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MICROBIOLOGICAL PROFILE OF VAGINAL INFECTIONS: THE EXPERIENCE OF THE AVICENNE MILITARY HOSPITAL IN MARRAKECH

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ABSTRACT

Introduction: 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. **Objective:** The aim of our study is to establish the microbiological profile of vaginal infections and to evaluate the resistance of isolated bacteria to antibiotics. **Methods:** We conducted a retrospective descriptive study over a period of 5 years, from January 15, 2013 to December 14, 2017, conducted by the Bacteriology Department of the Avicenne Military Hospital in Marrakech, on hospitalized or outpatients who presented symptoms of vaginal infection. The culture was done by aerobic germ isolation methods. Germ identification and antibiotic susceptibility testing were performed on the Becton Dickinson Phoenixi1000 automated system. **Results:** During the study period we took 393 samples of which 101 or 25.69% were positive. Direct examination showed the predominance of Gardnerella vaginalis in 33.89% of the samples, Gram-positive Coccis, yeasts and Gram-negative bacilli represented respectively 18.68%, 16.94% and 5.57% of the samples, with a rate of Trichomonas vaginalis of 2.1%. Abundant and polymorphic flora suggestive of anaerobes were found in 3.93% of cases. The culture was non-significant in 74.30%, monomicrobial in 23.15% of cases, and polymicrobial in 4.83% of cases. In total the number of isolated germs was 177, corresponding to 19 different species. The rates of isolation of Gram-positive Coccis and Gram-negative bacilli were 36.15% and 12.99% of cases. The distribution by species showed the predominance of Streptococcus agalactiae which represented 25.42% of the isolates, followed by Escherichia coli and Klebsiella pneumoniae with rates of 3.95%. The isolation rate was 22% for Chlamydia trachomatis, 14.35% for Ureaplasma urealyticum and 4% for Mycoplasma hominis. The sensitivity rates of the isolated enterobacteria strains were 86.36% for amikacin, 82% for aztreonam, 72% for imipenem, 68% for ciprofloxacin, 55% for cotrimoxazole and 41% for amoxicillin-ampicillin. The sensitivity rates of isolated Gram-positive Coccis were 100% for moxifloxacin, 97% for Peni G and vancomycin, 91% for clindamycin, 84,72% for erythromycin and cotrimoxazole, and 8% for tetracyclines. **Conclusion:** The results of this study show the frequency of vaginal infections, and the predominance of bacterial vaginosis with Gardnerella vaginalis. The germs isolated during bacterial vaginitis are represented essentially by Streptococcus agalactiae, E.coli and Klebsiella pneumoniae. Apart from treatment, genital infections, mainly bacterial vaginosis, can lead to complications involving the upper genital tract. For pregnant women, their role in the risk of chorio-amnionitis and premature deliveries is established.

INTRODUCTION

Vaginal infection is an inflammatory process localized in the vaginal cavity and can be caused by the presence of one or more associated infectious agents: bacteria, parasites, viruses. This process can be localized in the vaginal mucosa alone, in which case it is called simple vaginitis, or on the contrary, it can extend to the neighboring mucous membranes, in which case it is called vulvovaginitis or cervico-vaginal inflammation. Vaginitis can be primary or secondary. In primary vaginitis, the pathogen is in most cases of exogenous origin, its implantation and development in the vaginal cavity require very specific conditions, which may vary according to the agent in question, and generally cause an inflammatory reaction. This results in a discharge (leucorrhoea) in which the responsible "pathogen" is revealed. It is most often Trichomonas vaginalis or Candida albicans and sometimes even commensal germs. Secondary vaginitis is rather the consequence of a urethral or cervical infection due most often to a sexually transmitted pathogen (Neisseria gonorrhoeae, Chlamydiatrachomatis, urogenital mycoplasms).

