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Artículo original

Curable sexually transmitted infections among female sex workers in a population of Zulia State, Venezuela

Nailet Arráiz[a,b,*], María P. Sánchez[a], Eileen Sanz[a], Valmore Bermúdez[b], Baldimiro Urdaneta[a], Carem Prieto[b], Carolina Escalona[b], Andrea Mujica[b], Endrina Mujica[b]

[a]School of Bioanalysis. [b]Molecular Biology Research Laboratory of Research Center for Endocrine-Metabolic Diseases. Faculty of Medicine, University of Zulia. Maracaibo, Zulia State, Venezuela.

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***Abstract*:** Sexually transmitted infections (STIs) represent a public health problem worldwide. The aim of this study was to investigate the prevalence of curable STIs caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Ureaplasma urealyticum* in female sex workers in a population from Zulia State, Venezuela. Seventy eight (78) women attended a health monitoring sanitary controls were evaluated, and PCR amplification assays were used to detect the three microorganisms in endocervical samples. In 33.3% of the samples, at least one microorganism was detected: *U. urealyticum* was found more frequently (25.6%), followed by *N. gonorrhoeae* (18%), and *C. trachomatis* (12.8%). A significant association between *N. gonorrhoeae* and *C. trachomatis* was found ($p < 0.0001$). STIs cases represented 42.9% and 28% for symptomatic and asymptomatic groups, respectively. In the symptomatic group, *N. gonorrhoeae* was 2.4 times (28.6%) more frequent than in the asymptomatic one (12%) ($p=0.015$), particularly associated with mucopurulent discharge ($p=0.025$). No association was found between *C. trachomatis* ($p=0.078$), and *U. urealyticum* ($p=0.432$) with clinical manifestations. Prevalence of curable STIs in the study population was relatively low compared with other high-risk populations worldwide. The results support the possible association between *C. trachomatis* and *N. gonorrhoeae*, therefore, treatment would be indicated against both pathogens when one of them is detected in vulnerable populations.

Keywords: Curable STIs, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, female sex workers, cervicitis, PCR.

Infecciones de transmisión sexual curables en trabajadoras sexuales en una población del estado Zulia, Venezuela

***Resumen*:** Las infecciones de transmisión sexual (ITS) representan un problema de salud pública a nivel mundial. El objetivo de este estudio fue investigar la prevalencia de ITS curables causadas por *Neisseria gonorrhoeae*, *Chlamydia trachomatis* y *Ureaplasma urealyticum* en trabajadoras sexuales de una población del estado Zulia, Venezuela. Se evaluaron 78 mujeres que asistieron a jornadas de control sanitario y se utilizaron ensayos de amplificación por PCR para detectar los tres microorganismos en muestras endocervicales. En 33,3% de las muestras, se detectó al menos un microorganismo: *U. urealyticum* fue encontrado con mayor frecuencia (25,6%), seguido de *N. gonorrhoeae* (18%) y *C. trachomatis* (12,8%). Se encontró asociación significativa entre *N. gonorrhoeae* y *C. trachomatis* ($p<0,0001$). Los casos de ITS representaron porcentajes de 42% y 28% para los grupos sintomático y asintomático, respectivamente. *N. gonorrhoeae* fue 2,4 veces más frecuente en el grupo sintomático (28,6%) que en el asintomático (12%) ($p=0,015$), particularmente asociado con secreción mucopurulenta ($p=0,025$). No se encontró asociación entre *C. trachomatis* ($p=0,078$) y *U. urealyticum* ($p=0,432$) con manifestaciones clínicas. La prevalencia de ITS de la población en estudio fue relativamente baja comparada con otras poblaciones de alto riesgo a nivel mundial. Los resultados apoyan la posible asociación entre *C. trachomatis* y *N. gonorrhoeae*, por lo tanto se debería considerar el tratamiento contra ambos patógenos, cuando uno de ellos sea detectado en poblaciones vulnerables.

Palabras clave: ITS curables, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, trabajadoras sexuales, cervicitis, PCR.

