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Profile of Germs Involved in Cervico-Vaginal Infections in Women: Case of CHU of Mother and Child

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Abstract: ***Cervico-vaginal infections are one of the most common reasons for consultation in gynecology. These infections have a major impact on health. However, in the Chadian context, few studies have addressed this problem. The aim of this study was to determine the susceptibility of germs and factors associated with cervico-vaginal infections in women of childbearing age at the Mother and Child University Hospital. This was a prospective study with a longitudinal focus, which took place from October to March 2021 at the Mother and Child University Hospital in Ndjamena. Isolation and identification of strains were performed according to standard microbiology methods. The number of vaginal swabs taken in our study over a period of 6 months (October - March 2021) was 250, of which 91 cases of vaginal infections were diagnosed, representing a prevalence of 36.4%. The germs encountered were Candida albicans 41.75%, Candida spp 40.05%, Gardnerella vaginalis 12.08%, Staphylococcus spp 3.29%, and Escherichia Coli 2.19%. Candida albicans showed resistance to spiramicin 97.36% to 5fluorocytosine (5FC) 94.73% and to Amphotericime B 26 .31%. Econazole and Nystatin maintained a good sensitivity with 94.73% and 68.42% respectively. The most represented germs were Candida albicans, Candida spp and Gardnerella vaginalis. Health education of the population regarding these infections must be reinforced.***

Keywords: infection; cervico-vaginal; Candida; Gardnerella

1. Introduction

A localized inflammatory process in the vaginal cavity may be due to the presence of one or more associated infectious agents: bacteria, parasites, viruses. This process may be localized to the vaginal mucosa alone, in which case we speak of simple vaginitis, or on the contrary, it may extend to the neighboring mucous membranes, in which case we speak of vulvovaginitis or cervico-vaginal inflammation.

Cervico-vaginal infections are one of the most common reasons for consultation in gynecology. These infections have a major impact on health [1].

Sub-Saharan Africa has the highest rates of sexually transmitted infections (STIs) in the world [2].

In some sexually active women, these infections are recurrent for a number of reasons, including non-use of condoms and multiple partnerships [3].

These infections have a major impact on health, especially for mothers and their unborn babies [4]. They can cause severe complications such as ascending infections, cervical cancer and infertility in women of childbearing age. In pregnant women, they can lead to obstetrical complications such as premature rupture of fetal membranes, growth retardation, postpartum endometriosis and gynecological complications such as pelvic inflammatory disease, postpartum sepsis [5].

The notion of flora imbalance presupposes knowledge of a normal reference flora; however, this remains poorly defined. Moreover, the degradation of the genital flora is not univocal, hence the notions of flora imbalance without vaginosis, partial vaginosis or aerobic vaginitis and the need to define vaginosis on the basis of precise criteria [6].

In general, these infections are associated with significant morbidity, complications and costly treatments [7].

Infections of the female genital tract represent a public health problem in current practice. Initial treatment of vaginal infections is usually probabilistic. The benign nature of the condition and the safety of topical medications allow for immediate management, even if empirical, in order to respond quickly to the patient's discomfort.

However, in Chad, few studies have been conducted on this issue, which is of primary importance for the health of the mother and child. It is for this reason that this study entitled "Profile of germs involved in cervico-vaginal infections in women in the gynecological emergency department and at the prenatal consultation: case of the CHU of the mother and the child" was conducted.

2. Materials and Methods

Setting, type and period of study

This is a prospective study with a longitudinal aim, covering a period of 6 months, from October to March 2021,

conducted in the Bacteriology Department of the Mother and Child University Hospital of Ndjamena.

All outpatients and inpatients who received a vaginal swab for cytobacteriological study were included.

Before starting the diagnosis, the concordance between the name written on the swab and the voucher containing the necessary information of the patient was checked.

The quality of the samples and the clinical information determines the relevance of the bacteriological results.

The sample is taken after stopping any local or general antibiotic therapy and in the absence of local washing on the day of the examination. The patient must not have urinated for at least two hours. The sample should be taken outside the menstrual period and away from sexual relations.

On inspection, the macroscopic appearance is noted, i.e., the presence of leukorrhea, its color, odor, and the appearance of the cervix.

The sites of sampling are dictated by clinical signs and include the vagina-exocervix and the endocervix, depending on the context. The specimen may be taken from the vulva in a young girl.

The approach used standard techniques and therefore included:

All patients had a fresh examination of cervico-vaginal secretions from the cul de sac of Douglas in search of flagellated and motile Trichomonas, the presence of yeasts, highly motile germs, leukocytes, epithelial cells, red blood cells.