Bacterial vaginosis (in principle without inflammation) is a complex process resulting from an imbalance in vaginal antibiosis, usually exerted by certain species of lactobacilli which play an inhibitory role for other bacteria, in particular Gardnerella vaginalis. For a long time, it was thought that these lactobacilli produced lactic acid, from the glycogen contained in the intermediate cells of the vaginal squamous epithelium. The acid produced would maintain an acidic PH unfavorable to the survival of other bacteria. In fact, the role of these lactobacilli as a factor stimulating glycogenesis has never been established with certainty. It would seem that their protective effect is rather linked to the action of certain enzymes (endopeptidases) produced by these bacteria and of toxic metabolites which accumulate during the multiplication and survival of these bacteria. The lactobacillary flora is itself controlled by the presence of bacteriocins called Lactocins produced by a majority of the species usually found in the natural cavities of humans.

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 [1]. It is clear that "bacterial vaginosis" is the result of a complex phenomenon in which numerous cofactors are involved that are not yet fully recognized (hormonal variations, antibiotic therapy, foreign bodies), resulting in a temporary disturbance of the vaginal ecosystem; it is therefore more accurately a "vaginal bacteriosis".

MATERIALS AND METHODS

This is a retrospective descriptive study, covering a period of 5 years, from January 15, 2013 to December 14, 2017, conducted in the Bacteriology Department of the Avicenne Military Hospital in Marrakech. All patients followed as outpatients or hospitalized and having benefited from a vaginal swab for cytobacteriological study were included in our study. The sample was taken after stopping any local or general antibiotic therapy and in the absence of local cleansing 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 intercourse. On inspection, the macroscopic appearance is noted, i.e. the presence of leucorrhoea, its color, odour and the appearance of the cervix. Vaginal sampling is most often done by swabbing, in which case a maximum amount of serum and cells must be collected. They are done at the level of the vaginal cul de sac to look for infectious agents responsible for vaginosis or vaginitis. After the speculum is placed, two swabs are "loaded" by sweeping the vagina from top to bottom, one is "discharged" onto two slides for direct examination, the other will be used for culture. In case of cervicitis, suspected chorio-amniotitis or upper infection, after cleaning the ectocervix, the endocervix should be swabbed with a suitable swab for Chlamydia trachomatis, which is performed by immunochromatography from the first urine stream. The direct examination after Gram staining gave information on the morphology of the bacteria, their grouping and their staining affinity In case of anaerobic infection, it showed an abundant and polymorphic bacterial flora. Culturing was done on mannitol agar (Chapman), columbia agar with 5% sheep's blood and on cooked horse's blood agar with a vitamin mixture. Each of these media was streaked and incubated at 37°C in a 5% aerobic atmosphere for 24-48 hours in the 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 the bacteria (genus and species) was carried out by automated method on Phoenix i1000 (Becton Dickinson) which allows at the same time the determination of the sensitivity to a panel of antibiotics by the method of the minimal inhibitory concentrations (MIC) or with the help of galleries Api 20 E (Biomérieux).

The data collected were entered and processed using Microsoft Excel 2010 software. The qualitative variables were expressed as numbers and percentages, and the quantitative variables were expressed as averages.

RESULTS

The number of vaginal swabs performed in our study over a period of 5 years (2013-2017) was 393, of which 101 cases of vaginal infections were diagnosed (26%). In our study the majority of patients were outpatients with a rate of 92%. While patients hospitalized in the general surgery department represented 4.95% with a rate of 2.97% in the internal medicine department (Figure 1).

