Network di Microbiologia e Virologia
del Nord Est

LE INFEZIONI DA MICOPLASMI UROGENITALI

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Incontro di Aggiornamento

LE INFEZIONI DELL'APPARATO GENITALE E LE MALATTIE A TRASMISSIONE SESSUALE (non HIV): ASPETTI CLINICI E DIAGNOSTICI
25 gennaio 2013
Auditorium Ospedale S. Chiara
Trento

ECOSISTEMA

MICROBICO

DELLA VAGINA

Ecosistema vaginale

La vagina può essere considerata uno dei modelli più completi ed interessanti per lo studio dei rapporti tra ospite e flora microbica residente

La simbiosi mutualistica
tra ospite e flora lattobacillare
è l'aspetto caratterizzante l'ecosistema vaginale

L'equilibrio dell'ecosistema vaginale
costituisce il principale fattore di difesa
contro le infezioni delle basse vie genitali

La flora vaginale
è dominata dalla presenza
di differenti specie di lattobacilli,
che insieme costituiscono
“la flora di Doderleïn”

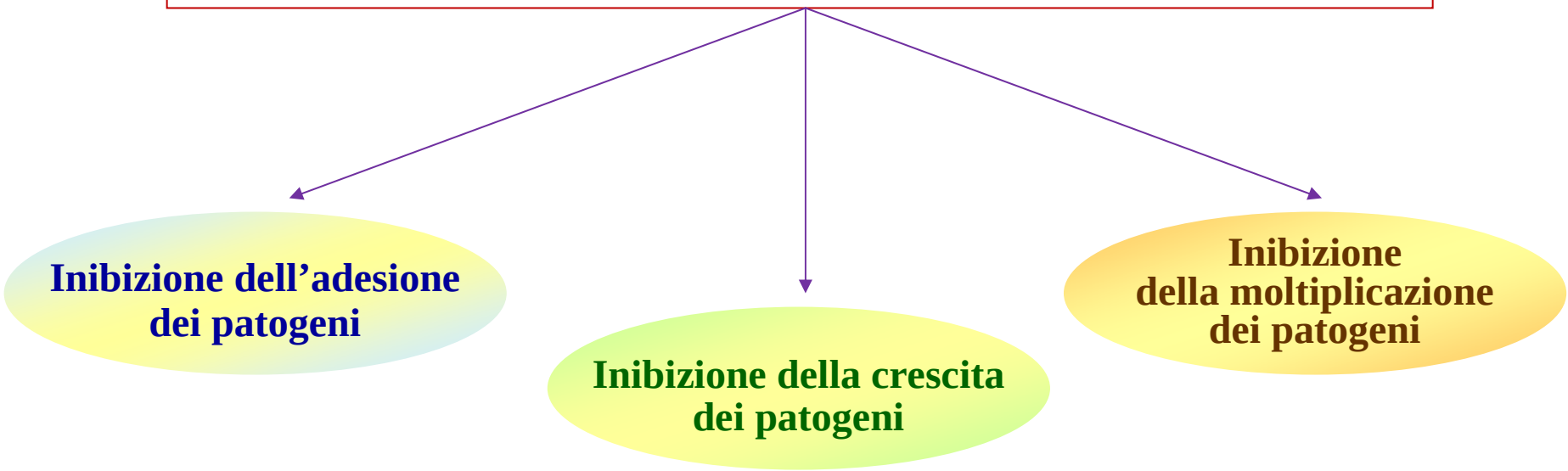
- *Lactobacillus acidophilus*
- *Lactobacillus fermentum*
- *Lactobacillus plantarum*
- *Lactobacillus brevis*
- *Lactobacillus jensenii*
- *Lactobacillus casei*
- *Lactobacillus cellobiosus*
- *Lactobacillus leichmanii*
- *Lactobacillus delbrueckii*
- *Lactobacillus salivarius*

J. P. Lepargneur et al. - *J. Gynecol. Biol. Reprod., 31: 485-494,2002*

J. P. Lepargneur et al. - *J. Gynecol. Biol. Reprod., 31: 485-494,2002*

Ruolo protettivo della flora di Doderleïn

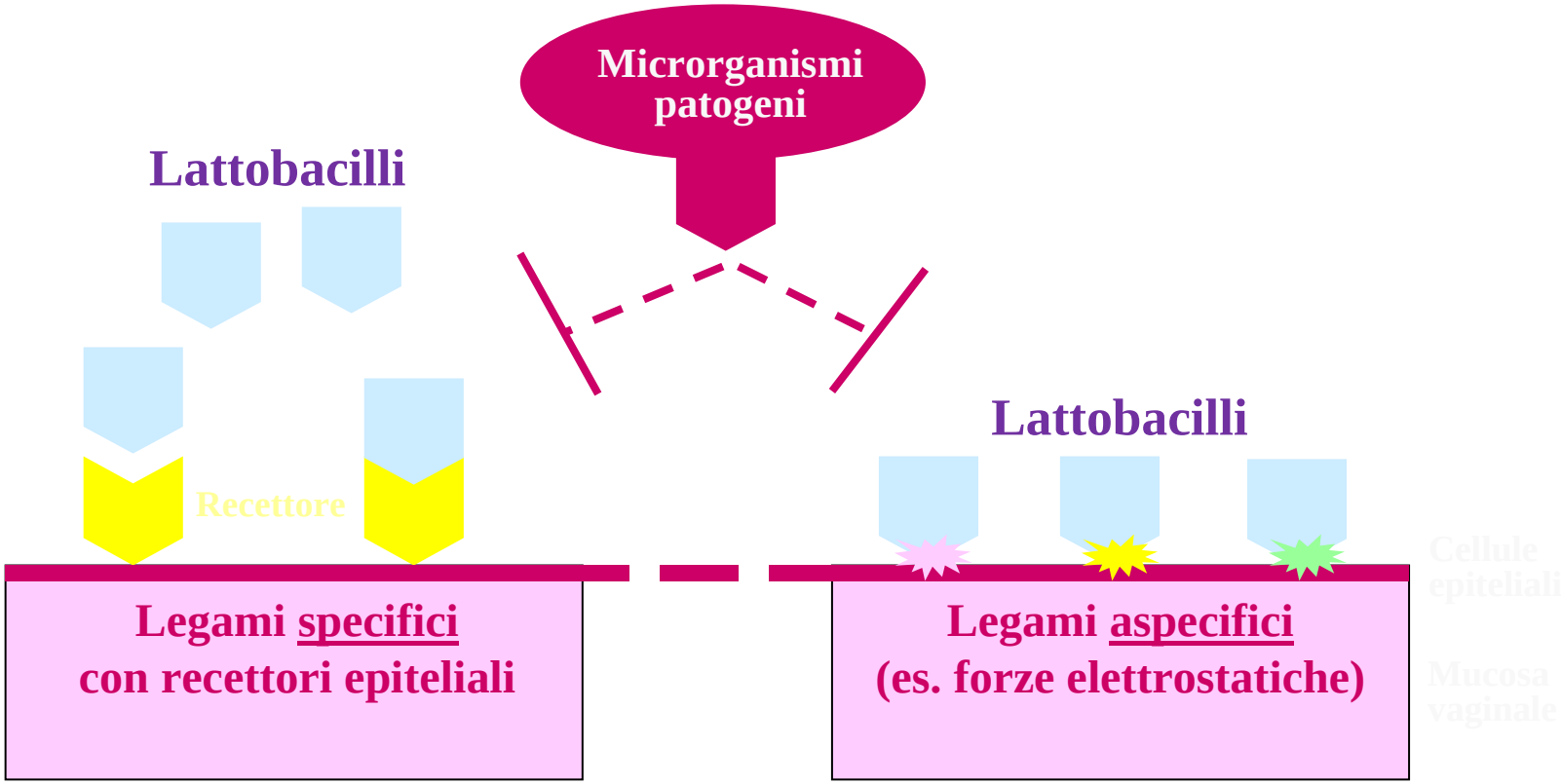
I lattobacilli mettono in atto
una serie di meccanismi
per svolgere un effetto protettivo
a difesa della mucosa vaginale
dall'aggressione dei microbi patogeni



J. P. Lepargneur et al. - *J. Gynecol. Biol. Reprod., 31: 485-494,2002*

Inibizione dell'adesione dei patogeni alla mucosa vaginale

I lattobacilli si legano in modo specifico e aspecifico
alle cellule epiteliali della mucosa vaginale...

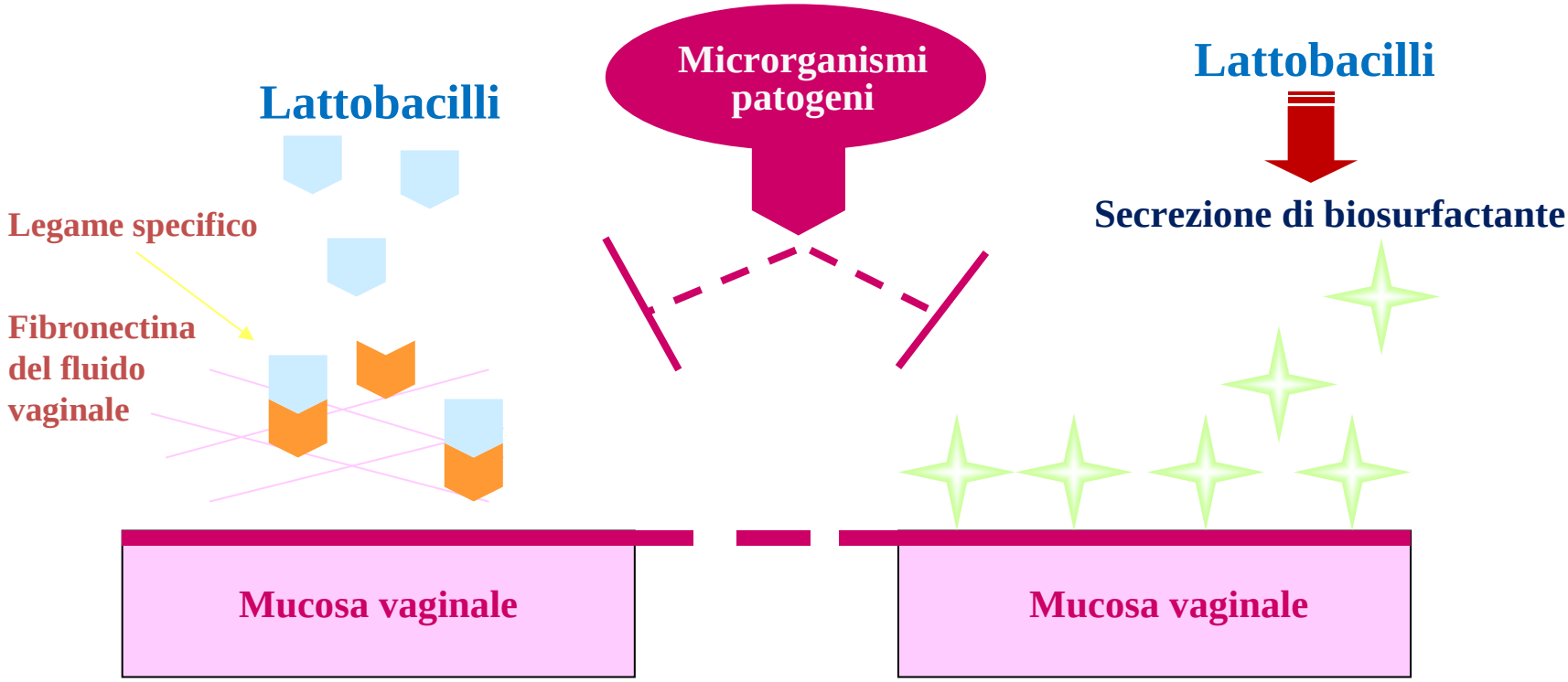


… ed impediscono ai microrganismi patogeni
di trovare liberi i siti di legame e quindi aderire alla mucosa vaginale

J. P. Lepargneur et al. - *J. Gynecol. Biol. Reprod., 31: 485-494,2002*

Inibizione dell'adesione dei patogeni alla mucosa vaginale

I lattobacilli si legano alla fibronectina del fluido vaginale
e producono biosurfattante…



… ulteriori meccanismi che impediscono ai microrganismi patogeni
di aderire alla mucosa vaginale

J. P. Lepargneur et al. - *J. Gynecol. Biol. Reprod., 31: 485-494,2002*

Inibizione della crescita dei patogeni

I lattobacilli sintetizzano sostanze fondamentali
per il mantenimento di un corretto equilibrio
nell'ecosistema vaginale...

