ORIGINAL ARTICLE

Diagnosis of cervicovaginal pathogens in symptomatic pregnant women hospitalized since the second trimester

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ABSTRACT

Introduction: infections of the female genital tract constitute an important health problem in medicine. Pregnancy is a predisposing factor for the acquisition and suffering of these infections.
Objective: to characterize cervicovaginal pathogens in symptomatic pregnant women hospitalized from the second trimester onwards.
Methods: a descriptive and cross-sectional research was conducted in the Microbiology Laboratory of the "Mariana Grajales" Hospital in the period from 2018 to 2019. The sample consisted of 86 symptomatic pregnant women hospitalized from the second trimester in the Perinatal Care Service. From the descriptive statistics, absolute numbers and percent and the two-stage clustering and cross-table technique were used within the subgroups obtained.
Results: there was a predominance of positive results for pathogenic microorganisms in 58.13% of the samples studied, mainly *Candida* spp (19.76%) and *Ureaplasma* spp (44.19%). Their antibiotic resistance to tetracycline predominated in 26.32% of their detections, but not to erithromycin (5.26%). Of the 14 coinfections diagnosed, *Ureaplasma* spp. was present in 13 and of these, 6.98% were with *Candida* spp.
Conclusions: there was a predominance of positive samples, with prevalence of *Candida* spp. and *Ureaplasma* spp, the latter resistant to tetracycline and sensitive to erythromycin and as the only integral microbiological diagnosis and in almost all positive diagnoses with coinfection.
Key words: cervicovaginal pathogens; obstetric infections; pregnancy

RESUMEN

Introducción: las infecciones del aparato genital femenino constituyen un importante problema de salud en Medicina. El embarazo es un factor predisponente para la adquisición y el padecimiento de estas infecciones.

Objetivo: caracterizar los patógenos cervicovaginales en embarazadas sintomáticas hospitalizadas a partir del segundo trimestre.

Métodos: se realizó una investigación descriptiva y transversal en el Laboratorio de Microbiología del Hospital "Mariana Grajales" en el período de 2018 a 2019. La muestra estuvo constituida por 86 embarazadas sintomáticas hospitalizadas a partir del segundo trimestre en el Servicio de Cuidados Perinatales. De la estadística descriptiva se emplearon los números absolutos y el por ciento y la técnica de conglomerados en dos fases y de tablas cruzadas dentro de los subgrupos obtenidos.

Resultados: hubo predominio de resultados positivos a microorganismos patógenos en el 58,13% de las muestras estudiadas, fundamentalmente *Candida* spp. (19,76%) y *Ureaplasma* spp. (44,19%). Su resistencia antibióticaa la tetraciclina predominó en el 26,32% de sus detecciones, no así a la eritromicina (5,26%). De las 14 coinfecciones diagnosticadas el *Ureaplasma* spp. estuvo presente en 13 y de ellas el 6,98% con *Candida* spp.

Conclusiones: hubo predominio de muestras positivas, con prevalencia de *Candida* spp. y *Ureaplasma* spp*.*, esta última resistente a tetraciclina y sensible a la eritromicina y como diagnóstico microbiológico integral único y en casi la totalidad de los diagnósticos positivos con coinfección.

Palabras clave: patógenos cervicovaginales; infecciones obstétricas; embarazo

INTRODUCTION

Pregnancy leads to an imbalance of the vaginal microenvironment, favors the proliferation of microorganisms and increases the severity and recurrence of infections. This is due to increased cervicovaginal secretions, with decreased local response, associated with the progestin action on T lymphocytes and on the anti-candida activity of polymorphonuclear cells.[1]

54.5% of the reports of patients with vaginal infections correspond to pregnant women, with a predominance of candidiasis vaginitis and bacterial vaginosis and with a risk of acquiring vaginal infections 67 times higher, and significantly so, with respect to non-pregnant women, which is associated with a large number of gynecobstetric complications such as preterm delivery, premature rupture of membranes (PROM) and low birth weight newborns, and even maternal complications such as chorioamnionitis and puerperal infection.[2]