* Correspondencia:
E-mail: narraiz1@yahoo.com

Introduction

Sexually transmitted infections (STIs) represent a public health problem worldwide, estimating about 340 million new STI cases annually [1]. The gonorrheal and chlamydial infections are responsible for a high incidence

of reproductive morbidity; *Ureaplasma urealyticum* has been implicated as the etiological agent of non gonoccocal urethritis in some studies; however, the vast majority of STIs are asymptomatic or subclinical, which contributes to the persistence and transmission of these infections in the population [1-4]. Several countries have used health strategies for the active research of STI cases, in fact, in recent years, the information on the prevalence of gonococcal and non gonococcal infections has increased [4-7]; however, it must keep going on emphasizing the need to investigate several sexually transmitted pathogens simultaneously at the population level [3,4,8,9].

Early diagnosis of curable STIs is a necessary strategy to prevent the development of reproductive sequelaes and to reduce the spread of the pathogen [7-10]. Sex workers are a highly vulnerable group to acquire and transmit STIs and Human Immunodeficiency Virus (HIV-1), therefore, it has been suggested that control of STIs should be strengthened in these high-risk individuals [10-12].

In our population, epidemiological surveillance of HIV infections has increased [13], while the diagnosis and treatment of curable STIs cases are still being managed with a syndromic approach, a partial reason for the ignorance of the actual prevalence of these pathogens. This study represents a preliminary strategy to characterize the epidemiological situation regarding these agents, and provide diagnostic tools that could be applied in large-scale population. The aim of this study was to investigate the prevalence of curable STIs caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *U. urealyticum* in a population of female sex workers from Zulia State, Venezuela, the relationship between the three organisms and the presence of clinical manifestations.

Materials and methods

Type of study: It was designed a descriptive and correlational study, since its purpose was to determine the prevalence of curable infections caused by *C. trachomatis*, *N. gonorrhoeae*, and *U. urealyticum* in female sex workers, the relationship between the three organisms, and the presence of a particular pathogen with clinical manifestations.

Population and sample: Endocervical swab samples from 78 women, aged between 18 and 42 years, who work as sex workers, and attended a health monitoring sanitary controls of sex workers, at health centers in Zulia State from January 2009 until November 2009, were tested. The control evaluation of these sex workers included serological tests for diagnosing infections by *Treponema pallidum* and by HIV. Patients with an infection diagnosis from these agents were excluded from the study. One hundred and three (123) women attended, but only 78 signed a consent document for participation, after they were informed of the nature and purpose of the study. The guidelines of the Declaration of Helsinki, as revised in October 2000 were followed. During clinical and gynecological evaluation, patients were classified as symptomatic and asymptomatic. The symptomatic group included patients with clinical manifestations such as abnormal vaginal discharge, cervicitis, post-coital bleeding, dysuria, pelvic pain, dysmenorrhea, or any other aspect considered by the medical specialist. The asymptomatic patient group included those women who showed no clinical symptoms at the moment of the study.

An endocervical swab sample was taken from the patients by a specialized physician in gynecology and obstetrics. Later, the swab was introduced into a 15 mL tube containing 1mL of phosphate saline buffer PBS (0.12 M NaCl, 0.01 M Na2HPO4, 5 mM KH2 PO_4 pH 7.5) as a transport media. The samples were kept refrigerated before being transported to the laboratory for processing.

Molecular Diagnostics:

DNA Extraction: The DNA extraction of *C. trachomatis*, *N. gonorrhoeae*, and *U. urealyticum* from clinical samples was carried out using an enzymatic lysis protocol previously used [14], with some modifications. The samples were incubated at 100 ºC for 15 minutes, then were transferred to 1.5 mL tubes and centrifuged at 14,000 rpm for 20 minutes. The sediment was re-suspended in 400 µL of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8) and 20 µl of lysozyme (20 mg/mL). After incubating the samples for 2 hours at 37 ºC, 40 µL of SDS 1% and 10 µL of proteinase K (20 mg/mL) were added, followed by incubation at 45°C for 3 hours. An organic extraction took place using phenol-chloroform, and the aqueous phase was transferred to another tube and DNA was precipitated with 2 volumes of absolute ethanol. A final wash with 70% ethanol was made, and then the sediment was dried up and re-suspended in 30 µL of TE buffer. Five µl of DNA was used in amplification assays.