Lattobacilli

***estrogeni* ±**

Acido lattico	H_2O_2	Batteriocine	Competizione per fonti nutrizionali
Bassi valori di pH 3,5 - 4,0	Effetto battericida su alcuni ceppi	Proprietà antibiotiche	Ad es. privazione di arginina (anaerobi)

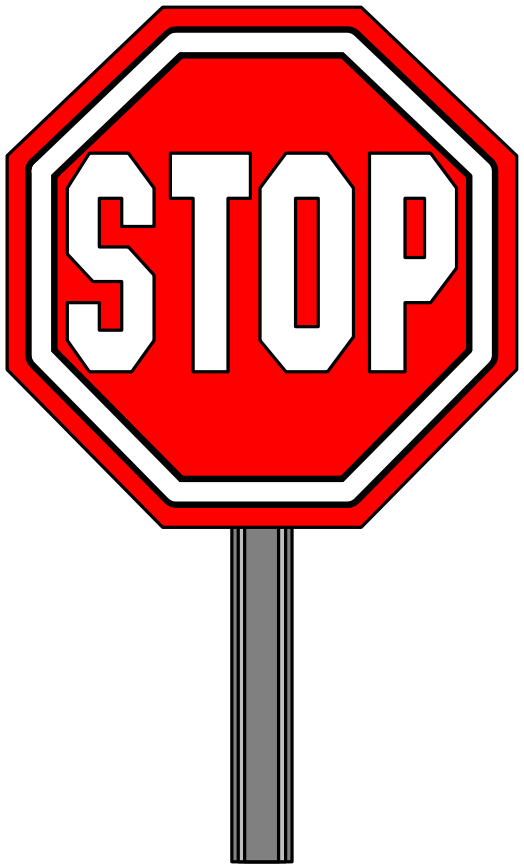
…in quanto agiscono come fattori di difesa
contro l'insorgenza di infezioni microbiche

NORMALE FLORA MICROBICA NELLE DONNE IN ETA' FERTILE

- **Lattobacilli**
- *S. epidermidis*
- Difteroidi
- Peptostreptococchi
- *Bacteroides* spp.
- *E. coli*
- *G. vaginalis*
- *Candida* spp.
- altri (**Micoplasmi**, ...)

FATTORI DI RISCHIO DELL'OMEOSTASI VAGINALE

DIFESE VAGINALI DELL'OSPITE



- **ANATOMIA DEI GENITALI ESTERNI**
- **STRUTTURA E SPESSORE EPITELIO**
- **pH VAGINALE ACIDO**
- **FLORA MICROBICA**
- **IMMUNOGLOBULINE**

Eventi fisiologici o patologici possono determinare

↓

una consistente diminuzione
dell'azione antagonista dei lattobacilli

↓

con alterazione dell'equilibrio dell'ecosistema vaginale
favorendo infezioni di tipo opportunistico

↓

da parte di microrganismi
non solo alloctoni ma anche autoctoni

Alcune condizioni rappresentano “fattori di rischio” di alterazioni dell’ecosistema vaginale

- *Età*
- *Attività sessuale*
- *Ciclo mestruale*
- *Gravidanza*
- *Trattamenti con antibiotici*
- *Trattamenti ormonali*
- *Abitudini sessuali*
- *Uso di contraccettivi*
- *“Abuso” di igiene intima*

BIOLOGY

Characteristic of *Mycoplasma* and *Ureaplasma*

General

- prokariotic
- small size 150-250 nm
- no cell wall

a. insensivity to beta-lactam antibiotics
b. no Gram staining
c. pleomorphic form

- trilayered cell membrane
- most are aerobic
- fastidious growth requirements

Characteristic of *Mycoplasma* and *Ureaplasma*

Differentiation from bacteria and L forms

- sterols in membrane
- no DNA homology with known bacteria (probably devolved from gram positive bacteria, *Lactobacillus* or *Clostridium*, through reductive evolution)
- low guanine + cytosine content
- low molecular weight genome (580 to 1170 kb)
- no reversion to walled forms

Differentiation from virus

- presence of both DNA and RNA
- division by binary fission
- free living-cell free growth in defined media in vitro
- extracellular parasitism in vivo

HISTORY

1898 Nocard and Roux

First to isolate mycoplasmas in a case of bovine pleuropneumonia. Microorganism descibed as **“d’une estreme tenuité, de dimensions trés inferieures à celles plus petits microbs connus” (PPLO, PleuroPneumonia-Like Organisms).**

1937 Diens and Edsall

From a Bartholin’s gland abscess, probably *M. hominis*

1954 Shepard

First description of T-strain mycoplasmas, later known as ureaplasma, isolated from the urethra of men with nongonococcal urethritis

1960s

Class ***Mollicutes*** established to include the mycoplasmas and related organisms and subdivided in **4 orders, 5 families, 8 genera and more than 200 known species**, detected in humans, vertebrate animals, arthropods and plants

1981 Taylor Robinson

M. genitalium from the urethra of men with nongonococcal nonchlamydial urethritis

1999 Kong

New species in *Ureaplasma* genus: *Ureaplasma parvum*

TAXONOMY

Class: ***Mollicutes*** "soft skin"

Order: ***Mycoplasmatales*** "fungus form"

(plant, bird and animal Mycoplamas)

Family: ***Mycoplasmataceae*** (humans and animals)

Genera: ***Mycoplasma and Ureaplasma OR***

Genital Mycoplasmas (humans)

Ecological niche: mucosal surface in humans

1. Respiratory tract
2. GU tract

EPIDEMIOLOGY

Trasmission

By direct contact between hosts:

- venereally though genital or oral-genital contact
- vertically from mother to offspring either at birth or in utero

Prevalence of genital Mycoplasmas

Apart from sexual contact, is influenced by age, race, socio-economic status, contraception, menstruation, menopausal change and pregnancy

M. hominis and ureaplasmas:

most studied because of their relative ease of cultivation

Ureaplasmas:

prevalent with the onset of sexual activity in both males and females, but highly prevalent in the genital tracts of healthy sexually active women, with infection rates of

60 to 70% *U. parvum* strains account for 70% of ureaplasma detected.

Lower the prevalence of ureaplasmas in **male urethra** (10-20%).

Infants can be infected at birth. The organism persist for a short time and disappear by age 2 years.

It is not known how long an individual ureaplasmal serovar or biovar persists in carriers or if there are changes in types over time.

Human Mycoplasmas

SPECIES	PREVALENCE IN GENITAL TRACT OF HEALTHY PEOPLE
Genital Mycoplasmas	
U. urealyticum	**++**
U. parvum	**++++**
M. hominis	**+++**
M. genitalium	**unknown**
M. fermentans	**+?**
M. penetrans	**Rare**
M. primatum	**Rare**
M. spermatophilum	**Rare**

Mandell, Douglas and Bennet “Principles and Practice of Infectious Diseases”, 2010

Colonisation of Genital Tract by Mycoplasmas

- MH & U belong to commensal vaginal flora (U ++) and their prevalence increase in pregnancy
- Yet colonisation of endocervix and uterus is anormal and can induce obstetrical complications
- MG does not belong to normal flora and is a STI agent (epidemiology similar to C. trachomatis). Its actual role in pregnancy abnormal outcome not well established but could be responsible for termination of pregnancy*

*Lawton BA, Contraception, 2008; 78: 294-8

Features of Mycoplasmas

- *M. hominis* (MH)
- Ureaplasma
  - *U. urealyticum* (UU)
  - *U. parvum* (UP)

Can be part of commensal vaginal flora

- *M. genitalium* (MG), always a pathogen (STI)
- *M. fermentans* ?? 1 study: 4/232 positive – 2 chorioamnionitis*

* Blanchard A., Clin Infect Dis, 1993; 17 (Suppl 1): S272-9

Taken as a whole, the high prevalence of ureaplasma and *M. hominis* in healthy people indicates that they are not prime pathogens.

What role in human disease?

TABLE 1. Mollicute flora of humans[a]

Organism	Primary site of colonization		Role in human disease[b]
	Respiratory tract	Urogenital tract	
Acholeplasma laidlawii	+	−	No
Mycoplasma amphoriforme	+	−	?
Mycoplasma buccale	+	−	No
Mycoplasma faucium	+	−	No
Mycoplasma fermentans	+	+	Yes?
Mycoplasma genitalium	−	+	Yes
Mycoplasma hominis	−	+	Yes
Mycoplasma lipophilum	+	−	No
Mycoplasma orale	+	−	No
Mycoplasma penetrans	−	+	?
Mycoplasma pirum	?	?	No
Mycoplasma pneumoniae	+	−	Yes
Mycoplasma primatum	+	+	No
Mycoplasma salivarium	+	−	No
Mycoplasma spermatophilum	−	+	No
Ureaplasma parvum	−	+	Yes
Ureaplasma urealyticum	−	+	Yes

[a] Mollicute species listed are those for which humans are presumed to be the primary host. The table does not include occasional isolates likely to be of animal origin that have been recovered from humans on rare occasions.

[b] In immunocompetent persons.

Waites KB et al, *Clin Microbiol Rev*, 2005

TABLE 2. Diseases in adults associated with or caused by *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma* species[a]

Disease	*Ureaplasma* spp.	*M. hominis*	*M. genitalium*[b]
Male urethritis	+	−	+
Prostatitis	±	−	±
Epididymitis	±	−	−
Urinary calculi	+	−	−
Pyelonephritis	±	+	−
Bacterial vaginosis	±	±	−
Cervicitis	−	−	+
Pelvic inflammatory disease	−	+	+
Infertility	±	−	±
Chorioamnionitis	+	±	−
Spontaneous abortion	+	±	−
Prematurity/low birth weight	+	−	−
Intrauterine growth retardation	±	−	−
Postpartum/postabortion fever	+	+	−
Extragenital disease (including arthritis)	+	+	+

[a] −, no association or causal role demonstrated; +, causal role; ±, significant association and/or strong suggestive evidence, but causal role not proven.