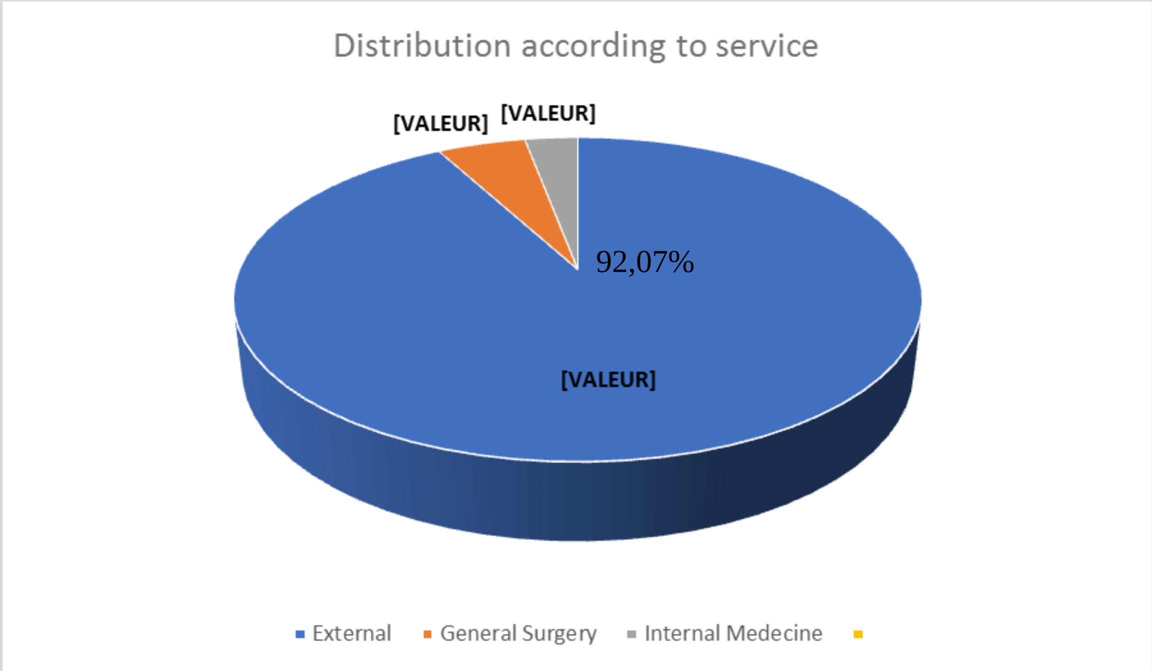

Figure 1: Distribution of patients by service.

On fresh examination, the presence of many leukocytes was noted, with the presence of Trichomonas vaginalis in 10 samples, i.e. 2.1%. Direct examination after Gram staining showed Gram-positive bacilli in 60.32%, clue cells suggestive of Gardnerella vaginalis in 33.89%, Gram-positive Coccis in 18.68%, yeasts in 15.38%, and Gram-negative bacilli in 5.57%, with the absence of the germ in 11.47% of the specimens taken (Figure 2).

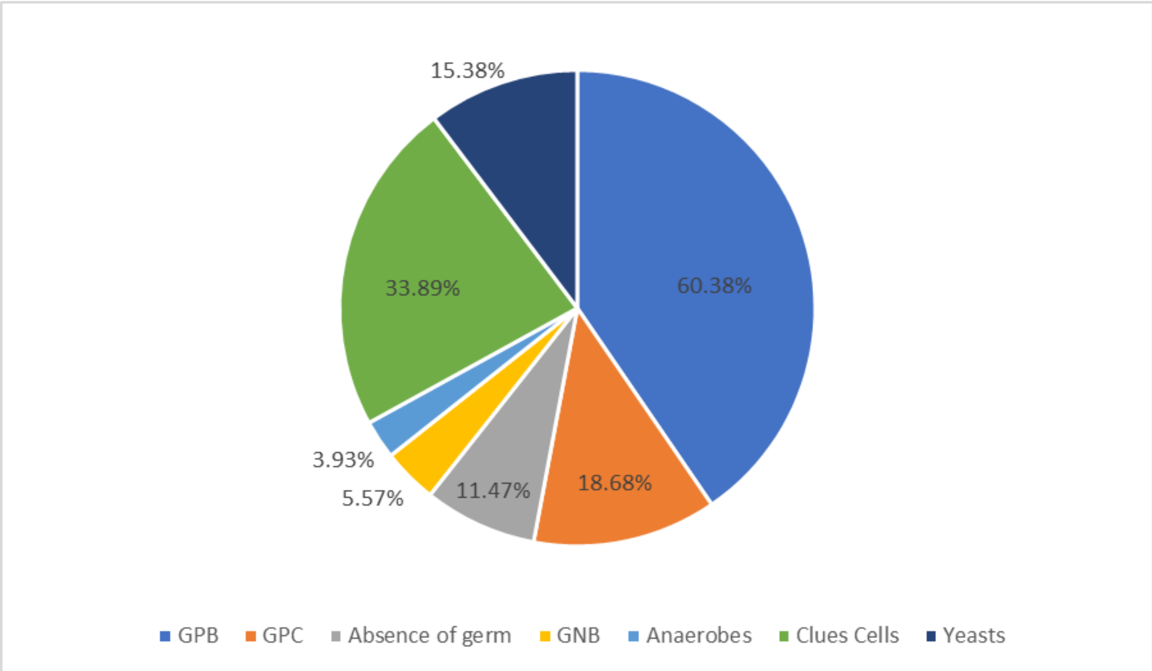

Figure 2: Direct examination results after Gram stain.

