ARTÍCULO ORIGINAL

Diagnosis of non-viral lower genital infections in patients from the Pediatric and Adolescent Gynecology Clinic

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ABSTRACT

Introduction: non-viral lower genital infections are one of the most frequent reasons for medical consultation in Gynecology. Youth is a vulnerable period for the development of this type of infections. The microbiological diagnosis by the various existing techniques guarantees to determine the specific etiology causing the genital infection.
Objective: to characterize non-viral lower genital infections in young females from the infantile and juvenile Gynecology Clinic.
Methods: a prospective longitudinal descriptive research was carried out in the Microbiology Laboratory of the "Mariana Grajales" Hospital from January to December 2019. The sample consisted of 91 patients who met the inclusion requirements. Their diagnosis was made according to the algorithm established in the reproductive tract infection section.
Results: vulvovaginitis was present in 41.8% of patients, *Candida spp.* was isolated in 63.2% of young women with vulvovaginitis, *Ureaplasma spp.* was diagnosed in 22% of cases, pathogenic bacteria were not isolated in 76.9% of patients and in the polymerase chain reaction diagnostic kit the most detected microorganism was *Mycoplasma genitalium* in 16.5% of cases.
Conclusions: vulvovaginitis due to *Candida spp.* was the most frequent clinical presentation of non-viral lower genital infections among young women. *Ureaplasma spp.* was the most frequently detected microorganism using the Myco Well D One commercial kit. No pathogenic bacteria were isolated in the majority of patients. The most detected microorganism by polymerase chain reaction was *Mycoplasma genitalium*.
Key words: non-viral lower genital infections; young females; microbiological diagnostics

RESUMEN

Introducción: las infecciones genitales bajas no virales constituyen uno de los motivos más frecuentes de consulta médica en Ginecología. La juventud constituye un período vulnerable para el padecimiento de este tipo de infecciones. El diagnóstico microbiológico por las variadas técnicas existentes garantiza precisar la etiología específica que causa la infección genital.
Objetivo: caracterizar las infecciones genitales bajas no virales en jóvenes femeninas procedentes de la Consulta de Ginecología infantojuvenil.
Métodos: se realizó una investigación descriptiva longitudinal prospectiva en el Laboratorio de Microbiología del Hospital "Mariana Grajales" en el período de enero a diciembre de 2019. La muestra quedó constituida por las 91 pacientes que cumplieron con los requisitos de inclusión. Su diagnóstico se realizó según el algoritmo establecido en la sección de infección del tracto reproductor.
Resultados: la vulvovaginitis estuvo presente en el 41,8% de las pacientes, en el 63,2% de las jóvenes con vulvovaginitis se aisló *Candida spp.*, se diagnosticó *Ureaplasma spp.* en el 22% de los casos, en el 76,9% de las pacientes no se aislaron bacterias patógenas y en el estuche diagnóstico de reacción de la cadena polimerasa el microorganismo más detectado fue el *Mycoplasma genitalium* en el 16,5% de los casos.
Conclusiones: la vulvovaginitis por *Candida spp.* fue la forma clínica más frecuente de presentación de las infecciones genitales bajas no virales entre las jóvenes. *Ureaplasma spp.* fue el microorganismo más detectado mediante el estuche comercial Myco Well D One. En la mayoría de las pacientes no se aislaron bacterias patógenas. El microorganismo más detectado por reacción de la cadena polimerasa fue *Mycoplasma genitalium*.

Palabras clave: infecciones genitales bajas no virales; jóvenes femeninas; diagnósticas microbiológicas

INTRODUCTION

Non-viral lower genital infections are one of the most frequent reasons for medical consultation in Gynecology and are an important cause of morbidity, including infections of the cervix, vagina, vulva and their adnexal glands. Among the most frequent microorganisms of non-viral etiology are *Candida spp*. causing bacterial vaginosis, Trichomonas vaginalis, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and some species of *Mycoplasma*.[(1)]
Youth is the period in which the psychological, social and biological characteristics of each person are consolidated; it is a stage of life in which there is a lack of the necessary knowledge to carry out organized sexual practices, in addition to the development of sexual characteristics in both sexes, which makes them feel the need to experience new ways of relating to each other. These conditions make this stage a vulnerable period for the contagion and suffering of genital infections.[(2)]
According to the World Health Organization in 2016 every day more than one million people contract a sexually transmitted infection (STI). Annually, an estimated 376 million people contract one of the following four: chlamydiasis (127 million), gonorrhea (87 million), syphilis (six million) or trichomoniasis (156 million).[(3)]
Infection by Chlamydia trachomatis is the second most frequent genital infection in the world and the most common in young women.[(4)]