[b] In the case of *M. genitalium*, lack of disease association may reflect the fact that insufficient studies using appropriate detection techniques have been attempted since this mycoplasma is much more fastidious and difficult to detect than *M. hominis* and *Ureaplasma* spp.

Waites KB et al, *Clin Microbiol Rev*, 2005

Sexually Transmitted Infections

- **Defined as -** infectious diseases spread from person-to-person through direct body contact with infected body fluids. Specifically any disease acquired primarily through sexual contact.

- ***Chlamydia trachomatis –*** Non-gonococcal urethritis; most common reportable STD ; estimated to have a true prevalence among sexually active population of 5 – 10%; 60% and 40% of men have no symptoms
- ***Neisseria gonorrhoeae –*** gonorrhoae and gonococcal pelvic inflammatory disease (PID) ; asymptomatic in 11.5% of men and 32.6% of women; incidence rate of ~1.7%
- ***Mycoplasma genitalium*** – Urethritis, cervicitis, endometritis PID; infected person may have some or all symptoms, or may be asymptomatic; incidence rate of 1~5%
- ***Mycoplasma hominis*** – non-gonococcal urethritis, PID, pyelonephritis or infertility; incidence rate of 21-53%.
- ***Trichomonas vaginalis*** – infection rates between men and women are the same with women showing symptoms while infections in men are usually asymptomatic
- ***Ureaplasma urealyticum*** –incidence of 20~40% in sexually active humans
- ***Ureaplasma parvum*** – during delivery of a baby, if the mother is infected with *U. parvum*, it may be spread to the CNS or respiratory tract of the infant leading to pneumonia, meningitis and septicemia.

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2010, p. 2715–2723 Vol. 48, No. 8
0095-1137/10/$12.00 doi:10.1128/JCM.01877-09

Detection and Characterization of Human *Ureaplasma* Species and Serovars by Real-Time PCR▽

Li Xiao,[1] John I. Glass,[2] Vanya Paralanov,[2] Shibu Yooseph,[2] Gail H. Cassell,[3] Lynn B. Duffy,[1] and Ken B. Waites[1]*

Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama[1]; J. Craig Venter Institute, Rockville, Maryland[2]; and Eli Lilly and Company, Indianapolis, Indiana[3]

NO CONFIRMED CORRELATION BETWEEN SEROTYPES AND BIOVARS AND PATHOGENICITY

Epidemiology in Pregnant Women

- MH & U most frequent bacteria isolated after PPROM
- Prevalence depends on age, race, socio eco status, hormonal status and pregnancy ++
- Pregnancy: Influence of estrogen & progesteron that increase prevalence of colonisation
- Pregnancy: Prevalence varies / studies:
  - MH: 23 – 50% (Annency: 2,3%)
  - U: 20 – 80% (Annency: 29%)
  - MG: UK 3%

PATHOGENESIS

- Mucosally associated organisms residing in the respiratory or urogenital tracts of their hosts in close association with epithelial cells.

- In some species, *M. fermentans, M. penetrans, M. genitalium,* and *M. pneumoniae* and *M. hominis* in some cases, invasion of host cell occur and the organisms reside intracellularly.

- Adherence is prerequisite for pathogenicity. Some mycoplasmas, such as *pneumoniae, penetrans* and *genitalium* have special attachment organelles containing adhesin molecules, *fermentans* and *hominis* not.

Pathogenicity of Mycoplasmas

- Adherence to host cells is prerequisite (membrane adhesin protein)
- Variation in proteins in Myc membrane enables them to evade host immune response
- Invasion of cells & biofilms formation protect them of antibiotics
- Myc activate macrophages & monocytes => pro-inflammatory cytokines (TNF, IL-1, -8, -12, -16, IF-γ)
- Systemic & local inflammation important in inducing abnormal outcomes of pregnancy.

Pathogenicity of Mycoplasmas - 2

- Bacterial endotoxins + memb lipoprot => activate fetal membranes (chorio-amnionitis) and decidua to produce inflammatory factors
- Endotoxins + cytokines stimulate synthesis & release of prostaglandins => proteases + … => uterine contractions & ROM
- Intraamniotic infection with Myc (PPROM) induces more intense inflammatory response than with other pathogens (chron vs acute??)*

* Oh KJ, *Am J Obstet Gynecol*, 2010, 203: e1-8

Materno-Fetal Transmission

- 45-65% especially high in case of:
  - vaginal delivery (direct contact with infected cervix vagina)
  - Infection by Ureaplasmas
- Vertical transmission of MG uncommon
- Neonatal colonisation tends to be provisional

Bacterial vaginosis

- Depletion of lactobacilli and anormal proliferation of other species, mostly anaerobic, and of MH in 60%
- Aerobic species have also a role (Donders G) and vaginal inflammation
- BV increases risks of preterm birth
- Actual role of MH not well known: co-pathogen or “bystander”: Metronidazole is effective in preventing PTB though not active on MH
- Recommendation for screening / Rx for BV in case of PTB history

Ectopic pregnancy

- Post-infectious lesions of Fallopian tube are major cause of EP
- MG can induce PID yet it is not as frequent as *N. gonorrhoeae* or *C. trachomatis*

- Taylor-Robinson D., *Br J Obstet Gynecol,* 2011; 118: 164-74

Low Birthweight

- No conclusive data
- Some studies suggest that Myc infections increase risk of low birthweight ??
- Discussion on respective roles of BV and Myc in low birthweight
- UU mainly responsible ?

Preterm Birth and/or PPROM

- Preterm birth and late miscarriage are most frequent adverse obstetrical outcome
- Often associated with PPROM
- Myc induce important inflammatory response in amniotic fluid
- Role of chorioamniotitis
- Role of:
  - Ureaplasmas: UU & UP
  - BV ± MH
  - MG ??

Postpartum Fever

- Role of MH in postpartum/postabortum fever
- Myc isolated most frequently in blood than in genital tract: endometritis??
- Role of MG ??

Infection in Newborn

- Transient infection of Neonate: Prevalence of Myc <10% after 3 months

- U can induce neonatal pulmonary diseases:
  - Arterial pulmonary hypertension (phospholipases => thromboxane)
  - Bronchopulmonary dysplasia

- Role of MH? BV?

U. urealyticum

14 serovar, divisi in 2 biovar per le caratteristiche genotipiche:

Biovar 1: serovar (parvo) 1,3,6,14
Biovar 2: serovar (T960) 2,4,5,7,8,9,10,11,12,13

Attualmente diviso in 2 specie:

U. parvum (ex- *U. urealyticum* biovar 1), la maggioranza degli isolati

U. urealyticum (ex- *U. urealyticum* biovar 2)

Eur J Clin Microbiol Infect Dis (2009) 28:641–646
DOI 10.1007/s10096-008-0687-z

ARTICLE

Detection of *Ureaplasma* biovars and polymerase chain reaction-based subtyping of *Ureaplasma parvum* in women with or without symptoms of genital infections

M. A. De Francesco · R. Negrini · G. Pinsi · L. Peroni · N. Manca

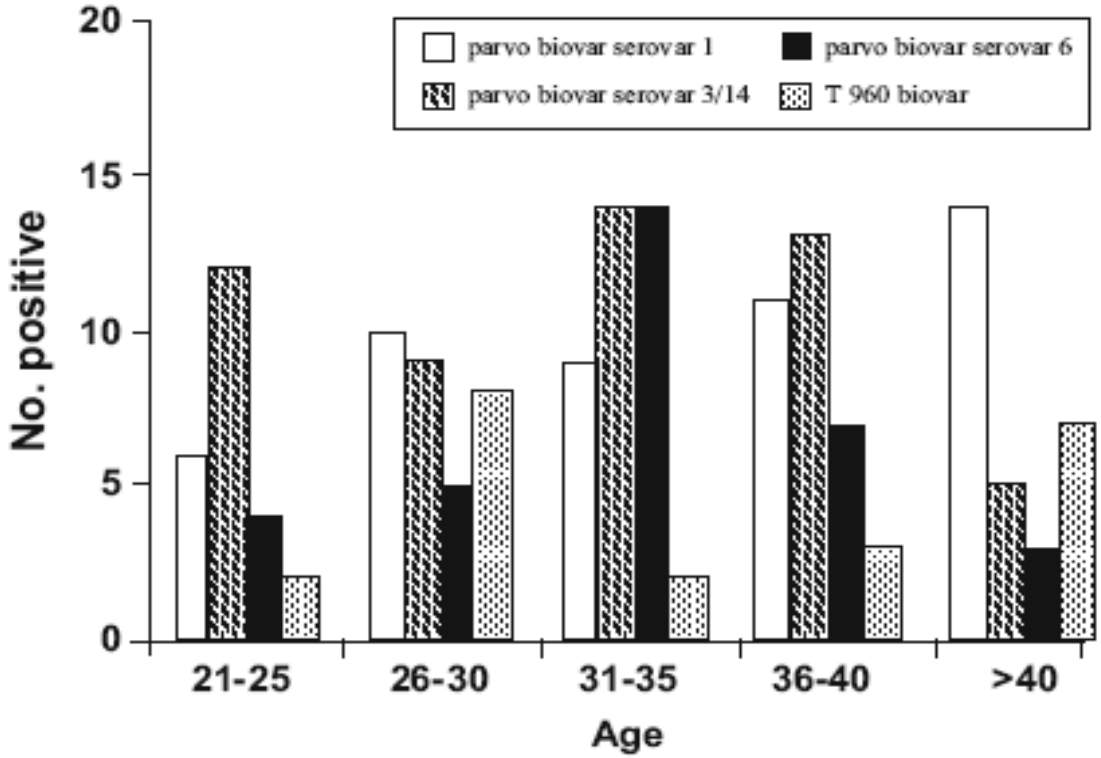


Fig. 1 *Ureaplasma parvum* serovars and T960 biovar distribution by age. The numbers of genital *Ureaplasma* isolates were 24, 32, 39, 34 and 29 for each year age group, respectively

Table 2 Relationship between vaginal flora changes and *Ureaplasma* biovars and serovars

Vaginal flora	*Ureaplasma* biovars and serovars			
	Parvo biovar Serovar 1	Parvo biovar Serovar 3/14	Parvo biovar Serovar 6	T960 biovar
Normal	31/94 (33%)	26/94 (28%)*	28/94 (30%)**	9/94 (9%)*
Absence of lactobacilli	13/45 (29%)	21/45 (47%)	3/45 (2%)	10/45 (22%)

*$P<0.05$; **$P<0.01$

Table 3 Relationship between clinical symptomatology and *Ureaplasma* biovars and serovars

	Ureaplasma biovars and serovars			
	Parvo biovar Serovar 1	Parvo biovar Serovar 3/14	Parvo biovar Serovar 6	T960 biovar
Symptomatic subjects	26/80 (32%)	35/80 (44%)*	3/80 (4%)**	16/80 (20%)*
Asymptomatic subjects	18/59 (31%)	12/59 (20%)	26/59 (44%)	3/59 (5%)

*$P<0.05$; **$P<0.01$

M. hominis

***M.hominis* patogenicità controversa**

Nella donna la colonizzazione è associata con

- uretriti
- Cerviciti

Nell'uomo la colonizzazione è associata con

- Prostatiti
- Prostatovescicoliti subacute (25-50% dei casi)
  - spesso accompagnate da emospermia

sono stati descritti anche casi di associazione con

- Epididimiti
- Balaniti

M. hominis

***M.hominis* patogenicità controversa**

Nella donna la colonizzazione è associata con

- Endometriti
- Infezioni corion amnios
- Rottura precoce delle membrane
- Basso pesa alla nascita

Nell'uomo la colonizzazione è associata con

- infertilità

Kim 2003
Taylor-Robinson 2007

U. urealyticum

Patogenicità controversa

Nella donna sono associati

- **Uretriti?**
- **Cerviciti?**

Nell'uomo sono associati

- **uretriti NG?**
- **Prostatiti?**

Inter J of Urol, 2004

U. urealyticum

Nella donna sono associati

- **Parto pretermine (produzione fosfolipasi)**
- **Natimortalità**
  - **Meningiti neonato**
  - **Batteriemie neonato**
- **aborto**

Nell'uomo sono associati

- **Infertilità (alto tropismo)**

Waites 2005
Olomu 2009

DIAGNOSIS

Micoplasmi

Mycoplasmas and ureaplasmas are fastidious, difficult to culture as they are very susceptible to adverse environmental conditions including desiccation, osmotic change, toxic metabolites and fluctuations in temperature (Duffy and Waites, 2008). Poor recovery of *Ureaplasma* spp. from clinical samples has been documented (Waites *et al.*, 2001).