The Gram stain showed an imbalance of the vaginal flora, the normal flora being composed only of Doderlein lactobacilli.
Among other things, the complete disappearance of the Doderlein flora, the appearance of an abundant, polymorphic substitute flora, and the presence of variable Gram coccobacilli were observed.

Microbiological analysis
Culturing was done on sabouraud, a 5% sheep blood Columbia agar, and on cooked horse blood agar (CHB) with added vitamin mixture. Each of these media was streaked and incubated at 37°C in an aerobic atmosphere for 24 to 48 hours in an oven.

The identification of bacterial strains was based on the study of the bacterial family, their morphological, cultural and biochemical characteristics. The precise identification of bacteria (genus and species) was carried out using Api 20 E galleries (Biomérieux). Detection of resistance phenotypes was completed by the conventional method of disk diffusion in agar medium. Reading and interpretation criteria were those of the French Microbiology Association Antibiogram Committee (CASFM/EUCAST 2018) [8].

Isolated germs were subjected to antibiotic susceptibility testing using the Mueller Hinton agar diffusion method, based on the use of impregnated disks [9]. After inoculation of Muller Hinton agar by flooding with a bacterial suspension standardized to 0.5 on the Mac Farland scale, and left to incubate for 15min, the antibiotic discs were placed on top of the agar spaced from each other and from the edges of the Petri dish. The choice of the disc used was made according to the nature of the germ isolated (Gram-negative bacilli, Gram-positive cocci and yeasts).

For this purpose, depending on the germs isolated, several classes of antibiotics and antifungals were used.

One or more plates, depending on the case, containing the Mueller-Hinton medium specifically intended for this method, are inoculated by flooding with the previously calibrated bacterial suspension. Antibiotic-impregnated discs are then placed on the agar surface and the antibiotic diffuses very rapidly in a concentric manner around each disc. The plates can then be incubated at 37°C under the required conditions (ambient atmosphere, reduced O_2 tension, anaerobic conditions...).

The reading consists of measuring the inhibition diameters of the culture around each disc manually

Data analysis
The collected data had been entered using a Microsoft Excel 2010 software, analyzed in a SPSS version 20 software. The elements of the descriptive statistics had allowed to calculate the frequencies and the proportions.

The results were presented in tables and figures. Chi-square and Student's t-tests were used to determine the association between dependent and independent variables according to whether they were quantitative or qualitative.
Differences were considered significant for P less than 0.05.

Ethical Considerations
The study received authorization from the Ministry of Public Health and the Dean of the Faculty of Medicine of Chad. In addition to authorizations, individual consent was obtained for the collection of samples for research.

3. Results

Microbiological data
The number of vaginal swabs taken in our study over a period of 6 months (October 2020 - March 2021) was 250, of which 91 cases of vaginal infections were diagnosed, a prevalence of 36.4%, (Figure 1). If possible to reverse the colors of figure to show clearly that the red is positive will give a nice picture.

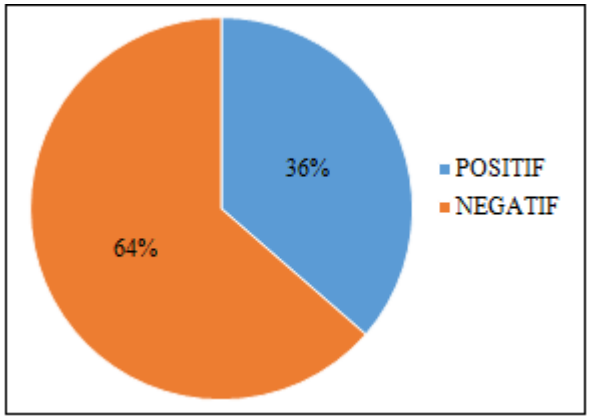

Figure 1: Distribution of vaginal samples

In our study, the majority of patients were in gynecological emergency with a rate of 60.8%. While outpatients represented 39.2% (Table I).

Table I: Distribution of patients according to department

Services	Effectives	Percentage (%)
Gynéco obstetrique	152	60.8
Externe	98	39.2
Total	250	100

Table I shows us that the percentage in the obstetrical gynecological service is much higher than in the outpatient department.

The analysis of the results presented in table II, indicates that 60 strains were isolated in obstetrics and gynecology (39.3%) and 31 strains in outpatient departments (31.5%).

Table II: Proportion of strains identified according to services.