Amplification by Polymerase Chain Reaction (PCR): DNA samples were analyzed by PCR amplification using sequence specific primers for each organism. A multiplex PCR amplification was performed, using the MPCR detection kit, CTR/UU/NG from Maxim Biotech, Inc., USA. The kit includes primers targeting specific sequences of DNA from each organism, generating amplification products of 364 bp, 298 bp, and 218 bp for *C. trachomatis*, *N. gonorrhoeae*, and *U. urealyticum*, respectively. The reaction mixture and PCR amplification conditions were reproduced from the instructions given by the supplier manufacturing.

The results were confirmed through PCR tests using primers targeting species-specific sequences reported previously, with amplification conditions previously standardized in the laboratory. For *C. trachomatis*, primers SERO 1A y SERO 2A, that recognizing specific sequences of the *omp1* gene encoding outer membrane protein OMP1 (1.1 Kb), were used [15]. To detect the genome of *U. urealyticum*, U4 and U5 primers, directed to the specific urease gene that generates a product of 429 bp, were used [16]. For *N. gonorrhoeae,* a 390 bp fragment of the *cppB* gene, located in the cryptic plasmid of this organism, was amplified [17].

The PCR products were analyzed on 2% agarose gels,

and stained with ethidium bromide at a final concentration of 0.5ug/mL. The bands were visualized on an ultraviolet transilluminator and photographed with a digital camera Olympus C-4000 (photodocumentation system Digi Doc, UVP).

Statistical analysis: The results were analyzed using SPSS Version 12 for Windows. Through the descriptive analysis, the respective frequency percentages for each of the variables were obtained. The possible association between variables was estimated by comparing observed and expected values using contingency tables, Chi-square test, and Pearson correlation test. A p value <0.05 was considered statistically significant.

Results

Through this study, the prevalence of urogenital infections caused by *C. trachomatis*, *N. gonorrhoeae* and *U. urealyticum,* in endocervical samples from 78 female sex workers, aged between 18 and 42 years (mean 32.7 years) from Maracaibo Municipality, Zulia State, Venezuela, was investigated. This population was incorporated into the study during a health monitoring sanitary controls practice of sex workers at two health centers in Zulia State, Venezuela. The population for this study included 50 (64%) asymptomatic women and 28 (36%) symptomatic women. HIV and *T. pallidum* infections in these patients were discarded by using serological tests, performed as a routine control by health authorities at the region.

For the genome detection of these microorganisms, DNA amplification assays by a multiplex PCR were used. In figure 1 the amplification pattern obtained from the PCR assay for 15 of the 78 samples studied is shown. In lanes 7, 11, and 15, fragments of 364, 298, and 218 bp, corresponding to segments of amplified DNA from *C. trachomatis, N. gonorrhoeae* and *U. urealyticum* respectively, were observed, revealing the presence of these three microorganisms in the samples. In lane 2, DNA fragments of *N. gonorrhoeae* and *U. urealyticum* were detected; in lanes 5, 12, and 14, a specific fragment of *U. urealyticum* is shown, and in lane 9 the two bands corresponding to plasmids of *C. trachomatis* and *N. gonorrhoeae* were observed. The results were confirmed by PCR standardized tests in the laboratory, using primers for each individual organism and 100% correlation between the two systems of diagnosis was obtained.

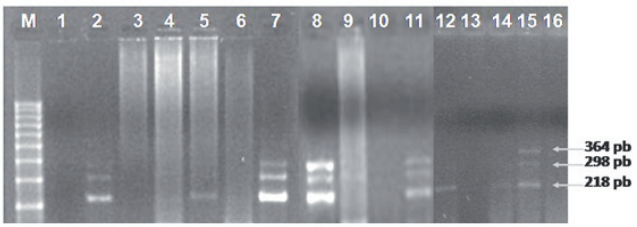

Figure 1. Results of PCR amplification from 15 samples. The arrows on the right indicate the molecular weight of the fragments expected for *C. trachomatis* (364 bp), *N. gonorrhoeae* (298 bp) and *U. urealyticum* (218 bp). M: molecular weight marker. Lane 8: positive control that contains the three genomes of microorganisms.