- Essicamento
- pH
- T°
- Fotosensibilità
- Metaboliti tox

5h a T°ambiente

24/48h +4°

Microbiological Methods

- Serologic methods of no use (≠ *M. pneumoniae*)
- **MH et UU**: cultures are possible but:
  - Threshold varies in literature ≥ 10^4 ucc/mL
  - PCR more sensible
- **UP, MG**: PCR
- Use of quantitative PCR or Ct (threshold cycle) PCR?
  - Correlation between microbial load and clinical/pathological infections*

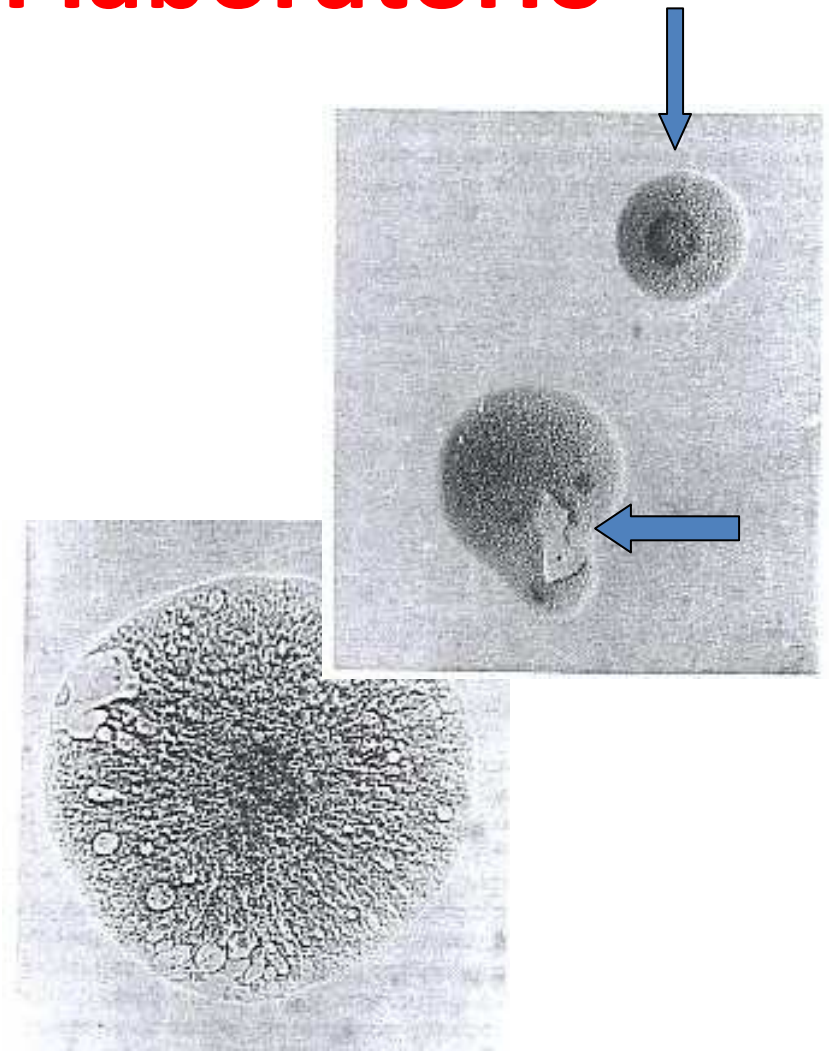
* Kacerovsky M., Am J Obstet Gynecol 2011; 205: e1-7

Diagnosi di laboratorio

- La ricerca di Mycoplasma/Ureaplasma viene eseguita su secreto cervicale o uretrale.
- Metodo di riferimento per l'identificazione è l'esame colturale in terreni selettivi, che comportano una lunga incubazione (in caso di negativi anche 25/30 gg.). L'identificazione deve essere sempre accompagnata dalla valutazione semiquantitativa del cut-off di carica (10.000 UFC / ml) , al di sopra del quale si può attribuire un ruolo patogeno al microrganismo.

Diagnosi di laboratorio

- Le colonie, cresciute su terreno di coltura selettivo, hanno la tipica forma ad uovo fritto, con una parte centrale più o meno grande di elementi granulari che penetrano in profondità nell'agar, contornate da ammassi cellulari più grossi che sfumano verso la periferia.



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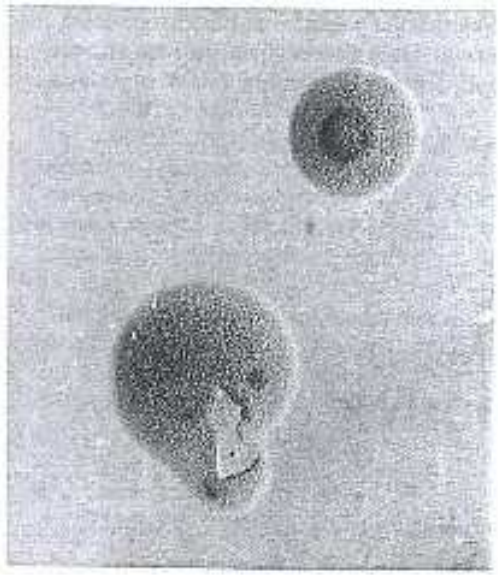


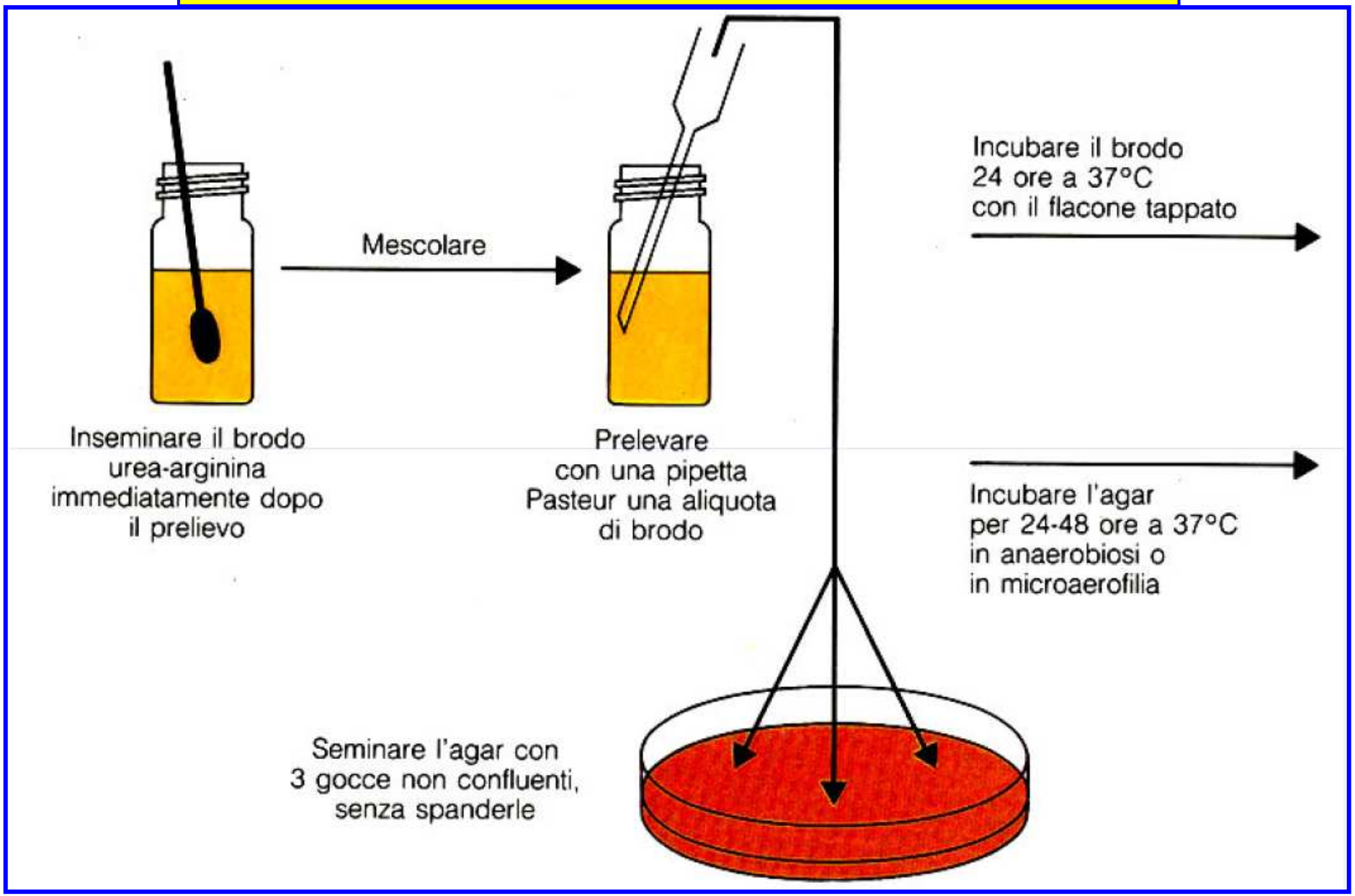
DIAGNOSI MICROBIOLOGICA

Campioni biologici: tampone vaginale e cervicale

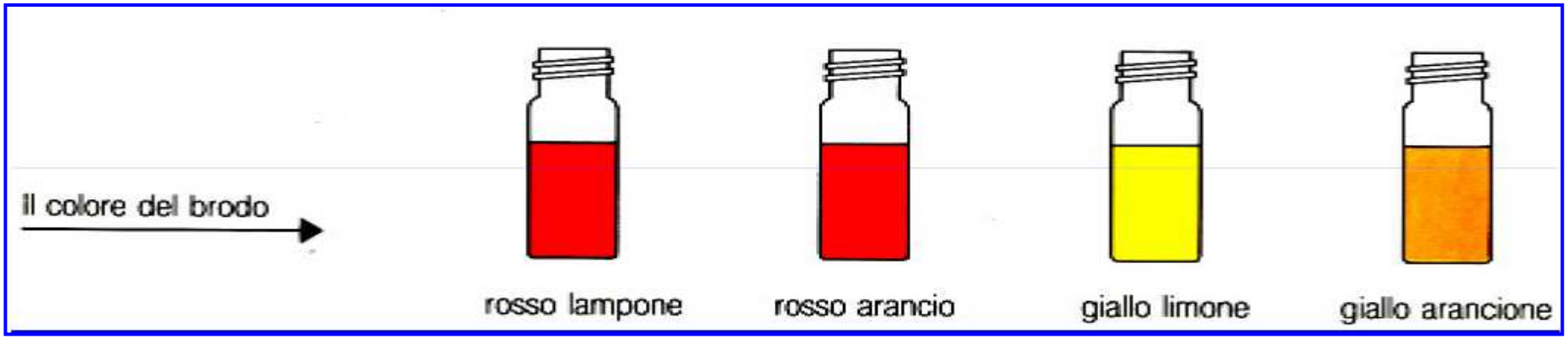
Esame colturale:

- inoculazione in brodo urea-arginina
- semina in piastra di terreno agarizzato A7
- incubazione del brodo a 37°C per 24 h
- incubazione delle piastre a 37°C per 48 h in anaerobiosi
  o microaerofilia
- esame del viraggio di colore del brodo
- osservazione microscopica delle colonie cresciute su terreno solido

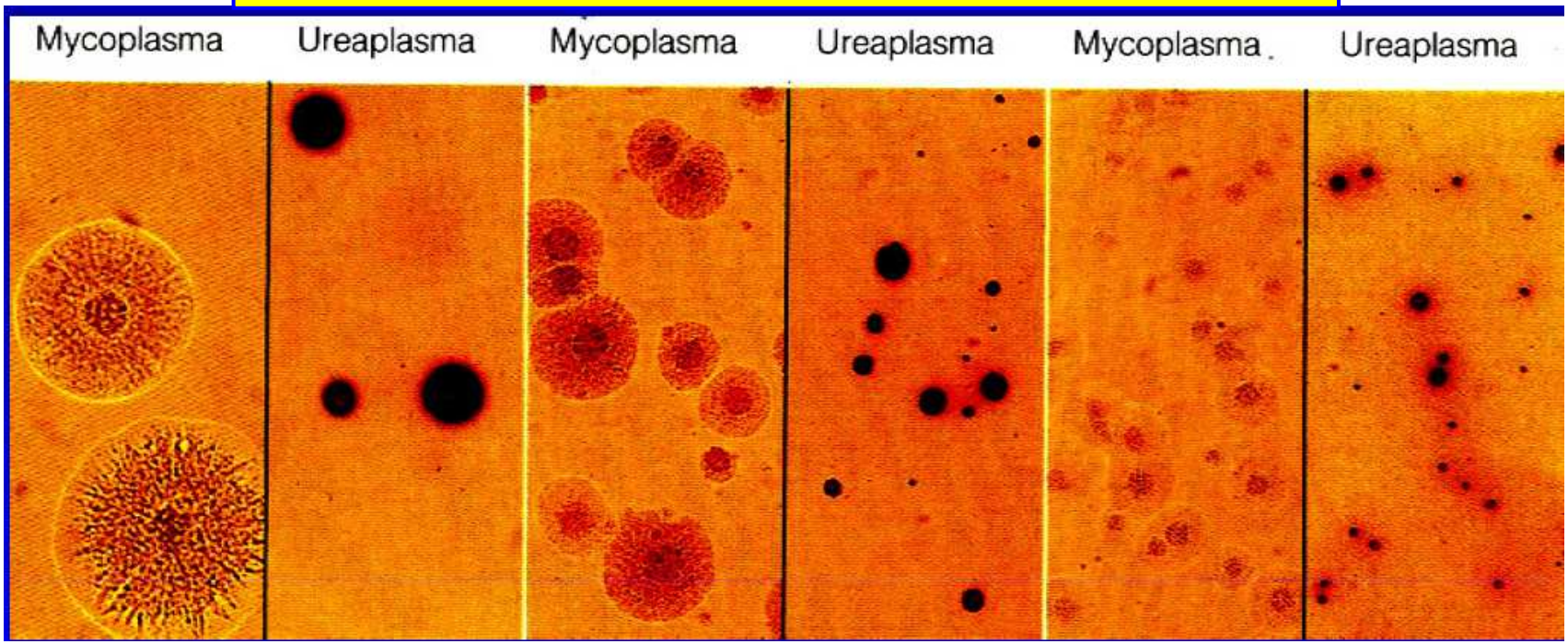
Coltura in brodo e isolamento su terreno solido selettivo



Osservazione del viraggio dell'indicatore presente nel brodo



Osservazione e conta delle colonie (10x)



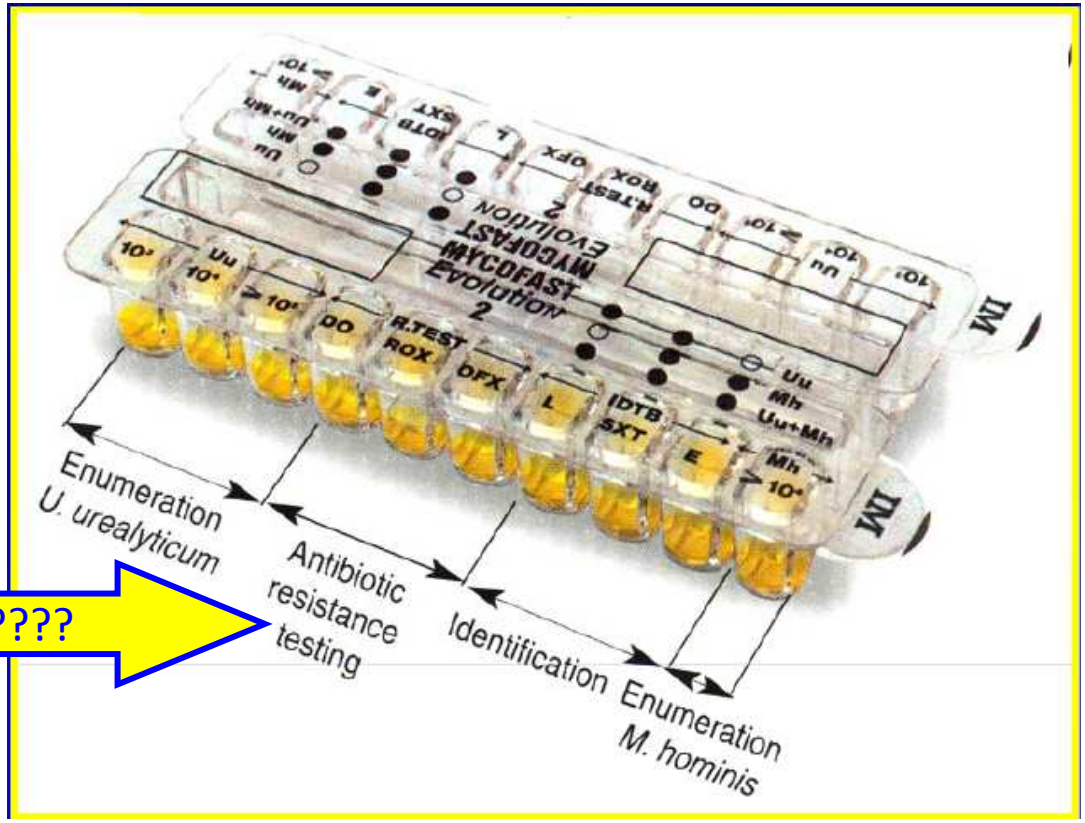
Numero medio di colonne per campo	1-5 colonie	5-15 colonie	> 15 colonie
Titolo dei prelievi	104 UFC	105 UFC	106 UFC

Diagnosi di laboratorio

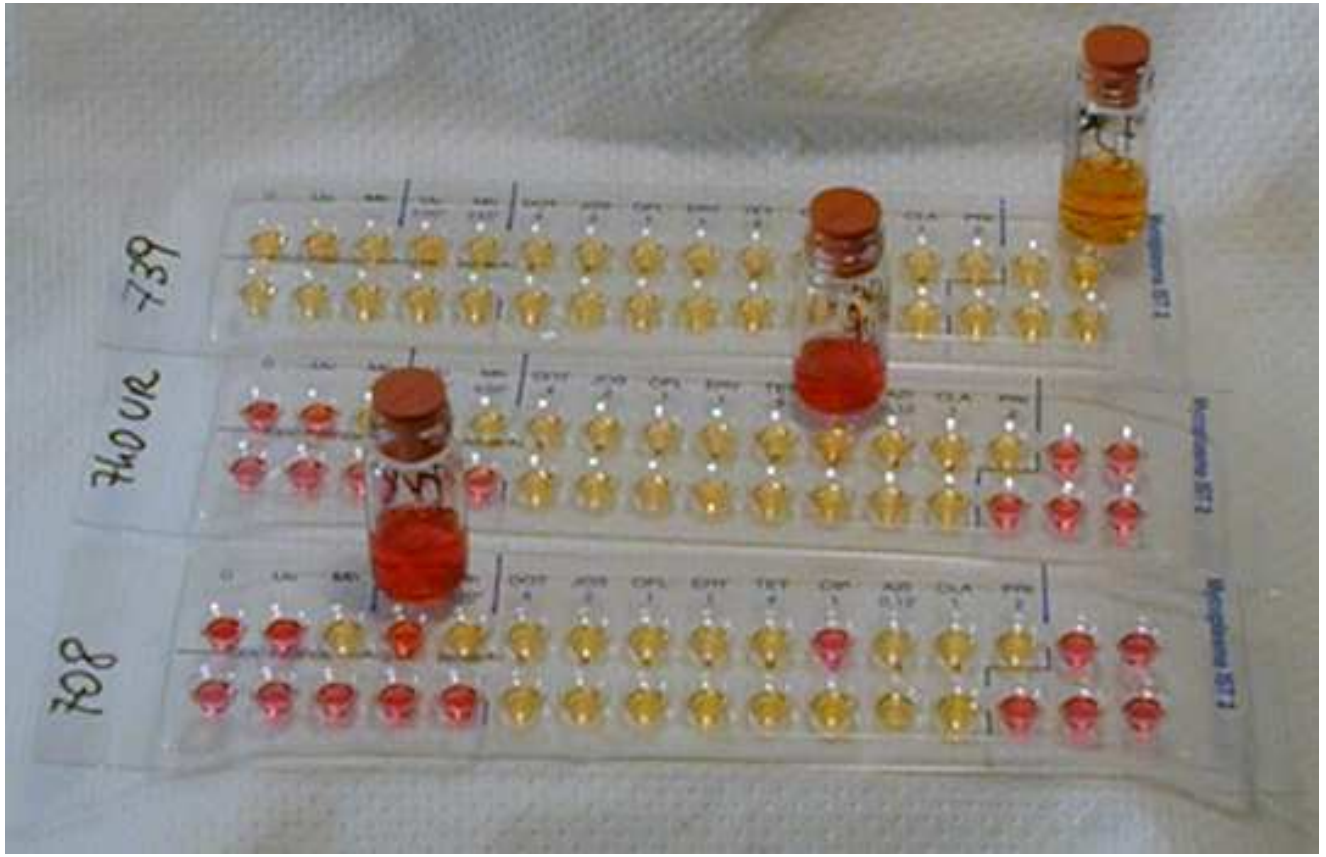
- Kit disponibili in commercio per l'identificazione dei Mycoplasmi/Ureaplasmi, permettono una lettura rapida (48 h), accompagnata sempre dalla carica batterica del microrganismo e il relativo antibiogramma.