The culture was non-significant in 292 cases (74.30%), monomicrobial in 31 cases (23.15%), and bimicrobial in 19 cases (4.83%) (Figure 3).

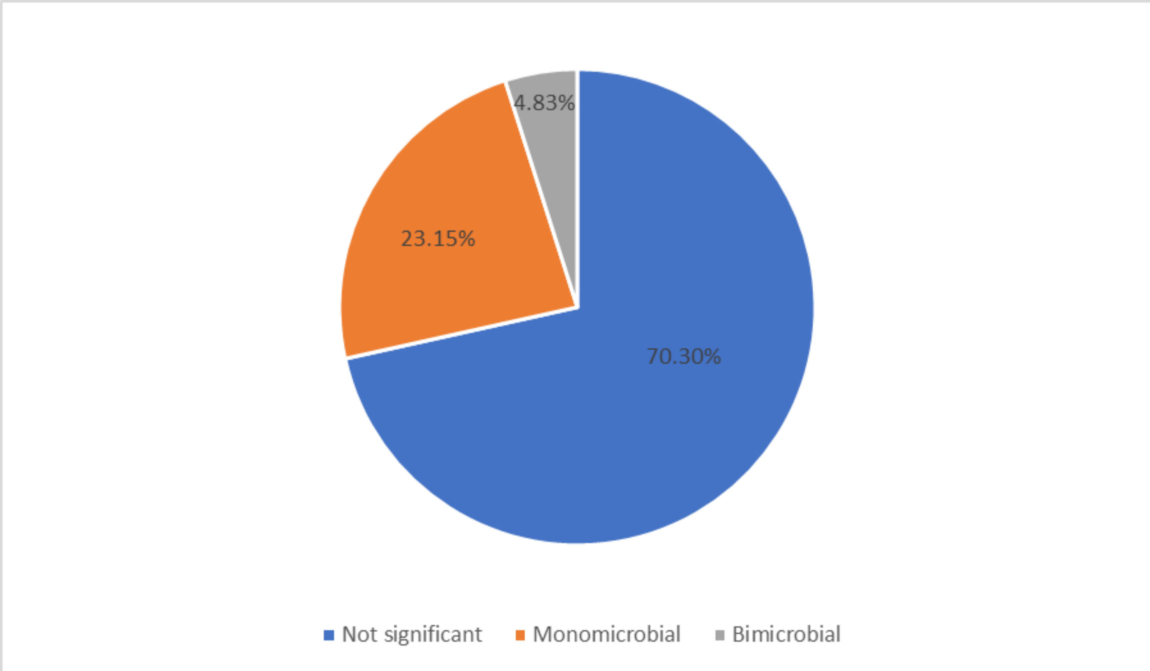

Figure 3: Distribution of culture results according to the number of germs isolated

The number of positive cultures was 25.69% with 177 germs isolated, distributed over 19 different species. The rates of isolation of Gram-positive Coccis and Gram-negative bacilli were 36.15% and 12.99% respectively. The distribution by families showed the predominance of Gardnerella vaginalis which represented 33.89% of the isolates, followed by Streptococci (33.33%) and Yeasts (16.94%) and then Enterobacteriaceae which occupied the fourth place with a rate of (12.42%) (Figure 4).