In 26 (33.3%) of the evaluated samples at least one microorganism was found. Ten cases (12.8%) of infection with *C. trachomatis,* 14 (18%) by *N. gonorrhoeae*, and 20 (25.6%) by *U. urealyticum* were detected, including single and mixed infections by two or by all the microorganisms studied (Table 1).

Table 1. Microrganisms detected by PCR in endocervical secretion samples from 78 female sex workers in Zulia State, Venezuela (2010).

Presence or absence	*C. trachomatis*	*N. gonorrhoeae*	*U. urealyticum*
Negative	68 (87,2%)	64 (82,1%)	58 (74,4%)
Positive	10 (12,8%)	14 (18%)	20 (25,6%)

The table includes cases of single infection for each organism or co-infection with two or all the microorganisms investigated.

Infections by a single organism were recorded in only 13 cases (16.7%), corresponding 12 (15.4%) to *U. urealyticum* and only 1 to *N. gonorrhoeae* (1.3%) (Table 2). *C. trachomatis* was not detected individually, but in 5 mixed infections with *N. gonorrhoeae* (6.4%) and in 5 cases of infection by the three microorganisms (6.4%). Co-infection by *N. gono*rrhoeae and *U. urealyticum* (3.8%) was also observed. A highly significant association between the presence of *N. gonorrhoeae* and C. *trachomatis* (p <0.0001) was found.

U. urealyticum was the most frequently detected microorganism in the symptomatic group (14.3%), with only 16% in the asymptomatic one. Considering cases of coinfection, this organism reaches values of 32.1% and 22% in symptomatic and asymptomatic groups (p=0.325), respectively. *N. gonorrhoeae* was 2.4 times more frequent in the symptomatic group (28.6%) than in the asymptomatic one (12%) (p=0.015) (Table 2).

In the symptomatic group (n=28) the predominant clinical manifestations were cervicitis and mucopurulent discharge, clinically documented in 13 (46.4%) and 8 women (28.6%), respectively, and both conditions accounted for 26.9% of the total studied population (n=78).

An evaluation of infected individuals was performed considering particular clinical findings: Twenty eight percent (14 cases) of infections was related to the group without clinical manifestations, 6 infection cases in 13 women with cervicitis (46.2%), and 5 (62.5%) cases of infection in women with mucopurulent discharge. Cases of infection were more frequent in the group with clinical manifestations (42.9%) than in the asymptomatic group.

When analyzing the possible association between single infections or co-infections with particular clinical manifestations, the cases of infection caused by the three microorganisms was 7 times lower in the asymptomatic group (2%) than in symptomatic one (14.3%), specifically in cases of cervicitis (Table 3). Cervicitis and mucopurulent discharge was significantly associated with the presence of *N. gonorrhoeae* (p=0.025), and no association was found between *C. trachomatis* (p=0.078), and *U. urealyticum*

Table 2. Individual and mixed infection by *C. trachomatis*, *N. gonorrhoeae* and *U. urealyticum* in symptomatic and asymptomatic groups of female sex workers.

Microorganism	Symptomatics Cases (%)[a] (%)[b]	Asymptomatics Cases (%)[c] (%)[d]	Total Cases (%)[e]
None	16 (57.1) (20.5)	36 (72) (46.2)	52 (66.7)
Single infection by *N. gonorrhoeae* (NG)	1 (3.6) (1.3)	0	1 (1.3)
Single infection by *U. urealyticum* (UU)	4 (14.3) (5.1)	8 (16) (10.3)	12 (15.4)
Coinfection CT + NG	2 (7.1) (2.6)	3 (6) (3.9)	5 (6.4)
Coinfection NG + UU	2 (7.1) (2.6)	1 (2) (1.3)	3 (3.8)
Coinfection CT + NG + UU	3 (10.7) (3.9)	2 (4) (2.6)	5 (6.4)
Total	28 (100) (35.9)	50 (100) (64.1)	78 (100)