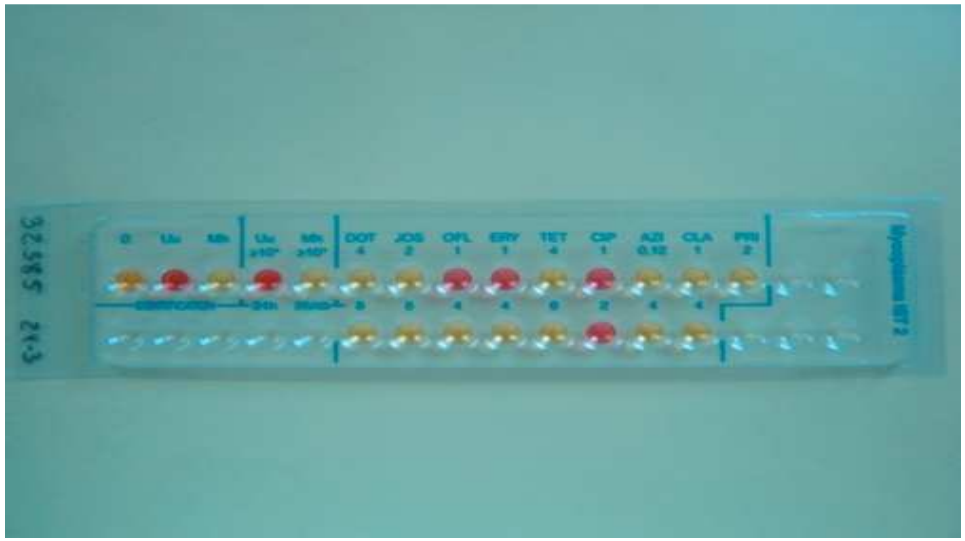
Semina in microgallerie contenenti substrati e antibiotici in forma liofila e incubate a 37°C per 48h.
Identificazione in base alle reazioni biochimiche e alla sensibilità agli antibiotici.

Affidabile????



Coltura , identificazione e test di sensibilità dei *Micoplasmi*





- **Lettura 48 h**

- **Falsi negativi** trasporto*

- **Falsi positivi** *Klebsiella* sp
  *P. mirabilis***

- **Positività U.u / U.p.**

*Teng et al. 1994

**
Rastawicki et al. 2004
Biernat-sudolska 2006

Comparison between culture and DNA amplification tests for the diagnosis of Genital Mycoplasma infection

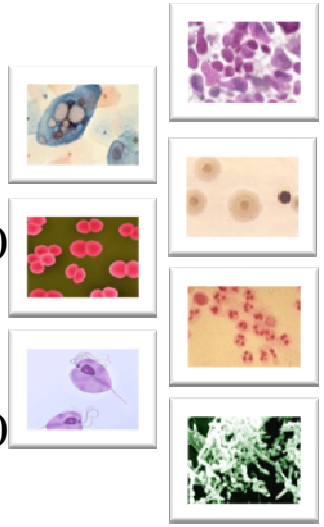
Characteristic	Culture	DNA Amplification
Detection time	2-4 days	Few hours
Species identification	*M. hominis* *Ureaplasma* spp.	*U. parvum* *U. urealyticum* *M. genitalium* *M. hominis*
Quantitation	Applicable	Real time PCR
Antibiotic susceptibility	Applicable	Not applicable
Cost	Low-moderate	High

Intended Use Statement

Anyplex II STI-7 Detection which is developed based on the DPO and TOCE technology detects 7 pathogens causing sexually transmitted infections (STIs) and an internal control (IC) for confirmation of DNA isolation and PCR inhibition simultaneous on the real-time PCR.

7 Analytes in one-tube

- **Internal control (IC)**
- ***Chlamydia trachomatis* (CT)**
- ***Neisseria gonorrhoeae* (NG)**
- ***Mycoplasma genitalium* (MG)**
- ***Mycoplasma hominis* (MH)**
- ***Trichomonas vaginalis* (TV)**
- ***Ureaplasma urealyticum* (UU)**
- ***Ureaplasma parvum* (UP)**



Specimens

- **Urine**
- **Swab specimens**

(urethral vaginal and cervical)

- **Liquid based cytology specimen**

(e.g., Thin-Prep® and SurePath™)

Data Interpretation

Specimen	Analyte CT, NG, MG, MH, UU, UP, TV	Internal Control	Data Interpretation
Case 1	Detected [insert pathogen name]	Detected	**Assay Valid**; Detected nucleic acid for [insert pathogen(s) name]
Case 2	Not Detected	Detected	**Assay Valid**; Sample negative
Case 3	Detected	Not Detected	**Assay Valid**; Detected nucleic acid for [insert pathogen name] (High titer of pathogens can lead to reduced or absent Internal Control signal)
Case 4	Not Detected	Not Detected	**Assay Invalid**; RE-TEST

Sensitive or more than singleplex real-time PCR

TOCE technology provides a level of sensitivity as good as or better than probe-based singleplex real-time PCR.

Method	Detection limit of analytes (copies/rxn)						
	CT	NG	TV	MH	MG	UU	UP
* TOCE-based 9-plex real-time PCR	10	10	10	10	10	10	10
Taqman probe-based singleplex real-time PCR	10	10	50	50	50	10	10

CT, Chlamydia trachomatis; NG, Neisseria gonorrhoeae; TV, Trichomonas vaginalis; MH, Mycoplasma hominis; MG, Mycoplasma genitalium; UU, Ureaplasma urealyticum UP, Ureaplasma parvum

*** 9-plex Real-time PCR: 7 analytes + dual target for CT + IC**

Confronto fra metodo Seegene e metodo DID su 171 campioni

Microrganismo	*M. hominis*		*U.urealyticum*		*U. parvum*
	STD4D	DID	STD4D	DID	STD4D
POSITIVI	6	2	6	11	7
TOTALE	STD4D vs DID 6-2		STD4D vs DID 13-11		

Prove di sensibilità

- Sistemi di riferimento????
- IOM (International Organisation for Mycoplasmology)
- stesura documento
- Microdiluizione in brodo
- MIC
  - Effetto inoculo — Kenny, Clin.Inf.Dis 1993
  - Effetto pH — Kenny, Antimic Agent Chemot,2011

Ureaplasma urealyticum: epidemiologia delle resistenze Mestre – 750 ceppi

	Sensibile (%)	Intermedio (%)	Resistente (%)
Azitromicina	51.5	35.5	13
Eritromicina	49.5	31	19.5
Claritromicina	74.4	8.4	17.2
Josamicina	93.9	5.6	0.5
Ofloxacina	14	74	12
Ciprofloxacina	2.3	33	64.7
Doxiciclina	96.4	1.2	2.4
Tetraciclina	93.8	1.9	4.3
Pristinamicina	98.9	0.3	0.8

Mycoplasma hominis:
epidemiologia delle resistenze
Mestre – 20 ceppi

	Sensibile (%)	Intermedio (%)	Resistente (%)
Azitromicina	5	10	85
Eritromicina	0	5	95
Claritromicina	0	0	100
Josamicina	95	0	5
Ofloxacina	45	45	10
Ciprofloxacina	45	25	30
Doxiciclina	85	5	10
Tetraciclina	70	0	30
Pristinamicina	95	0	5

Mycoplasma genitalium

- Identificato per la prima volta negli anni ’80
- Riscontrato anche nel tratto respiratorio
- Estremamente difficile la coltura e l’isolamento
- Diagnosi solo mediante amplificazione genica
- Ad oggi dimostrata l’associazione con:

  - uretrite non gonococcica
  - cervicite
  - MIP
  - endometrite
  - infertilità da fattore tubarico

M. GENITALIUM: prevalence and incidence

- **Prevalence**
  - **General population**
    - **M 1 – 4%**
    - **F 1 – 6%**
  - **Clinic population**
    - **M 4 – 26%**
    - **F 4 – 38%**
- **Incidence**
  - **University women: 0.9 per 100 WY**
  - **Kenya female sex workers: 23 per 100 WY**

Anagrius STI 2005, Hamasuna STI 2004, Ross STI 2009, Tosh JAH 2007, Oakeshott CID 2010, Cohen std 2007, Pepin STI 2005, Hancock STI 2010

M. genitalium: disease association

Men	Women
NGU	Urethritis
Epididymitis	Cervicitis
Prostatitis	Endometritis, Salpingitis (PID)
Proctitis (MSM)	Ectopic pregnancy
	Preterm birth
	Infertility

Association between *M. genitalium* and female disease
Clinical presentation

- **Frequently asymptomatic**

- **Similar to *Chlamydia trachomatis* with some exceptions**
  - **Mucopurulent discharge**
  - **Fewer PID symptoms**

- **Long duration of infection**
  - **Up to 21-23 months**

M. genitalium and upper genital tract disease
Endometritis / Salpingitis

- ***M. genitalium*** **was found in 9 of 58 women (16%) with histological endometritis and in 1 of 57 women (2%) w/o endometritis** (Cohen , 2002)
- **In the PEACH study, *M. genitalium* was found in 15% (CT 14%; NG 15%),** (Haggerty, 2008)
  - ***M. genitalium*** **found in endometrium of 60% of those positive in the cervix**
  - **Pelvic pain score, clinical symptoms, and signs were similar in MG and CT positive women** (Short *et al.*, CID, 2009)
- ***M. genitalium*** **was detected in 9 (7%) of women with laparoscopically confirmed PID but only in 1 specimen from the tubes** (Cohen et al, 2005)

Etiologies of Nongonococcal Urethritis: Bacteria, Viruses, and the Association with Orogenital Exposure

Catriona S. Bradshaw,[1,2] Sepehr N. Tabrizi,[3,4] Timothy R. H. Read,[1] Suzanne M. Garland,[3,4] Carol A. Hopkins,[1] Lorna M. Moss,[1] and Christopher K. Fairley[1,2]

[1]Melbourne Sexual Health Centre, The Alfred Hospital, [2]School of Population Health and [3]Department of Obstetrics and Gynaecology, University of Melbourne, and [4]Department of Microbiology and Infectious Diseases, The Royal Women's Hospital, Victoria, Australia

336 • JID 2006:193 (1 February) • Bradshaw et al.