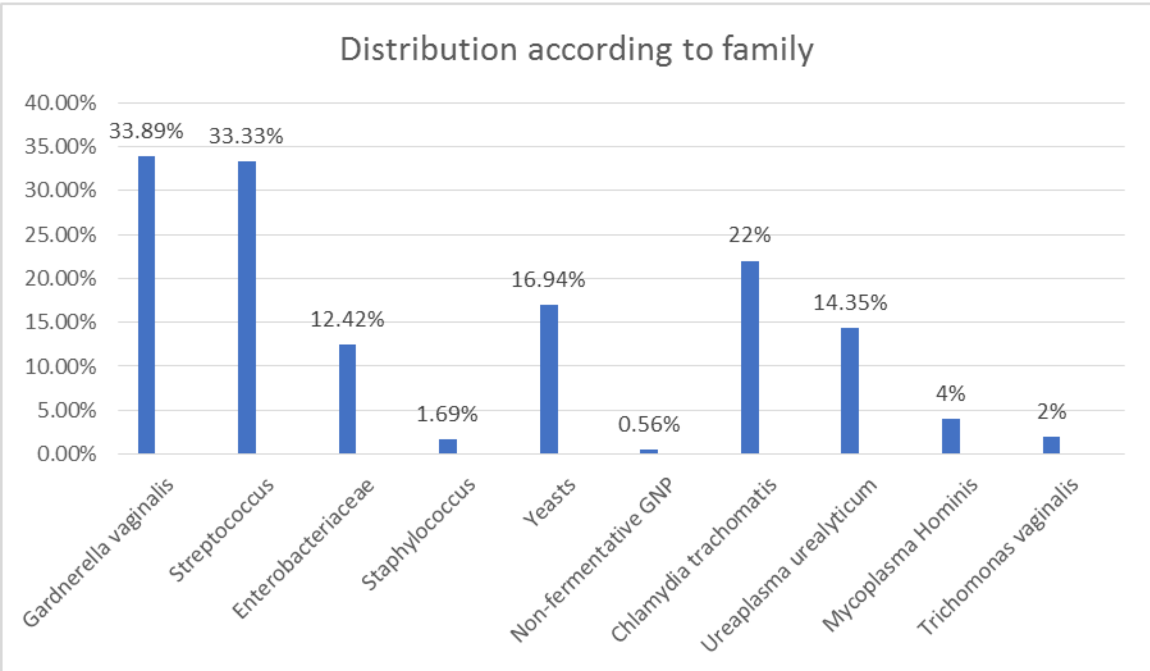

Figure 4: Distribution of isolated germs according to family.

The distribution by species showed the predominance of clues cells evoking Gardnerella vaginalis which represented (33.89%) of isolates, followed by Streptococcus agalactiae (25.42%), Candida albicans (9.03%), E.coli and Proteus mirabilis by rates of (3.95%) (Table I).

Table I: Distribution of isolated germs.

Germs	Number	Percentage (%)
Gardnerella vaginalis	60	33,89
Gram positive cocci	**64**	**36,15**
Enterococcus faecalis	2	1,12
Streptococcus	**59**	**33,33**
Streptococcus agalactiae (B)	45	25,42
Streptococcus uberus	5	2,82
Streptococcus A	4	2,25
Streptococcus acidominimus	1	0,56
Streptococcus anginous	1	0,56
Streptococcus bovis	3	1,69
Staphylococcus	**3**	**1,69**
Staphylococcus aureus	1	0,56
Staphylococcushaemolyticus	2	1,12
Gram-negative bacilli	**23**	**12,99**
Enterobacteriaceae	**22**	**12,42**
Escherichia coli	7	3,95
Enterobacter cloacae	1	0,56
Proteus mirabilis	7	3,95
Proteus vulgaris	1	0,56
Klebsiella pneumoniae	6	3,38
Non-fermenting Gram-negative bacilli	**1**	**0,56**
Pseudomonas aeruginosa	1	0,56
Yeasts	**30**	**16,94**
Candida albicans	16	9,03
Candida glabrata	**3**	**1,69**
Candida tropicalis	**1**	**0,56**
Total	**177**	**100**

In our study, we studied the sensitivity of the most frequently isolated bacteria. The rate of Streptococcus agalactiae (B) was 25.42%, and had a sensitivity rate of 100% to moxifloxacin, oxacillin and ceftriaxone. The combination amoxicillin-ampicillin, levofloxacin, linezolid, teicoplanin, vancomycin, Peni G and pristinamycin showed good activity on Streptococcus B isolates, while high rates of isolate resistance were noted for tetracycline (92%), cotrimoxazole and erythromycin (14%) (Figure 5).