Approximately 50,000 cases of STIs are reported annually in Cuba, with an upward trend in recent years. Among the most frequent are blennorrhagia, syphilis, non-gonococcal urethritis, condyloma acuminatum, trichomoniasis, genital herpes simplex and human immunodeficiency virus (HIV).[2]

There are no previous studies in Cuba or in Villa Clara Province that specifically address this issue in this age group, only the study conducted in Villa Clara by López Pérez and collaborators in women attended in the Gynecology outpatient clinic, in which 222 patients were analyzed: 18.9% showed yeast cells, 16.2% presented bacterial vaginosis and four patients had a diagnosis of *Trichomonas vaginalis*.[4]

Genital infections in Villa Clara Province represent a health problem due to the high incidence with which they are reported, the amount of resources used in their diagnosis and treatment, and the impact they have on the sexual and reproductive health of the young women who suffer from them.

Young women constitute a sensitive group due to the psychological and biological immaturity in this period of life, and at the same time it is also a stage in which proper diagnosis and timely treatment can turn around the evolution of the disease. Knowing the main clinical and microbiological characteristics of genital infections in young women in the province is a valuable tool for a better diagnostic and therapeutic approach and for the prevention of future complications. This work characterizes non-viral lower genital infections in young females from the Pediatric and Adolescent Gynecology Clinic.

METHODS

A prospective longitudinal descriptive research was carried out in the Microbiology Laboratory of the "Mariana Grajales" University Gynecological and Obstetric Provincial Hospital of Santa Clara City, Villa Clara Province, from January to December 2019.

The sample was selected by non-probabilistic purposive sampling by criteria and consisted of the 91 patients who met the inclusion criteria:

- Being between 12 and 20 years of age
- Clinical diagnosis of non-viral lower genital infections.
- Having sexual intercourse
- Accepting to be included in the study.

Patients with suspected lower genital infections referred from the infant and adolescent gynecology office, once the clinical diagnosis was confirmed, were referred to the microbiology laboratory for sample collection. These were collected, preserved and processed according to the diagnostic algorithm established in the reproductive tract infections section of the laboratory: vaginal exudate was performed to evaluate Amsel criteria (foul-smelling leucorrhea, presence of guide cells in the vaginal exudate, positive amine test and pH greater than 4.5), which allowed the diagnosis of bacterial vaginosis; bacteriological vaginal culture on Blood Agar and Mac Conkey Agar, used for diagnosis of other pathogenic bacteria; mycological culture on Saboureaud Dextrose Agar for diagnosis of *Candida* species; endocervical exudate for use of the Myco Well D One commercial kit in the diagnosis of *Mycoplasma hominis*, *Mycoplasma spp*. and *Ureaplasma spp*. and, finally, self-collectable

vaginal exudate collected together with 20 ml of urine sample was used to perform multiple reverse transcription-polymerase chain reaction (RT-PCR) at the Laboratory of Molecular Biology of the Provincial Center of Hygiene, Epidemiology and Microbiology of Villa Clara for the diagnosis of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis*.

An Observation Guide was prepared for the collection of the necessary data for the study based on the request for microbiological examination and the Laboratory's Record Book. The information obtained was entered into a database.

The variables used in the investigation were the clinical forms of presentation (vulvovaginitis, vaginosis, cervicitis and cervicovaginitis) and the diagnosis of *Candida spp.*, bacterial vaginosis, *urogenital Mycoplasmas*, other pathogenic bacteria and genital infections by multiple RT-PCR.

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

Descriptive statistics were used as summary measure for qualitative variables, absolute and relative frequencies (numbers and percent).

The test of independence based on the Chi-square distribution was applied; due to limitations in this, the result of Fisher's exact test was taken. The results were presented in tables.

The ethical standards established in the context and the bioethical principles of autonomy, beneficence, nonmaleficence and justice were taken into account. Informed consent was obtained in writing from the patients or, in the case of minors under 18 years of age, from their legal guardians.