Germes	Gynéco obstetrique (152) N (%).	Externes (98) N (%).
Candida albicans	27 (17.7%)	11(11.2%)
Candida spp	22(14.4%)	15(15.3%)
G.vaginalis	7(4.6%)	4(4.0%)
Staphyloccocus spp	2(1.3%)	1(1.0%)
E.coli	2(1.3%)	0(%)
TOTAL	60(39.3%)	31(31.5%)

Table III show the proportion of strains identified with 41.75% for Candida albicans followed by 40.65% for Candida spp then 12.08% for G.vaginalis and Staphyloccocus spp, E.coli have 3.29% and 2.19% respectively

Table III: Proportion of strains identified

Germs n=91	Percentage %
Candida albicans n=38	41.75%
Candida spp n=37	40.65%
G.vaginalis n=11	12.08%
Staphyloccocus spp n=3	3.29%
E.coli n=2	2.19%

In women aged 20-29 and over 40, Candida albicans and Candida spp were the most represented, with 41.75% and 40.65% respectively. In women aged under 20, G.vaginalis 12.08% was in the majority.

Sensitivity tests were carried out on the various germs isolated. Different classes of antibiotics and antifungals were used. The results showed that for the same germ, the sensitivity profile could vary.

For the Candida albicans strains isolated, Econazole was sensitive in 94.73% of cases and Nystatin in 68.42%, while resistance was observed with spiramicin 97.36% and 5FC 94.73% (Figure 2).

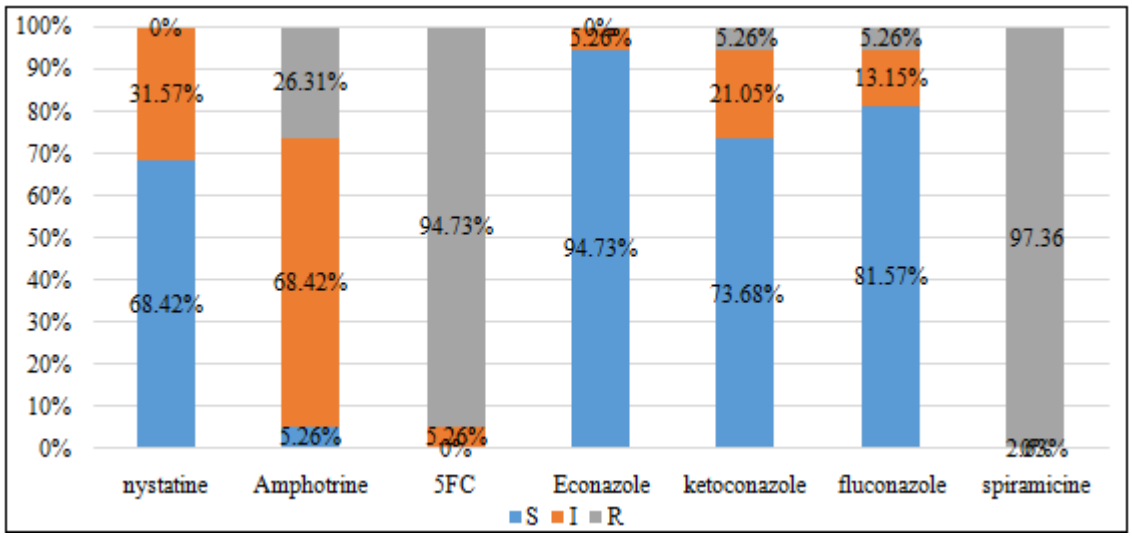

Figure 2: Susceptibility of Candida albicans strains to antifungal agents.

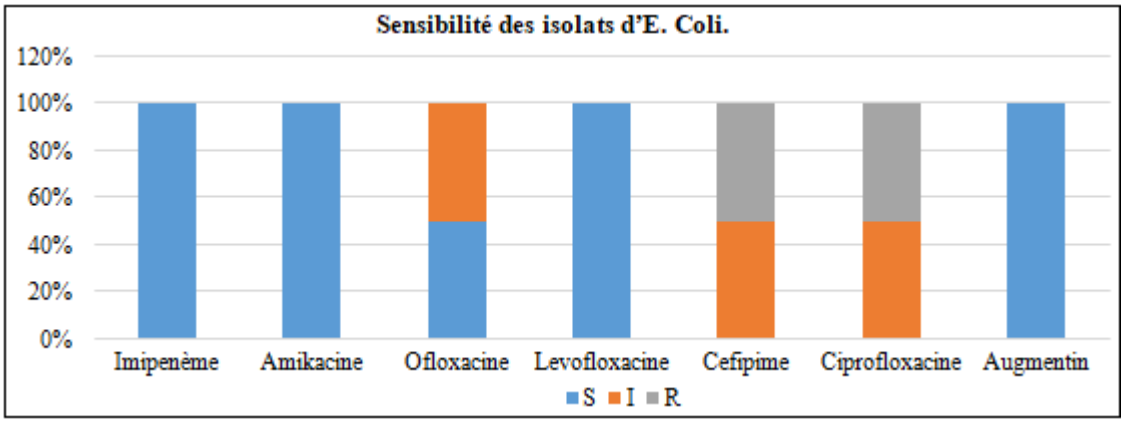

Figure 3: Sensitivity of E. coli isolates. (N=2)

4. Discussion

In our study, we worked on 250 samples taken over 6 months from October - March 2021, of which 91 were positive with a prevalence of 36.4%.