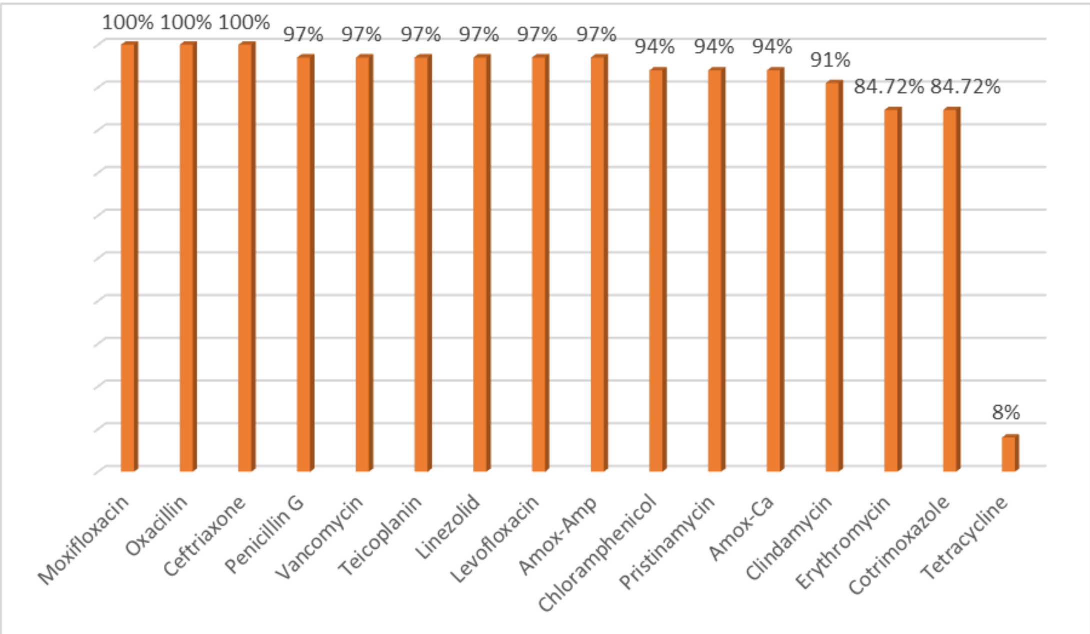

Figure 5: Susceptibility rate of Streptococcus agalactiae (B) isolates.

Enterobacteriaceae isolates represented 12.42%, and showed a high rate of resistance to Amox-Amp (68.18%), nitrofurantoin (45.45%) and cotrimoxazole (18.18%). Aztreonam, imipenem, amikacin, norfloxacin, ceftazidime, ciprofloxacin, ertapenem, fosfomycin, ceftriaxone were the most effective antibiotics on enterobacteria isolates. We isolated 11 multidrug-resistant bacteria, representing 6.21% of the isolates. Enterobacteriaceae resistant to third-generation cephalosporins (3GC) were predominant, representing 100% of MRB and 45.83% of enterobacteriaceae. ESBL-producing Enterobacteriaceae represented 5.64% and 90.90% of BMR, including 4 strains of E.coli and Klebsiella pneumoniae (4%), and 2 strains of Proteus mirabilis (2%). One cephalosporinase-producing Enterobacter cloacae strain was isolated representing 9.09% of the MRB. No methicillin-resistant Staphylococcus aureus (MRSA) was isolated. No strains of Acinetobacter baumannii multi-resistant to betalactam were isolated. No glycopeptide-resistant Enterococcus faecium or ceftazidime and/or carbapenem-resistant Pseudomonasaeruginosa were isolated.

DISCUSSION

Bacterial vaginosis is a very common infection. However, it is very difficult to determine its exact prevalence because the figures vary greatly depending on geographic location, patient age, socioeconomic background, type of consultation and pregnancy status. The prevalence of bacterial vaginosis is generally estimated to be between 15 and 30%, 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%.[2] In our study, vaginitis accounted for 26% of cases; similar results to those in our study have been reported in Cameron, France and China with respective rates of 28%, 30% and 24%.[3-5] The cultures were monomicrobial in 23.15% of cases and polymicrobial in 4.83% of cases. Similar results were reported in Cameron with a rate of 24.66% for monomicrobial cultures and 2% for polymicrobial cultures. In France, the rate was higher with 60.9% of monomicrobial cultures and 8.9% of polymicrobial cultures.[3-4]