Other microorganisms reported as a cause of cervicovaginal infections worldwide are the urogenital mycoplasma species, including *Mycoplasma hominis* and *Ureaplasma* spp.[3] They are nutritionally demanding cell wall-free bacteria. Both are part of the normal vaginal flora of sexually active individuals, so this is believed to be the source of intrauterine infection in most cases. They have been associated, among others, with neonatal infections such as bacteremia, congenital pneumonia, the development of bronchodysplasia, meningitis and abscesses, increased incidence in preterm delivery and vertical transmission or at birth.[4]

It is described that up to 20% of newborns are colonized with these bacteria, but colonization rates drop significantly over the next three months. Preterm infants are at the highest risk of colonization.[5]

International research reports an increase in infections with urogenital mycoplasmas.[3]

A study on the microbiological profile of pathogens isolated in vaginal and urethral secretions shows a predominance of *Candida albicans* (36.3%), *Gardnerella vaginalis* (25.7%) and *Trichomona vaginalis* (15.1%).[6,7]

With the introduction of advanced methods in Cuba, such as the MYCO WELL D-ONE® diagnostician for presumptive identification and determination of antimicrobial susceptibility of urogenital mycoplasmas in Microbiology Laboratories, it is possible to identify these species, which contributes to establish treatment strategies. It is a reliable, fast, sensitive, specific and less expensive method than molecular methods and can be used in a larger number of samples.

In the "Mariana Grajales" Hospital there have been no studies of cervicovaginal pathogens in hospitalized pregnant women with urogenital symptoms, so it is a challenge to know the implication of these microorganisms in maternal-fetal health. The aim of this research is to characterize cervicovaginal pathogens in symptomatic pregnant women hospitalized from the second trimester onwards.

METHODS

A descriptive and cross-sectional research was carried out in the Microbiology Laboratory of the University Gynecoobstetric Provincial Hospital "Mariana Grajales" of Santa Clara City, Villa Clara Province, in the period from January 2018 to December 2019.

The sample consisted of 86 pregnant women with cervicovaginal infections hospitalized in the Perinatal Care Service of the Hospital, selected by non-probabilistic purposive sampling by criterion.

Patients with cervical lesions, active endocervical bleeding and cervicovaginal treatment at the time of sampling were excluded.

Diagnostic procedures were governed by the norms established in the Microbiology Laboratory of the "Mariana Grajales" Hospital.

To obtain the vaginal exudate sample, the patient was placed in the gynecological position and a sterile, non-lubricated vaginal speculum was placed on her, with a first sterile cotton swab, preferably from the posterior sac fundus, the first sample was taken and, with a second applicator, another sample was taken for mycological culture.

Microscopy was used to look for yeast cells, guide cells, polymorphonuclear leukocytes, lymphocytes, *Trichomonas vaginalis* and *Mobiluncus* spp.

The diagnosis of bacterial vaginosis was established in at least three of the four clinical criteria of Amsel.

In the mycological culture in Sabouraud's Dextrose Agar, to look for the growth of colonies characteristic of the Candida genus, Higashide's criteria were applied to differentiate between colonization and growth compatible with an infectious process.[8]

A sample for Chlamydia spp. was obtained from the endocervical orifice using Chlamy-Check-1 and for urogenital mycoplasmas using MYCO WELL D-ONE®.

MYCO WELL D-ONE® is a test to detect urogenital mycoplasmas (Mycoplasma hominis and Ureaplasma spp. in single infections or coinfection - meaning color change in both wells of Mycoplasma hominis and Ureaplasma spp.-) and allows, by minimum inhibitory concentration, to establish antibiogram with tetracycline, levofloxacin, moxifloxacin and erythromycin.

Other co-infection criteria were established, such as *Ureaplasma* spp. combined with other agents and bacterial vaginosis - *Candida* spp.

The data corresponding to the study variables were entered into files and processed using SPSS version 22 and Microsoft Excel 2016.

From the descriptive statistics, absolute numbers and percent were used as summary measures for qualitative variables and it was necessary to make use of the two-phase clustering technique and cross tables within the subgroups obtained.