Results. *C. trachomatis* (20%), *M. genitalium* (9%), adenoviruses (4%), and HSV-1 (2%) were more common in cases with NGU ($n = 329$) after age and sexual risk were adjusted for ($P \leq .01$); *U. urealyticum*, *U. parvum*, and *G. vaginalis* were not. Infection with adenoviruses or HSV-1 was associated with distinct clinical features, oral sex, and male partners, whereas infection with *M. genitalium* or *C. trachomatis* was associated with unprotected vaginal sex. Oral sex was associated with NGU in which no pathogen was detected ($P \leq .001$). Fewer than 5 polymorphonuclear leukocytes (PMNLs) per high-power field (HPF) on urethral smear were present in 32%, 37%, 38%, and 44% of cases with *C. trachomatis*, *M. genitalium*, adenoviruses, and HSV, respectively.

Mycoplasma genitalium Compared to Chlamydia, Gonorrhea and Trichomonas as an Etiologic Agent of Urethritis in Men Attending STD Clinics

Charlotte Gaydos[1,*], **Nancy E. Maldeis**[2], **Andrew Hardick**[1], **Justin Hardick**[1], and **Thomas C. Quinn**[1,3]

Sex Transm Infect. 2009 October ; 85(6): 438–440.

Results—The overall prevalences of infection with *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium*, were 20.3%, 12.8%, 3.4%, and 15.2% respectively. Prevalences in men with urethritis were 32.7%, 24.2%, 5.2%, and 22.2% for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium*, respectively. Percentages of coinfections were high. All men with *N. gonorrhoeae* had urethritis. *C. trachomatis* and *M. genitalium* were found to be significantly associated with urethritis in univariate analysis and in multiple logistic regression analysis.

Conclusion—The association of *M. genitalium* with urethritis in this study provides confirmation of the importance of screening men for *M. genitalium* as a cause of non-gonococcal urethritis and supports treatment considerations for urethritis for agents other than gonococci and chlamydia.

Mycoplasma genitalium as a Contributor to the Multiple Etiologies of Cervicitis in Women Attending Sexually Transmitted Disease Clinics

Charlotte Gaydos, DRPH, Nancy E. Maldeis, PHD,† Andrew Hardick, MS,* Justin Hardick, MS,* and Thomas C. Quinn, MD*‡*

Sexually Transmitted Diseases • Volume 36, Number 10, October 2009

Results: Overall prevalence of infection with *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium* was found to be 11.1%, 4.6%, 15.3%, and 19.2%, respectively. Prevalence in women with cervicitis was 15.8%, 6%, 18.9%, and 28.6% for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium*, respectively. Percentages of coinfections were high. *C. trachomatis* and *M. genitalium* were significantly associated with cervicitis in univariate analysis, but only *M. genitalium* was significantly associated with cervicitis (AOR: 2.5) in multiple logistic regression models.

Management of Women with Cervicitis

Jeanne M. Marrazzo[1] and David H. Martin[2]

[1]Department of Medicine, University of Washington, Seattle; and [2]Louisiana State University Health Sciences Center, New Orleans

S102 • CID 2007:44 (Suppl 3) • Marrazzo and Martin

In the past several years, the collective understanding of cervicitis has extended beyond the recognition of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* as the prime etiologic suspects. *Trichomonas vaginalis* and herpes simplex virus cause cervicitis, and both *Mycoplasma genitalium* and bacterial vaginosis have emerged as new candidate etiologic agents or conditions. However, major gaps in our knowledge of this common condition remain. Putative etiologic agents have not been identified in many women with cervicitis. Moreover, cervicitis occurs in a relatively small proportion of women with chlamydia or gonorrhea. Finally, scant research has addressed the clinical response of nonchlamydial and nongonococcal cervicitis to antibiotic therapy, and there are no data on the benefit of sex partner treatment for such women. New research into the etiology, immunology, and natural history of this common condition is needed, especially in view of the well-established links between cervicitis and an increased risk of upper genital tract infection and human immunodeficiency virus type 1 acquisition.

Has the Time Come to Systematically Test for *Mycoplasma genitalium*?

Lisa E. Manhart, PhD

Sexually Transmitted Diseases • Volume 36, Number 10, October 2009

Certezze:

causa di uretrite maschile e di PID e infertilità femminile

Incertezze:

causa di cervicite (prevalenza troppo alta, imprecisa definizione di cervicite, diverse popolazioni esaminate)

Auspicio:

disponibilità di test commerciali per saggiare in modo sistematico tutte le donne sintomatiche

Detection of *Chlamydia trachomatis* and *Mycoplasma hominis, genitalium* and *Ureaplasma urealyticum* by Polymerase Chain Reaction in patients with sterile pyuria

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Advances in Medical Sciences
Vol. 53(1) - 2008 - pp 80-86
DOI: 10.2478/v10039-008-0020-1

ABSTRACT

Purpose: *Chlamydia trachomatis* and *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* are associated with various diseases of the urogenital tract, but they are usually not detected by routine microbiological diagnosis. To determine the occurrence of *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* in patients with sterile pyuria.

Material/Methods: Sterile pyuria urine samples collected during the period from February 2006 to April 2007 were tested by polymerase chain reaction (PCR) for the presence of *C. trachomatis*, *M. hominis*, *M. genitalium*, and *U. urealyticum* using specific primers for each species. A total of 200 sterile pyuria samples selected from about 2400 urine samples attending the genitourinary clinic at Al-Shifa hospital, Gaza, during the period February 2006 to April 2007 and were analyzed for routine urine examination and cultured on MacConkey agar, blood agar, and sabouraud agar to detect the presence of bacteria and *Candida*. The 200 samples (96 male, 104 female; aged ≥18 years) containing more than 10 leukocytes / HPF and negative for culture (showing no significant growth after 24 hr) were tested by PCR for *C. trachomatis* and *M. hominis*, *M. genitalium*, and *U. urealyticum*.

Results: *C. trachomatis* was detected in 20 samples (10%), *U. urealyticum* in 10 samples (5%), *M. hominis* in 6 samples (3%) and *M. genitalium* in 2 samples (1%). The difference in occurrence of *C. trachomatis* was statistically insignificant between males and females (P=0.509), but it was significant (P=0.008) for *U. urealyticum*. *M. hominis* was detected only in samples collected from female patients. On the other hand, *M. genitalium* was detected only in men.

Conclusion: PCR testing of sterile pyuria showed a significant number of *C. trachomatis*, *Mycoplasma*, and *Ureaplasma infections*. Consequently, PCR is recommended for the detection of those microorganisms in the urine samples of sterile pyuria patients.

Adv Med Sci. 2008; 53 (1): 80-86

Table 2. **Microorganisms identified in the 200 studied specimens.**

Microorganisms	male		female		total		P value
	n	(%)	n	(%)	n	(%)	
C. trachomatis	11	(5.5)	9	(4.5)	20	(10.0)	0.509
U. urealyticum	1	(0.5)	9	(4.5)	10	(5.0)	0.008*
M. hominis	0	(0.0)	6	(3.0)	6	(3.0)	0.005*
M. genitalium	2	(1.0)	0	(0.0)	2	(1.0)	0.085

"Evidence for a role of *Mycoplasma genitalium* in pelvic inflammatory disease"
Curr Opin Infect Dis 2008;21:65-69

Published in final edited form as:
Clin Infect Dis. 2009 January 1; 48(1): 41–47. doi:10.1086/594123.

Clinical Presentation of *Mycoplasma genitalium* Infection versus *Neisseria gonorrhoeae* Infection among Women with Pelvic Inflammatory Disease

Vanessa L. Short[1], **Patricia A. Totten**[2], **Roberta B. Ness**[1], **Sabina G. Astete**[2], **Sheryl F. Kelsey**[1], and **Catherine L. Haggerty**[1]

1*Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania*

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Presentazione clinica più simile alla PID da *C. trachomatis*

Mycoplasma genitalium is associated with symptomatic and asymptomatic non-gonococcal urethritis in men

H Moi,[1] N Reinton,[2] A Moghaddam[2]

Sex Transm Infect 2009;**85**:15–18. doi:10.1136/sti.2008.032730

Key messages

- *Mycoplasma genitalium* is associated with non-gonococcal urethritis (NGU) in men with and without clinical symptoms
- *M genitalium* is associated with the symptoms of NGU.
- *M genitalium* is associated with the severity of NGU.

Mycoplasma genitalium in women with lower genital tract inflammation

Sex Transm Infect 2009;**85**:10–14. doi:10.1136/sti.2008.032748

H Moi,[1] N Reinton,[2] A Moghaddam[2]

Key messages

- *Mycoplasma genitalium* is associated with lower genital tract inflammation in women.
- Cervical swabs have higher sensitivity than urine for detecting *M genitalium* by PCR.
- Urine is more specific than cervical swabs in the analysis of the association of *M genitalium* infection with lower genital tract inflammation.

JOURNAL OF CLINICAL MICROBIOLOGY, May 2009, p. 1469–1475 Vol. 47, No. 5
0095-1137/09/$08.00+0 doi:10.1128/JCM.01602-08

Analysis Identifying Common and Distinct Sequences among Texas Clinical Strains of *Mycoplasma genitalium*▽

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Mycoplasma genitalium **is a human bacterial pathogen linked to urethritis and other sexually transmitted diseases. Here, we assessed the incidence of *M. genitalium* infection in patients attending a sexually transmitted disease clinic in San Antonio, TX, by use of diagnostic real-time PCR. Overall, 16.8% of women and 15.1% of men were found *M. genitalium* positive. Regions of the *mgpB* gene, which encodes the MgPa adhesin, were amplified from positive clinical specimens and evaluated for sequence variability, which demonstrated transmission of the pathogen between sexual partners. Follow-up analysis of a subset of patient specimens revealed reinfection by a different strain of *M. genitalium*, indicating the absence of protective immunity. Eighteen DNA sequence variants were obtained and compared with all other available clinical sequences. Detailed analysis revealed silent mutations of six amino acid residues within the encoded region of the MgPa adhesin in numerous clinical strains. In addition, missense mutations of limited numbers of amino acids were observed. Alignment of putative amino acid sequences revealed the simultaneous occurrence of several mutations and the existence of identical or similar protein variants in strains from different locations.**

Sexually Transmitted Diseases, March 2008, Vol. 35, No. 3, p.250–254
DOI: 10.1097/OLQ.0b013e31815abac6

Mycoplasma genitalium *Detected by Transcription-Mediated Amplification Is Associated With* Chlamydia trachomatis *in Adolescent Women*

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JESSICA A. KAHN, MD, MPH,* KIMBERLY D. RICH, MPH,§ AND MARCIA M. HOBBS, PHD§

*From the *Division of Adolescent Medicine, †Laboratory Medicine, and ‡Emergency Medicine, Cincinnati Children's Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, Ohio; Departments of §Medicine and ‖Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina*

Objectives: **The clinical significance of *Mycoplasma genitalium* (MG) infection in adolescent women is poorly understood. We compared the prevalence of MG with that of other sexually transmitted organisms such as *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) and assessed the associations of MG with sexual behaviors, genitourinary symptoms, physical and laboratory findings.**

Study Design: **Women aged 14 to 21 years (n = 331) were recruited from an urban medical center. The subjects' sexual behaviors, genitourinary symptoms, and physical findings were recorded. Endocervical swabs were collected for CT and NG testing and vaginal swabs for wet mount, Gram stain, TV and MG testing. MG infection was identified by nucleic acid amplification using a transcription-mediated amplification assay.**

Results: **MG was detected in 74 (22.4%), CT in 79 (24.4%), TV in 60 (18.2%), and NG in 35 (10.7%) subjects. MG infection was not associated with vaginal symptoms, physical evidence of cervicitis, or findings on wet mount or Gram stain. In logistic regression, variables positively associated with MG were current CT [odds ratio (OR), 2.3; 95% confidence interval (CI), 1.4–4.4] and recent sexual contact (≤7 days) (OR, 2.0; CI, 1.1–3.2). Dysuria (OR, 0.44; CI, 0.2–0.96) and use of hormonal contraception (OR, 0.55; CI, 0.3–1.0) were negatively associated with MG infection.**

Conclusion: **In adolescent women, MG infection was as common as chlamydial infection and trichomoniasis and more common than gonorrhea. MG was associated with CT and recent sexual contact but not with vaginal symptoms or signs of cervicitis.**

ADOLESCENT WOMEN FREQUENTLY COMPLAIN of genitourinary symptoms and are at high risk for sexually transmitted infections (STI) with organisms such as *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV). However, clinicians often cannot establish a definitive diagnosis for vaginitis or cervicitis.[1] In addition, symptoms sometimes persist despite treatment. Infection with a sexually transmitted organism that is difficult to culture, such as *Mycoplasma genitalium* (MG), may explain genitourinary symptoms or poor response to treatment of other STI. Yet, little is known about the prevalence or significance of MG in adolescent women who are at high risk for STI.