The prevalence of bacterial vaginosis is generally estimated at 36.4%, but some studies show higher prevalences (61% in an STI consultation) or sometimes much lower (from 4.9% to 20% in pregnant women). It seems reasonable to consider that the prevalence in Europe is around 10%. [10].

In our study, vaginitis represented 36.4% of cases; results similar to those of our study have been reported in France by Bohbot et al was 30%. [11].

Higher results than our study (36.4%) were observed in Italy and Ethiopia with a respective rate of 51% and 48.6% [12] [13]. Also results lower than our work was shown by Koanga et al [14] and Aiping Fan et al [15] with a rate of 28% and 24% respectively.

Data from the literature show that vulvovaginal candidiasis is the second most common cause of vaginitis in women of childbearing age [16, 17] and in 80% of cases it is C. albicans [18,19]. Denney et al. found in their study that C. albicans was the second most common cause of vaginal infections [20].

This difference could be explained in our context by the low attendance of the hospital laboratory for this examination.

Bacterial vaginosis showed a frequency of 30.39% with Gardnerella vaginalis representing the second cause of cervico-vaginal infection.

Our study showed a frequency of 18.08% for *Gardnerella vaginalis.*

This prevalence is higher than that found in women of reproductive age in a rural community in China 6%, while in a poor community in northern Brazil it was 20% [21, 22].

Tibaldi et al. obtained lower results than our result on *Gardnerella vaginalis* with a rate of 8.9% [13].

Candida albicans was the most represented germ in the gynecological obstetrics department with a proportion of 41.75% in our study. But the difference was not significant in comparison with non-pregnant women. On the other hand, studies show that pregnant women are a risk factor for the development of vulvovaginal candidiasis caused by C. albicans [4, 23].

In Togo, Tchelougou et al. found that Candida albicans was the second most common cause with 29.4%. This difference could be explained by the disparity of the two samples, which consisted of both pregnant and non-pregnant women in this study.

Regarding Nystatin in our study, we noted a sensitivity of 68.42%. A study in China found the same sensitivity to Nystatin [24].

Our study showed the predominance of BGN bacteria was *Gardnerella vaginalis* with a rate of isolation of 12.08% followed by E. Coli with a rate of 2.19%.The most frequently isolated species for gram positive bacteria was Staphylococcus spp with a rate of 3.29%.

Studies carried out in Ethiopia by Adane et al obtained results clearly superior to our study with an E. Coli rate of 28.5% [12].

A study carried out in France by Bohbot et al showed results similar to those of our study, Gram positive bacteria were predominant with a rate of 60.54%. The rate of isolation of *Gardnerella vaginalis* was 46.7%. [25]

Among the enterobacteria, the sensitivity test showed that *E. coli* was 100% active on imipenem, Amikacin, Levofloxacin and Augmentin while less active on Cefixime and Ciprofloxacin.

Similar results were obtained by Adane et al and Koanga et al in which Amikacin and Imipenem were very active and Ciprofloxacin was less active [12,25].

5. Conclusion

Vaginitis is due either to bacteria, or to parasites, or more frequently to Candida.

Our study has highlighted the great etiological diversity of female genital infections with a predominance of bacterial vaginosis due mainly to Gardnerella vaginalis but also an important frequency of bacterial vaginitis represented essentially by *Staphylococcus spp* and *E.coli*.

The most represented germs were *Candida albicans, Candida spp* and *Gardnerella vaginalis*.

Health education of the population regarding these infections must be strengthened.

Acknowledgements
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Conflicts of interest: The authors declare no conflict of interest.

Author's contributions: this study was the fruit of the research work of a team of young researchers, whose conception, data collection, analysis and interpretation were carried out by AHMAT Mahamat Ahmat , BERTILLE Dewa and MOCTAR Abaya. The study was coordinated and the manuscript corrected by Pr Choua Oucheimi.

Scientific contribution: This article provides important scientific information on resistance in a global way, specifically on the resistance of P. aeruginosa at the CHU-ME.

Limitations of the study: this study was limited due to lack of means to a biochemical characterization of the strains, which was not able to differentiate the different subspecies of the isolated strains and a search for resistance genes could not be performed.

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