The results were presented in text and tables.

The ethical norms established in the context and the bioethical principles of autonomy, beneficence, non-maleficence and justice were taken into account.

RESULTS

In the comprehensive microbiological diagnosis from the vaginal and endocervical secretion samples studied, no pathogenic microorganisms were detected in 36 samples (41.87%), while the positive samples were 50 (58.13%). Of the positive samples, 36 (41.87%) had no coinfection and 14 (16.26%) had coinfection.

Of the microorganisms causing cervicovaginal infection, *Candida* spp. were diagnosed in 17 samples (19.76%), bacterial vaginosis in four (4.65%), *Chlamydia* spp. in two (2.32%) and *Trichomonas vaginalis* in one (1.16%) and *Mobiluncus* spp. were not detected.

The results of endocervical exudate samples are shown in Table 1. There was a predominance of negatives (53.49%), although the genus *Ureaplasma* spp. was identified in 44.19% (38).

Table 1. Results on endocervical exudate samples for mycoplasma diagnosis

Results	Patients (N=86)	%*
Negative	46	53.49
Positive to *Ureaplasma* spp.	38	44.19
Positive to *Ureaplasma* spp. and *Mycoplasma hominis*	2	2.32
Total	**86**	**100**

* Percent calculated in relation to N

Source: Microbiology Laboratory Record Book, Sexually Transmitted Infections (STI) Section

Table 2 shows the behavior of *Ureaplasma* spp. resistance to the antimicrobials available in the diagnostician. There was greater resistance to the tetracycline group (10, 26.32%), followed by levofloxacin (9, 23.68%). Resistance to erythromycin was found in only two patients (5.26%).

Table 2. *Ureaplasma* spp. resistance to antimicrobial agents

Antimicrobials	Microbial resistance (N=38)	
	Number	%*
Levofloxacin	9	23.68
Moxifloxacin	5	13.16
Tetracycline	10	26.32
Erythromycin	2	5.26

*Percent calculated in relation to N

Fuente: Libro de Registro del Laboratorio de Microbiología, Sección ITS

As for the positive microbiological diagnosis with coinfection, as shown in Table 3, the association of *Ureaplasma* spp. and *Candida* spp. predominated in six

cases (6.98%), and those of *Ureaplasma* spp. and *Mycoplasma hominis* and of *Ureaplasma* spp., *bacterial vaginosis* and *Candida* spp. in two cases each (2.32%).

Table 3. Distribution of co-infection in vaginal and endocervical secretion specimens

Positive diagnostic(s) with coinfection	Number (N=86)	%*
Ureaplasma spp.- *Candida* spp.	6	6.98
Ureaplasma spp.- *Mycoplasma hominis*	2	2.32
Ureaplasma spp.- *Bacterial vaginosis-Candida* spp.	2	2.32
Ureaplasma spp.- *Chlamydia* spp.	1	1.16
Ureaplasma spp.- *Trichomona vaginalis*	1	1.16
Ureaplasma spp.- *Bacterial Vaginosis*	1	1.16
Bacterial vaginosis - Candida spp.	1	1.16
Total	**14**	**16.26**

* Percent calculated in relation to N
Source: Microbiology Laboratory Record Book, ITS Section

DISCUSSION

The present investigation showed results similar to those reported in international and national literature because the diagnosis of *Ureaplasma* spp. predominated in about half of the endocervical samples studied. A study carried out in Venezuela[8] showed 60% positivity, while *Mycoplasma hominis* was detected in one case (4%) associated with *Ureaplasma* spp. In vaginal exudates of 46 pregnant women with preterm labor attended at the Gynecology and Obstetrics Service of the Fundación Hospital Infantil Universitario de San José de Bogotá, *Ureaplasma urealyticum* was detected in 21 (43.48%) and *Mycoplasma hominis* in nine (19.57%).[9]

In a research carried out in Havana, in 181 positive vaginal samples of pregnant women, 84 corresponded to the diagnosis of *Ureaplasma urealyticum* (46.4%).[10]

It is important to highlight the essential role of *Ureaplasma* spp. as a cervicovaginal infectious etiology in pregnant women. Its high frequency could result from the imbalance of the vaginal microenvironment with subsequent ascent of these microorganisms to the endocervix, where they colonize and invade, with the consequent risk of damaging the ovarian membranes and causing adverse perinatal outcomes.[11,12,13,14]

The analysis showed a predominance of *Ureaplasma* spp. resistance to the tetracycline group, followed by levofloxacin and moxifloxacin, leaving erythromycin (macrolides of choice) as a suggestion for treatment.