(a) Percentage of each type of infection expressed in relation to the symptomatic group (n = 28). (b) Percentage of each type of infection in the symptomatic group expressed in relation to the total population evaluated (n=78). (c) Percentage of each type of infection expressed in relation to the asymptomatic group (n=50). (d) Percentage of each type of infection in the asymptomatic group expressed in relation to the total population evaluated (n=78). (e) Percentage of each type of infection expressed in relation to total population evaluated.
Cases of coinfection CT + NG: ($p<0.0001$)
N. gonorrhoeae: symptomatic vs asymptomatic group ($p=0.015$)
U. urealitycum: symptomatic vs asymptomatic group ($p=0.325$)

Table 3. Association between signs, symptoms and the presence of infection by *N. gonorrhoeae*, *C. trachomatis* and *U. urealyticum*.

Infection	Absence of clinical findings Cases (%)[a] (%)[b]	Cervicitis Cases (%)[a] (%)[b]	Mucopurulent discharge Cases (%)[a] (%)[b]	Dysuria Cases (%)[a] (%)[b]	Total Cases (%)[c]
None	36 (72) (69.2)	7 (53.9) (13.5)	3 (37.5) (5.8)	6 (85.7) (11.5)	52 (66.7)
Single infection by *N. gonorrhoeae* (NG)	0	0	1 (12.5) (100)	0	1 (1.3)
Single infection by *U. urealyticum* (UU)	9 (18) (75)	2 (15.4) (16.7)	1 (12.5) (8.3)	0	12 (15.4)
Coinfection CT + NG	3 (6) (60)	1 (7.7) (20)	1 (12.5) (20.0)	0	5 (6.4)
Coinfection NG + UU	1 (2) (33)	0	1 (12.5) (33.3)	1 (14.3) (33.3)	3 (3.8)
Coinfection CT + NG + UU	1 (2) (20)	3 (23.1) (60)	1 (12.5) (20)	0	5 (6.4)
Total	50 (100) (64.1)[c]	13 (100) (16.7)	8 (100) (10.3)	7 (100) (9)	78 (100)

a) Percentage of each type of infection in relation to particular clinical manifestation. (b) Percentage of cases in relation to the groups without infection, with infection or with co-infection. (c) Percentage of the total population evaluated.
Association between cervicitis, mucopurulent discharge and NG: $p=0.025$.
Association between cervicitis, mucopurulent discharge and CT: $p=0.078$.
Association between cervicitis, mucopurulent discharge and Uu: $p=0.432$.

($p=0.432$) with particular clinical manifestations in women evaluated.

Discussion

In the past, the most relevant STIs were syphilis and gonorrhea. Nowadays, the clinical significance of other microorganisms that cause sexually transmitted infections, such as *C. trachomatis* and various species of the genus *Mycoplasma* [1,5-8,10] is recognized. Due to the syndromic management of STI cases in our population, the actual prevalence of these pathogens is unknown, and according to our knowledge, this is the first report of gonococcal and non gonococcal STIs that could represent a preliminary contribution to design diagnostic strategies, at larger scale, to determine the epidemiological situation of these pathogens in the region.

Prevalence of STI pathogens investigated in this population was similar to a recently report in an urban population at high risk of Nairobi, Kenya [12]; however, it could be

considered relatively low when compared to other groups of sex workers in other developing countries [18,19,21,23], which reported high prevalence of STIs by *N. gonorrhoeae* (35-55%) and *C. trachomatis* (25-45%). The relatively low prevalence of infection in the evaluated population could be explained by the regular use of condoms, which was informed by most of the participants, but with inaccurate frequency of use, so it was not possible to obtain conclusive results.

In the particular case of STIs caused by *C. trachomatis*, it is striking that the prevalence (12.8%) found in this high risk population, was not significantly higher than the reported in a recent study [14], in a group of women attending to the routine gynecologic control (10.4%); so, *C. trachomatis* appears to be a common pathogen in our region, and could be considered to have a medium to high prevalence according to the criteria of several authors [7,21,23].

