR assay.

All three assays tested were highly specific (98-99%) for the detection of *M. genitalium*. However, where q-PCR and TMA demonstrated high sensitivities (96% and 100%), the sensitivity of the conventional PCR assay was low (78%). One or more pathogens were detected in a total of 129 (43%) men with urethritis. *M. genitalium* was the most frequently detected pathogen in men with urethritis (129; 43%), and significantly more ($p= 0.04$) than in asymptomatic men (7; 9.0%).

There is a strong association with *M. genitalium* bacterial load and clinical urethritis. Patients with urethral discharge had significantly higher *M. genitalium* concentrations than those with only burning on micturition ($p<0.001$), and the bacterial concentrations in men with symptoms and/or signs of urethritis were significantly higher than that in asymptomatic men ($p=0.02$). As the number of organisms increased, the severity of the symptoms increased; an indication of the role that the organism plays in disease progression.

In conclusion, by applying the modified Koch postulates, it was shown that *Mycoplasma genitalium* is by no means a passenger, but rather an important cause of adult male urethritis that should be taken into account when making diagnosis and when designing treatment strategies.

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ABBREVIATIONS USED:

A:	adenine
ATCC:	American Type Culture Collection
BOM:	burning on micturition
bp:	base pair
C:	cytosine / concentration
CDC:	Centers for Disease Control and Prevention
cDNA:	copy deoxyribonucleic acid
CFT:	complement fixation test
CI:	confidence interval
cPCR:	conventional polymerase chain reaction
Ct:	threshold cycle
CT:	<i>Chlamydia trachomatis</i>
<i>C. trachomatis:</i>	<i>Chlamydia trachomatis</i>
dAs:	deoxyadenosines
DKA:	dual kinetic assay
DNA:	deoxyribonucleic acid
dNTP:	deoxyribonucleotide
DOH:	Department of Health
dTTP:	deoxythymine triphosphate
dTs:	deoxythymidines
dUTP:	deoxyuracil triphosphate
eg:	for example
EIA:	enzyme immuno assay
EM:	electron microscope
<i>et al:</i>	and other

Fig:	figure
FL:	fluorescein
FRET:	fluorescent resonant energy transfer
FVU:	first void urine
fwd:	forward
g:	gram / gravitation
G:	guanidine
<i>gap</i> :	<i>glyceraldehyde-3-phosphate dehydrogenase gene</i>
GAPDH:	glyceraldehyde-3-phosphate dehydrogenase
gDNA:	genomic DNA
geq:	genome equivalents
GUM:	genitourinary medicine
IC:	internal control
ICSB:	International Committee for Systemic Bacteriology
i.e.:	that is
kbp:	kilo base pairs
kDa:	kilo Dalton
l:	liter
LAMP:	Lipid-Associated Membrane proteins
LC:	LightCycler
LCR:	ligase chain reaction
m:	meter / mass
M:	molar
mg:	milligram
MG:	<i>Mycoplasma genitalium</i>
MgCl ₂ :	magnesium chloride

<i>M. genitalium</i> :	<i>Mycoplasma genitalium</i>
<i>MgPa</i> :	<i>Mycoplasma genitalium</i> adhesion gene
MIC:	minimum inhibitory concentration
ml:	milliliter
MSM:	men having sex with men
MsrA:	methionine sulfoxide reductase
MW:	molecular weight
MUS:	male urethritis syndrome
n:	number / genome size
NAAT:	nucleic acid amplification test
ng:	nanogram
NG:	<i>Neisseria gonorrhoeae</i>
<i>N. gonorrhoeae</i> :	<i>Neisseria gonorrhoeae</i>
NGU:	non gonococcal urethritis
NCNGU:	non chlamydial non gonococcal urethritis
NIH:	National Institutes of Health
NTPs:	nucleotides
OR:	odds ratio
ORF:	open reading frame
out:	outer
P:	Protein
³² P:	Radioactively charged phosphate
PAGE:	polyacrylamide gel electrophoresis
PCR:	polymerase chain reaction
PID:	pelvic inflammatory disease
PMNLs/hpf:	polymorphonuclear leukocytes per high power field
q-PCR	real-time polymerase chain reaction

rev:	reverse
RFLPs:	restriction fragment length polymorphisms
RLUs:	relative light units
RNA:	ribonucleic acid
rRNA	ribosomal ribonucleic acid
s:	second
S:	Svedberg
SDS:	sodium dodecyl sulphate
SEM:	scanning electron microscope
SM:	size marker
SNPs:	single nucleotide polymorphisms
SP:	sucrose phosphate
spp:	species
STD:	Sexually Transmitted Disease
STI:	Sexually Transmitted Infection
STR:	short tandem repeat
T:	thymine
TBE:	Tris-borate- Ethylenediaminetetraacetic acid
TCS:	target capture system
TEM:	transmission electron microscope
T _m :	melting temperature
TMA:	transcription mediated amplification
Tris-EDTA:	
TTU:	ten tube unit
TV:	<i>Trichomonas vaginalis</i>
<i>T. vaginalis</i> :	<i>Trichomonas vaginalis</i>
U:	units
UNG:	uracil-N-glycosylase

USA: United States of America

U. urealyticum: *Ureaplasma urealyticum*

V: volume

vs: versus

Symbols:

% percentage

μ micro

° degrees