RESULTS

Vulvovaginitis was diagnosed in 38 patients (41.8%), followed by cervicitis (24, 26.3%) and vaginosis (17, 18.7%). A clinical diagnosis of cervicovaginitis was made in only 12 (13.2%) of the cases studied.

When evaluating the results of the different diagnoses applied, it was observed that 37.4% of the patients had *Candida spp.*, bacterial vaginosis was diagnosed in 24.2%, urogenital mycoplasmas in 25.3% and other pathogenic bacteria in 23.1% of the cases. A 39.6% positivity rate was also detected using the real-time multiple PCR technique for genital infections.

Table 1. Diagnosis of *Candida spp.* according to the clinical forms of non-viral lower genital infections

Diagnosis of *Candida spp.*	**Clinical forms**								**Total**	
	Cervicitis		**Vulvovaginitis**		**Vaginosis**		**Cervicovaginitis**			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Positive	4	16.7	24	63.2	2	11.8	4	33.3	34	37.4
Negative	20	83.3	14	36.8	15	88.2	8	66.7	57	62.6
Total	**24**	**100**	**38**	**100**	**17**	**100**	**12**	**100**	**91**	**100**

χ^2=20.040; p=0.000

Table 1 shows the diagnosis of *Candida spp.* with the clinical forms of presentation and it was found that, significantly, there is a relationship of dependence between these two variables, and it was observed that 63.2% of

patients with vulvovaginitis had a diagnosis of *Candida spp.*; this microorganism was also isolated in 33.3% of patients with cervicovaginitis and, to a lesser extent, among patients with cervicitis and vaginosis.
The diagnosis of bacterial vaginosis according to the clinical forms of presentation of lower genital infections is shown in Table 2. Significantly, 94.1% of patients with symptoms of vaginosis were diagnosed with bacterial vaginosis based on the Amsel criteria.

Table 2. Diagnosis of bacterial vaginosis according to clinical forms of non-viral lower genital infections

Diagnosis of bacterial vaginosis	Clinical forms								Total	
	Cervicitis		Vulvovaginitis		Vaginosis		Cervicovaginitis			
	No.	%	No.	%	No.	%	No.	%	No.	%
Positive	1	4.2	3	7.9	16	94.1	2	16.7	22	24.2
Negative	23	95.8	35	92.1	1	5.9	10	83.3	69	75.8
Total	**24**	**100**	**38**	**100**	**17**	**100**	**12**	**100**	**91**	**100**

χ^2=49.474; p=0.000

Table 3 showed the relationship between the diagnosis of urogenital *Mycoplasmas* and the clinical forms of presentation and it was observed that the most frequently detected species was *Ureaplasma spp.* present in 33.3% of the cases, followed by *Mycoplasma hominis* and only one case diagnosed with Mycoplasma spp. There was no significant relationship between the clinical forms of lower genital infections and the detection of these microorganisms.

Table 3. Diagnosis of urogenital *Mycoplasmas* according to clinical forms of non-viral lower genital infections

Diagnosis of ***Mycoplasmas*** urogenital	Clinical forms								Total	
	Cervicitis		Vulvovaginitis		Vaginosis		Cervicovaginitis			
	No.	%	No.	%	No.	%	No.	%	No.	%
Mycoplasma hominis	1	4.2	1	2.6	0	0.00	0	0.00	2	2.2
Mycoplasma spp.	0	0.00	1	2.6	0	0.00	0	0.00	1	1.1
Ureaplasma spp.	8	33.3	4	10.5	5	29.4	3	25	20	22
Negativo	15	62.5	32	84.2	12	70.6	9	75	68	74.7
Total	**24**	**100**	**38**	**100**	**17**	**100**	**12**	**100**	**91**	**100**

χ^2=9.078; p=0.360

The diagnosis of other pathogenic bacteria related to the clinical forms of presentation was shown in Table 4, which showed that in 76.9% of the patients no pathogenic bacteria were isolated. There is no significant relationship between these isolates and the clinical forms of presentation. Despite this, of the total number of positive cases, the first type of bacteria, in order of frequency, were enterobacteria, followed by gram-positive cocci and only two cases in which non-fermenting gram-negative bacilli were isolated.
Table 5 showed the relationship between the clinical forms of presentation and the diagnosis of genital infections by multiple RT-PCR. It was observed that there is no significant relationship between these two variables; however, the most detected microorganism was *Mycoplasma genitalium* (16.5%), followed by Chlamydia trachomatis (13.2%) and *Mycoplasma genitalium-Chlamydia trachomatis* coinfection.