In this study, *C. trachomatis* was found only on co-infection conditions with *N. gonorrhoeae*, which is consistent with several reports indicating an interdependence of these microorganisms in high-risk subjects [4,23-25], while other authors suggest an unidirectional relationship, in which the infection with *N. gonorrhoeae* predisposes a infection with *C. trachomatis*. Because a hierarchical character of infection has not been well elucidated, it is recommended to indicate treatment against the two pathogens, even though only one is detected, particularly for people at high risk [12,20].

Another important finding was the association between *N. gonorrhoeae* and the presence of clinical manifestations, particularly in cases of cervicitis and mucopurulent discharge. Although infections by *C. trachomatis* and *U. urealyticum* may also occur with cervicitis [5,7,15], the presence of *C. trachomatis* or *U. urealyticum* was not associated with clinical findings, suggesting that co-infection cases of cervicitis with these pathogens is attributable to the presence of *N. gonorrhoeae*. Antimicrobial therapy for these patients should be prescribed to both pathogens, because both *N. gonorrhoeae* and *C. trachomatis* can develop pelvic inflammatory disease and tubal obstruction, increasing the risk of sterility.

A key issue to discuss is that the increased frequency of infections, by the pathogens investigated, was observed in the group of symptomatic women (42.9%) hence the therapeutic syndromic management may gain importance and contribute to the decrease of these pathogens prevalence in our region. However, to support this hypothesis is necessary to assess populations of sex workers on a large scale. The limitation of the syndromic pattern is that 28% of cases of infection detected in asymptomatic women remain untreated; therefore, it is necessary to highlight diagnostic strategies that allow early and appropriate therapeutic guidance.

The absence of clinical manifestations in these infections has been explained by several reasons. It has been estimated that over 50% of infections produced by *N. gonorrhoea*e and *C. trachomatis* do not cause enough inflammation to reveal obvious clinical manifestations [20]. Furthermore, the asymptomatic nature has been explained by poor adherence to the treatment [12], because the lesions of cervical-vaginal tract in women treated may heal, but treatment failure may lead to episodes of endogenous re-infection.

This study also found a high proportion of cervical-vaginal infections caused by *U. urealyticum*, similar to the reported in other populations [16,25], but no association was found between infection by this organism and particular signs or symptoms. The role of *U. urealyticum* as urogenital pathogen has been controversial, because there is insufficient evidence of the pathogenic potential of this organism and high detection rate in asymptomatic patients [26]. However, several studies report a strong association between infection by *U. urealyticum* with gynecologyc and obstetric complications, such as pelvic inflammatory disease, abortions and infertility [27,28], and some authors warn that this organism should be considered as the responsible of pathologies in the absence of gonorrheal and chlamydial infection [16,25,28]. The cases of cervicitis and mucopurulent secretion, in which single infection by *U. urealyticum* was identified, could be classified under these criteria but requires better characterization of the role of this organism as urogenital pathogen in our environment, through the evaluation of a large-scale population.

The investigation of *C. trachomatis*, *N. gonorrhoeae*, *T. pallidum* and HIV has been strongly recommended in high risk patients [20]. This study meets these criteria, because the first two pathogens were investigated, and the information of the last two pathogens was collected by official health agencies.

Since asymptomatic infections are common and many of them can remain without symptoms for many years, patient's education is crucial, particularly for sex workers. The physician should alert about their risk behavior and emphasize the importance of developing or strengthening safer sexual practices such as condom use, the need to attend health monitoring sanitary controls, and adhere to the prescribed treatments [29].

In conclusion, although *U. urealyticum* was the most prevalent organism, its presence was not associated with clinical manifestations. *N. gonorrhoeae* was the second most frequently encountered pathogen, particularly in cases of cervicitis and mucopurulent discharge, whereas *C. trachomatis* infection was detected only in co-infection with *N. gonorrhoeae*, supporting the need to prescribe antibiotic treatment against both pathogens, even though only one is detected.

It is necessary to assess populations of female sex workers on a larger scale to confirm these findings; therefore, the diagnostic strategies used in this study could be of great value.

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