Bacterial vaginosis is one of the most frequent causes of leukorrhea in genitally active women. Since the description of the syndrome by Gardner and Dukes in 1955, this condition has been the subject of numerous studies. Several studies have drawn attention to the pathogenic role of Gardnerella vaginalis. This bacterium is present in small amounts in healthy women (< 105 CFU/ml exudate); it becomes the dominant bacterium in more than 80% of women with bacterial vaginosis (10 7 ~t 109 CFU/ml exudate) [6]. Our study showed the predominance of Gram-positive bacteria (GPB) with a rate of 60.32%; the rate of isolation of GNB was 5.57%, the most frequently isolated species was Streptococcus agalactiae with a rate of 25.42%, with the second ranking for Gardnerella vaginalis by an isolation rate of 33.89%, and 16.94% for Candida. 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% and Streptococcus agalactiae was the predominant pathogenic species with a rate of 29.72%. The isolation rate of Gardnerella vaginalis was 46.7%.[3] Among the GNB we noted the predominance of enterobacteria representing 12.99% of isolates, Escherichia coli and Proteus mirabilis were the most frequently isolated species with rates of 3.95%. These results are close to those reported by Bohbot et al where enterobacteria were isolated in 11% of cases.[3] The existence of bacterial vaginitis facilitates the acquisition of STIs such as Neisseria gonorrhoeae and Chlamydia trachomatis infections.[2] The most frequent STIs are manifested mainly by cervicitis (gonorrhoeae, Chlamydia), the clinical picture of which is different from that of vaginitis. In contrast, trichomoniasis often presents as vaginitis (associated with cervicitis). It should be noted that cases of STI are associated with other infections (vaginosis or mycotic vaginitis).[3] In our study Trichomonas vaginalis infection represented a rate of 2.1% close to that described by Tibaldi et al where trichomoniasis represents 1.6%.[7]

In women, gonococcal infection is asymptomatic in 70% of cases.[8,9] When it is symptomatic, it is most often manifested by a discrete cervicitis with a normal-looking cervix or sometimes an inflamed cervix with pus coming from the cervical orifice.[8-10] In our study, no case of gonococcus was identified because the woman was an asymptomatic carrier and the germ was fragile. Chlamydia infection is most often asymptomatic in 50-90% of cases in women.[9] In our study the rate of chlamydia was 22%, a slightly lower rate of 13% was described in the United States by Bautista et al.[11] The rate of Ureaplasma urealyticum and Mycoplasma hominis were respectively 14.35% and 4%, similar results were reported by Bitew et al by rates of 16.9% Ureaplasma urealyticum and 1.7% for Mycoplasma hominis.[12]

In our study, Gram-positive bacteria expressed a high rate of resistance to tetracycline (92%). Moxifloxacin, clindamycin, vancomycin, erythromycin were the most sensitive antibiotics. Clindamycin was sensitive to all gram-positive bacteria, and erythromycin was sensitive to 84.72% of Streptococci which is in line with the study of Mulu et al, where clindamycin was sensitive to all strains and erythromycin was sensitive to gram-positive bacteria.[13]

Table II compares the susceptibility profile of Gram-positive bacteria in vaginal infections between different studies.

Table II: Comparison of GPB susceptibility profile in vaginal infections between different studies.

Authors of the study (year)	Country	Gram positive bacteria	
		Most Active Antibiotics	**Least active antibiotics**
Bitew et (2017)[12]	Ethiopia	Cefoxitin Tobramycin	Peni G Tetracycline Erythromycin
Mulu et al (2015)[13]	Ethiopia	Clindamycin Ciprofloxacin Erythromycin Norfloxacin	Ampicillin Cotrimoxazole Amoxicillin
Our Study	Morocco	Clindamycin Vancomycin Erythromycin Moxifloxacin	Cotrimoxazole Tetracycline Peni G

Concerning GNB, the strains of enterobacteria isolated in our study expressed a high rate of resistance to the combination amoxicillin-ampicillin 58%. Amikacin and imipenem were the most active antibiotics with a sensitivity rate of 86.36% and 72% on isolates. In the study by Bitew et al amikacin and tobramycin were the most active antibiotics on GNB, these bacteria showed a high rate of resistance to the combination amoxicillin-ampicillin.[12]

In vaginal infections, ciprofloxacin and cotrimoxazole are the most frequently prescribed antibiotics.