Table 4. Diagnosis of other pathogenic bacteria according to clinical forms of non-viral lower genital infections

Diagnosis of other pathogenic bacteria	Clinical forms								Total	
	Cervicitis		Vulvovaginitis		Vaginosis		Cervicovaginitis			
	No.	%	No.	%	No.	%	No.	%	No.	%
Non pathogenic bacteria	18	75	29	76.3	15	88.2	8	66.7	70	76.9
Gram-positive cocci	3	12.5	2	5.3	0	0.00	3	25	8	8.8
Enterobacteriaceae	3	12.5	6	15.8	1	5.9	1	8.3	11	12.1
Gram-negative bacilli	0	0.00	1	2.6	1	5.9	0	0.00	2	2.2
Total	**24**	**100**	**38**	**100**	**17**	**100**	**12**	**100**	**91**	**100**

χ^2=8.430; p=0.416

Table 5. Diagnosis of genital infections by PCR according to clinical forms of non-viral lower genital infections

Diagnosis of genital infections by PCR	Clinical forms								Total	
	Cervicitis		Vulvovaginitis		Vaginosis		Cervicovaginitis			
	No.	%	No.	%	No.	%	No.	%	No.	%
Mycoplasma genitalium	4	16.7	3	7.9	4	23.5	4	33.3	15	16.5
Chlamydia trachomatis	3	12.5	5	13.2	1	5.9	3	25	12	13.2
Trichomonas vaginalis	1	4.2	0	0.00	0	0.00	1	8.3	2	2.2
Neisseria gonorroehae	0	0.00	1	2.6	0	0.00	0	0.00	1	1.1
Chlamydia trachomatis+ Trichomonas vaginalis	0	0.00	1	2.6	0	0.00	1	8.3	2	2.2
Micoplasma genitalium+ Chlamydia trachomatis	1	4.2	2	5.3	1	5.9	0	0.00	4	4.4
Negative	15	62.5	26	68.4	11	64.7	3	25	55	60.4
Total	**24**	**100**	**38**	**100**	**17**	**100**	**12**	**100**	**91**	**100**

χ^2=18.960; p=0.240

DISCUSSION

The diagnosis of genital infections among young women requires a clinical and microbiological analysis that allows a better therapeutic approach. Knowing the main clinical forms and the microorganisms that cause them represents a valuable tool in medical practice.

The research showed that vulvovaginitis is the most frequent clinical form of presentation among young women. When performing the microbiological diagnosis it was found that the highest positivity was obtained by the multiple PCR technique for the detection of genital infections, this diagnostic test has a high sensitivity and allows the detection of Mycoplasma genitalium, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomona vaginalis*; however, when particularizing the diagnosis of each microorganism separately, *Candida spp.* and bacterial vaginosis are the most frequent in the population studied.

Vulvovaginitis due to *Candida spp.* is the most frequent infectious cause in adolescence and a common problem associated with high morbidity rates.[5,6] These results coincide with those of the present investigation in which *Candida spp.* was found as the microorganism causing vulvovaginitis in more than half of the young women.

It is valid to clarify that *Candida spp.* can be isolated in patients with other clinical forms of presentation because it can be found associated with other

microorganisms and even be part of the normal flora without causing the symptoms commonly caused when it is associated with vulvovaginitis.

Bacterial vaginosis is also a frequent disease in sexually active women, so young women who are already sexually active are not exempt from this group. In the present investigation, the diagnosis of this disease was made based on the Amsel criteria, which relates different clinical aspects, although most of them are evaluated in practice in the laboratory.

Bacterial vaginosis is the first cause of genital infection in sexually active women,[7,8,9] which does not coincide with the results of the present investigation, in which vaginosis occupies the second place in order of frequency. This study presents results that demonstrate the great variability that the diagnosis of bacterial vaginosis can have worldwide and refers to the problem of overdiagnosis by the use of the Amsel criteria which, although it presents lower sensitivity and specificity than other methods used for the diagnosis of bacterial vaginosis, constitutes a useful tool to be used due to its low cost, accessibility and easy implementation in the Microbiology Laboratories of the health areas.