MG has been identified as an etiologic agent of urethritis in men.[2] However, in women, the results from studies that examined the associations between MG and genitourinary symptoms or signs are inconsistent. In one study using stored specimens that were

Diagnosis of *M. genitalium* infections

- Only a direct diagnosis
- Culture extremely fastidious
- By nucleic amplification tests:
  - a lot of in house PCRs, real-time PCR++
  - some specimens better than others

    FVU>urethral swabs in men

    vaginal swabs>cervix>FVU in women
  - a few multiplex tests commercialized

    (Bio-Rad, Seegene)
- No serology commercialized

M. genitalium: antibiotic susceptibility testing

- **Intrinsic resistance**

ß-lactams and other antibiotics targeting the cell wall

- **No susceptibility testing done in routine**

- Active antibiotics

TABLE 2. Activities of various antibiotics against *M. genitalium* and *M. pneumoniae*

Antimicrobial agent	MIC range (µg/ml)	
	M. genitalium	*M. pneumoniae*
Erythromycin	≤0.01	≤0.01
Spiramycin	0.5	0.5
Josamycin	0.02	≤0.01–0.02
Miocamycin	≤0.01	≤0.01
Roxithromycin	≤0.01	≤0.01
Azithromycin	≤0.01	≤0.01
Clarithromycin	≤0.01	≤0.01–0.05
Pristinamycin	≤0.01–0.02	0.02–0.05
Lincomycin	1–8	4–8
Clindamycin	0.2–1	1
Tetracycline	≤0.01–0.05	0.05
Doxycycline	≤0.01–0.05	0.02–0.05
Minocycline	≤0.01–0.02	0.02–0.05
Nalidixic acid	16–32	64–128
Ofloxacin	1–2	0.5–1
Ciprofloxacin	2	1
Lomefloxacin	2–4	2–8
Sparfloxacin	0.05–0.1	0.1
Rifampin	32–64	64–128
Chloramphenicol	0.5–4	4
Amikacin	16	16

MYCOPLASMA GENITALIUM

- Difficult to monitor antibiotic sensitivity in an organism so difficult to culture
- PCR for the resistance sequences
- Usually resistant to tetracyclines, ofloxacin and (?) azythromicin
- Moxifloxacin 400 mg a day for 7 days
- Since resistance for the drugs commonly used for chlamydial infection, mycoplasma genitalium will increase
- In the absence of resistance extended course of azythromicin (500 mg start followed by 250 mg daily for 4 days).

Mycoplasma genitalium treatment

M. genitalium treatment
European guidelines, 2009

- **Acute NGU – cervicitis**

  Azithromycin 1 g single dose

  or

  Doxycycline 100 mg x 2, 7 d

- **Chronic NGU**

  1) Extendend 1.5 g azithomycin (5 d)

  2) Moxifloxacin 400 mg, 10 d

M. genitalium male NGU treatment studies

- **Open Scandinavian multicenter trial (Biörnelius, 2008)**
  - **Doxycycline 200 mg + 100 x 8 d: 22% cure rate (n=103)**
  - **Azithromycin 1 g x 1 d: 86% cure rate (n=23)**
  - **Azithromycin 500 mg + 250 mg x 4 d: 97% cure rate (n=60)**
- **Randomized US trial (Mena, CID, 2009)**
  - **Doxycycline 200 mg x 7 d: 45% cure rate (n=31)**
  - **Azithromycin 1 g x 1 d: 87% cure rate (n=23)**

  Clinical cure in DOX group at 2-3 weeks but subsequent recurrence
- **Randomized US trial (Schwebke, CID, 2011)**
  - **Doxycycline 200 mg x 7 d: 49% cure rate (n=149)**

    ***M. genitalium* clearance rate 30.8%**
  - **Azithromycin 1 g x 1 d: 43.6% cure rate (n=156)**

    ***M. genitalium* clearance rate 66.7%**
- **Moxifloxacin 400 mg for 7-10 d in treatment failure after AZM: 100% cure rate (Bradshaw, 2006; Jernberg, 2008)**

M. genitalium: emergence of macrolide resistance

- **8 clinical strains AZM-R** (Bradshaw, EID, 2006)
  - MIC AZM >32 mg/l, ERY >64 mg/l
  - mutations 2058, 2059 in 23S rRNA

- **19 patiens: Mg positive specimens for a strain AZM-R**
  - mutations 2058, 2059 in domain V of 23S rRNA (Jensen, CID, 2008)

- **Azithromycin 1 g single dose ➡ 13-33% therapeutic failures**
  (Bradshaw, EID, 2006; Jensen, CID, 2008; Ito, STI, 2011; Shimada, EID, 2011)

  - selection of resistant mutants during AZM treatment

  - Therapeutic failure if patient infected with a mutated strain

M. genitalium: emergence of macrolide resistance

- Emergence of macrolide resistance in 2005 for M. genitalium in Australia, Scandinavia, New-Zealand, Japan and France
- According to the primary treatment used in countries

  - Sweden: **DOX = 1st line TT for NGU and cervicitis**

    181 M. genitalium (+) STD-clinic attendees

    3 (1.6%) had 23S rRNA mutations

  - Danemark : **AZM = 1st line TT for NGU and cervicitis**

    415 M. genitalium (+) GP and STD-clinic attendees

    162 (39%) had 23S rRNA mutations

  - France : **AZM and DOX = 1st line TT for NGU and cervicitis**

    123 M. genitalium (+) STD-clinic attendees

    13 (10.6%) had 23S rRNA mutations

M. genitalium : acquired resistance

- **Acquired resistance to fluoroquinolones**
  - **Few reports, Japan ++**
  - **Target mutations (gyrase and topo IV)**
- **Description of multidrug resistance**
  - **Resistance to macrolides and fluoroquinolones**
  - **1st strain described for a Chinese patient:**

    AZM and MXF MIC > 16 mg/l
  - **Few other cases desribed in Australia and Norway**

M. genitalium treatment studies
Response to treatment in men

- Insufficient treatment leads to persisting of recurring symptoms
- Persistence of symptoms

  - Patients having Mg eradicated: 17%

  - Patients with Mg treatment failure: 91% (p<0.0001)
  (Bradshaw et al, pLoS One, 2008)
- Of 78 men with persistent NGU after doxycycline treatment, 41% were *M. genitalium* positive (Wikström & Jensen, STI, 2006)
- Patients failing azithromycin 1g single dose cannot be treated successfully with extended 1.5 g AZM (Jerneng, STI, 2008)

Conclusion (1)

- *M. genitalium*, an emerging STI pathogen, a new chlamydia ?
- An accepted cause of male NGU and female cervicitis
- Probably associated with sequelae in women
  - PID
  - Infertility
  - Preterm birth?
- Relatively low general population prevalence
  - screening programs not appropriate
  - Testing and treating in high risky populations

Conclusion (2)

- **Commercially available nucleic acid amplification test,** multiplex PCRs for detection of STI pathogens
- **Treatment of *M. genitalium* infections**
  - Tetracyclines not useful, AZM single dose better
    Extended 1.5 g AZM 95% effective
  - Emergence of resistance to macrolides
    Huge local differences in resistance rates
  - Moxifloxacin 10 d in case of AZM failure

Treatment & Management

- Potentially active antibiotics: cyclines, macrolides & azithromycin, clindamycin and fluoroquinolones
  - Cyclines theorically active on MH but resistance++
  - Transplacental transfer of macrolides: 3%
- Clindamycin active on MH
- Indication for fluoroquinolones in U infections?
  - Smorgick N., Fetal Diagn Ther, 2007; 22: 90-3
- MG: azithromycin or moxifloxacin (+Rx partner)

MYCOPLASMA GENITALIUM
AND PID
IT IS THE RIGHT TREATMENT ?

Recommended Parenteral Regimen A

Cefotetan 2 g IV every 12 hours
OR
Cefoxitin 2 g IV every 6 hours
PLUS
Doxycycline 100 mg orally or IV every 12 hours

Recommended Parenteral Regimen B

Clindamycin 900 mg IV every 8 hours
PLUS
Gentamicin loading dose IV or IM (2 mg/kg of body weight), followed by a maintenance dose (1.5 mg/kg) every 8 hours. Single daily dosing (3–5 mg/kg) can be substituted.

Alternative Parenteral Regimens

Ampicillin/Sulbactam 3 g IV every 6 hours
PLUS
Doxycycline 100 mg orally or IV every 12 hours

CDC - 2010

2009 European Guideline on the Management of Male Non-gonococcal Urethritis

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Table 1 Prevalence of the most common pathogens detected from patients with NGU

Microorganism	Prevalence	Reference
C. trachomatis	11–43%	7,8,11,12,16,19,23–25,27,82
M. genitalium	9–25%	5–7,12,13,16,22,23,25,27,38,83,84
Adenoviruses	2–4%	27,32
T. vaginalis	1–20%	28,82,85–87
Herpes simplex virus	2–3%	27,33

Tetracyclines and azithromycin are generally effective against *C. trachomatis* though sporadic reports of treatment failure have been reported with tetracyclines.[54] While in general treatments that are effective against *C. trachomatis* appear to be also effective in NGU, tetracyclines and azithromycin in the doses used do not consistently eradicate *M. genitalium*[55–58] (IIa, B).

Prevention of recurrent PTB

- In patients with history of PTB
- Screening for BV . If positive:
  - Rx metronidazole po or clindamycin
  - Screening at 2nd & 3rd trim. and treat if positive

McDonald H., Cochrane Database Syst Rev, 2005: CD000262

Conclusion

- Roles of Myc in abnormal pregnancy outcome better known
- Ureaplasmas, MH with/without BV are responsible for chorioamnionitis and important inflammatory that can induce PPROM and PTB (& late miscarriage)
- Role of MG has still to be assessed

GRAZIE PER L'ATTENZIONE