The table III compares the sensitivity profile of GNB in vaginal infections between different studies.

Table III: Comparison of GNB sensitivity profile in vaginal infections between different studies.

Authors of the study (year)	Country	Gram negative bacteria	
		Most Active Antibiotics	**Least active antibiotics**
Bitew et al (2017)[12]	Ethiopia	Amikacin Tobramycin	Amoxicillin-Ampicillin
Mulu et al (2015)[13]	Ethiopia	Ciprofloxacin Norfloxacin Gentamicin	Cotrimoxazole Amoxicillin
Koanga et al (2015)[4]	Cameron	Imipenem Amikacin Fosfomycin	Ciprofloxacin 3rd generation cephalosporins
Our Study	Morocco	Amikacin Imipenem Ciprofloxacin Aztreonam	Amoxicillin-Ampicillin Cotrimoxazole

According to these results, it would be preferable to avoid prescribing amoxicillin-ampicillin and tetracycline in the treatment of vaginal infections, as the rate of resistance for these antibiotics was high for GNB, which could be at the origin of the emergence and diffusion of MRB (Multi-resistant bacteria). The evolution of community resistance of enterobacteria to antibiotics is a real phenomenon. It exposes difficulties in the therapeutic management of infections. The current control of this phenomenon is a real emergency and requires the involvement of public authorities.[14]

In our study, we isolated 11 MRB representing 6.21% of isolates, on the other hand the study of Sbiti et al reported 12.2% of MRB in 2017.[15] According to the results of our study, 95.8% of MRB identified were E.coli and Klebsiella spp strains which is consistent with the study of Sbiti et al.[15] The multidrug resistance observed in some pathogens is of particular concern. It greatly reduces and sometimes completely eliminates the effective therapeutic arsenal against infections caused by these pathogens with a consequent negative impact on clinical outcomes.[16]

CONCLUSION

Bacterial vaginitis and vaginosis are more likely to occur as a result of a vaginal imbalance in the patient, the causes of which are multiple. The precise analysis of the factors, which can be of endogenous origin, linked to the patient, or exogenous, linked to the environment, must lead to a personalized treatment. These are the frequent infectious problems in women. Vaginitis is due either to bacteria or parasites, or more frequently to Candida. Whitish, creamy, abundant discharge and severe itching are often the signs of candida vaginitis. Yellowish, foul-smelling, little itching discharge is more likely to be a sign of bacterial vaginitis. The mucous membranes of the vagina are normally protected in an acidic environment, an environment generated by lactobacillus acidophilus which acidifies the vaginal environment. A poor diet and an unbalanced intestinal flora, a weak immune system, insufficient local hygiene or, on the contrary, an overly aggressive one, will provide the bed for vaginitis.

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 Streptococcus agalactiae, E.coli and Klebsiella pneumoniae. The low rate of sexually transmitted infections (STIs) must be confirmed by other studies (immunochromatography) to look for Chlamydia trachomatis, Neisseria gonorrhoeae, Ureaplasma urealyticum, Mycoplasma hominis and others.

Antibiotic treatment is not the miracle solution for a vaginal infection, all factors must be taken into account to resolve the imbalance of the flora responsible for recurrent infections. It is sometimes necessary to wait several weeks before obtaining a good result and insist on controlled hygiene measures in prevention.

Abreviations

3GC : Third generation cephalosporin
Amox : Amoxicillin
Amp : Ampicillin
CA : Clavulanic Acid
ESBL : Extended spectrum beta lactamase
E.Coli : Escherichia
GNB : Gram-negative bacilli
GPB : Gram-positive bacilli
GNC : Gram-negative coccis
GPC : Gram-negative coccis
MIC : Minimum inhibitory concentrations
MRB : Multi resistant bacille
MRSA : Methicillin-resistant Staphylococcus aureus
Peni G : Pénicillin G
STIs : Sexually transmitted infections

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