Mycoplasma hominis, *Ureaplasma spp.* and *Mycoplasma genitalium* have been definitively associated with disease and potential involvement in urogenital tract disease. Colonization of the urogenital tract by *Mycoplasma hominis* and *Ureaplasma spp.* poses difficulties in the assessment of their isolation from lower genitourinary tract samples, requiring the use of quantitative culture methods and the establishment of cut-off points to differentiate colonization from infection.[10]

For diagnosis, a large number of methods based on selective media cultures have been developed for the detection of genital *Mycoplasma*, such as the Myco Well D One diagnostic kit. This commercial kit allows the detection of *Mycoplasma hominis*, *Ureaplasma spp.* and *Mycoplasma spp.*

Three studies show that *Ureaplasma spp.* is the most frequently isolated *Mycoplasma* in 70%, 56.4% and 15.3% respectively of the patients studied in each study, results that coincide with those of the present investigation.[4,10,11]

There are other specific pathogenic bacteria which are of greater value in the case of pre-pubertal girls where contamination occurs more frequently due to the proximity of the anus to the vaginal introitus and contamination, in many cases, with respiratory secretions. In the case of the present investigation, in which young girls and adolescents are studied, these isolates should be interpreted as etiology of vulvovaginitis when the growth is confluent and is generally caused by inadequate cleaning of the genitals, imbalances of the normal microbiota of the vagina or before some procedure or previous surgical intervention.

According to the article "Vaginal flora in patients attending gynecological consultations", gram-positive cocci (*Enterococcus sp*, Streptococcus group B, *Staphylococcus aureus* and *coagulase-negative Staphylococcus*) and *Escherichia coli* were the microorganisms isolated from patients with aerobic vaginitis: out of a total of 18 patients 27.7% had isolation of enterobacteria and the rest, 72.2%, were gram-positive cocci,[12] which does not coincide with the results of the present investigation in which, in order of frequency, enterobacteria occupy the first place, followed by gram-positive cocci.

In a study of 440 women, 75 patients with *Escherichia coli* vaginitis were isolated, representing 17% of those studied.[13] This result coincides with the present investigation in which enterobacteria are in first place among this group of pathogenic bacteria.

The multiple PCR diagnostic kit for genital infections used in this study allows the detection of four microorganisms (Mycoplasma genitalium, *Chlamydia trachomatis*, *Trichomona vaginalis* and *Neisseria gonorrhoeae*). Its use in young women and adolescents is a valuable tool because it allows the detection of four of the most important sexually transmitted pathogenic microorganisms, with often irreversible consequences on the sexual and reproductive health of women. Mycoplasma genitalium, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are currently the main causes of cervicitis, pelvic inflammatory disease and infertility.

Mycoplasma genitalium is significantly associated with cervicitis and is detected by PCR in 25% of women with this diagnosis, although co-infections are frequent in a logistic regression analysis.[14] This statement differs, in part, from the present investigation in which, although the majority of *M. genitalium* isolates occurred in patients with cervicitis, there is no significant relationship between PCR diagnosis and clinical forms of presentation.

Chlamydia trachomatis is present in 9% of sexually active women under 25 years of age; screening with self-sampling and detection by nucleic acid amplification are the most effective forms of health control.[15] It agrees with the present investigation in the use of self-sampling for the diagnosis of *Chlamydia trachomatis* by PCR, as well as in the frequency of its detection, because it does not differ much from that found.

CONCLUSIONS

Vulvovaginitis due to *Candida spp.* is the most frequent clinical presentation of non-viral lower genital infections among young women, followed by the diagnosis of bacterial vaginosis. *Ureaplasma spp.* was the most frequently detected microorganism using the Myco Well D One commercial kit. Pathogenic bacteria were not isolated in the majority of patients. The microorganism most detected by PCR was *Mycoplasma genitalium*, followed by *Chlamydia trachomatis*, both of which are difficult to diagnose and related to the sexual and reproductive health of the young woman.

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CONFLICT OF INTEREST

There is no conflict of interest in relation to the article presented.

CONTRIBUTION OF THE AUTHORS

RAD: conceptualization, data curation, research, methodology, project management, resources, supervision, original drafting, writing (proofreading and editing).
MLP: conceptualization, research, writing the original draft, writing (proofreading and editing).
ETT: research, resources, writing the original draft, writing (proofreading and editing).
NDM: formal analysis, methodology, writing (revision and editing).
ARR: research, writing the original draft, writing (proofreading and editing).