This result does not coincide with those presented in a research[8] in which no resistance to the tetracycline group was found but to the fluoroquinolones group (81.25%), nor with those determined in another[12] which indicates higher resistance percentages to macrolides (from 91.9% to 88.4%) and to fluoroquinolones (from 94% to 55.3%). Both used the Biomerieux Mycoplasma IST 2 diagnostician in samples of vaginal secretions from pregnant women.

Nor do the results coincide with those reported by Vázquez Niebla JC et al[10] who found greater resistance of *Ureaplasma urealyticum* to macrolides (from 100% to 6.5%) and of *Mycoplasma hominis* to macrolides (from 100% to 32.6%) and to tetracycline (32.6%).

These data are important because they reflect how mycoplasmas are beginning to evolve in the face of the selective pressure exerted by empirical antimicrobial therapies using tetracycline, which are frequently applied to treat various nonspecific infections, although its prescription during pregnancy is limited due to its teratogenic effects; nevertheless, the abuse and self-medication of this antibiotic by the population is a problem with important clinical and therapeutic implications.[15,16,17]

The associations of pathogenic or potentially pathogenic microorganisms in the genital secretions of pregnant women at term constitute a major cause for concern for the Specialists who take care of this vulnerable group of the population due to the therapeutic difficulties involved, the increased risk of invasive ascending infections towards the endocervix and the ovular membranes and the consequent appearance of serious maternal and infant complications.

In cervicovaginal infections in which two or more microorganisms concomit, different combinations can be seen, the most common being *Ureaplasma* spp.-*Candida* spp., *Ureaplasma* spp.-*Mycoplasma hominis* and *Ureaplasma* spp.-*bacterial vaginosis-Candida* spp.

Mucci[18] reached similar conclusions when he identified 5.2% of coinfection between *Ureaplasma* spp. and *Candida* spp.; Quartara[19] differs because he identifies *Ureaplasma* spp.-*Mycoplasma hominis* as the most common association of coinfection (6.75%) in five cases.

In a study carried out in Santiago de Cuba, a high percentage had more than one agent in their genital secretions (67%);[20] the most common microbial associations are those between *Mycoplasma* and *Chlamydia* (29.1%) and between *Mycoplasma*, *Chlamydia* and *Candida* (18.4%), although it has similarities with the present study in finding 5.6% of coinfection *Ureaplasma* spp.-*Candida* spp.

Other research does not agree, obtaining higher numbers of coinfections between *Mycoplasma hominis* and *Ureaplasma* spp. (45.8%) and between *Candida* spp. and *Ureaplasma* spp. (37.5%). Although he also reports two patients (4.1%) who presented a combination of *Ureaplasma* spp, *bacterial vaginosis* and *Candida* spp.[11]

The fact that there are co-infections with proven repercussions on health increases the risk even more in susceptible groups such as pregnant women, in whom the disease has a direct impact on the term of gestation, delivery and the newborn, and constitutes an additional cause for concern for specialists.

CONCLUSIONS

There was a predominance of positive samples studied, with prevalence of *Candida* spp. and *Ureaplasma* spp, the latter resistant to tetracycline and sensitive to erythromycin and as the only integral microbiological diagnosis and in almost all positive diagnoses with coinfection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

EGÁ: conceptualization, data curation, research, project management, validation, original drafting and writing (review and editing).
LMC: data curation, research.
MLP: research, supervision, writing the original draft, and writing (reviewing and editing).
NDM: formal analysis, research, methodology, validation.
DGG: research, validation.
RAD: research, methodology.