World Journal of
Biological and Pharmaceutical Research

Journal homepage: https://zealjournals.com/wjbpr/

ISSN: 2799-0338 (Online)

(RESEARCH ARTICLE)

A study on vaginal infections among reproductive-aged women in the region of Marrakech, Morocco

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World Journal of Biological and Pharmaceutical Research, 2022, 03(02), 010–017

Publication history: Received on 05 October 2022; revised on 16 November 2022; accepted on 18 November 2022

Article DOI: https://doi.org/10.53346/wjbpr.2022.3.2.0042

Abstract

Purpose: The principal aim of this study was to establish the microbial epidemiology of vaginal infections and to study the susceptibility of strains isolated to antibiotics. The secondary aim was to orient probabilistic antibiotic therapy.

Methods: This is a retrospective descriptive study, covering a period of 9 years, from 2013 to 2022, conducted in the Bacteriology Department of the Avicenne Military Hospital in Marrakech.

Results: The number of vaginal swabs taken in our study over a period of 9 years (2013-2022) was 693, of which 131 cases of vaginal infections were diagnosed, i.e. 19%.

On fresh examination the presence of *Trichomonas vaginalis* was observed in 15 samples or 2.1%.

Direct examination after Gram staining showed the presence in 33.4% of cases of clue cells suggestive of *Gardnerella vaginalis*; Gram-positive Coccis were present in 22.1%, yeasts in 17%, and Gram-negative bacilli in 5%.

The distribution by species showed the predominance of clues cells evoking *Gardnerella vaginalis* which represented 35% of the isolates, followed by *Streptococcus agalactiae* at 28%, *Candida albicans* at 10%, and *Escherichia coli* and *Proteus mirabilis* by 5%.

Conclusion: Our study showed the great microbial 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, Escherichia coli* and *Klebsiella pneumoniae*.

Keywords: Bacterial vaginosis; Trichomonal vaginitis; Vaginitis; Reproductive-aged women; Morocco

1. Introduction

Almost all women are affected by a vulvovaginal infection at some point in their lives, and some even experience it recurrently. This results in painful or embarrassing physical symptoms that can affect their quality of life and self-esteem [1].

While a balanced vaginal microbiota is associated with good vaginal health [2]. Increased diversity can paradoxically be associated with dysbiosis, such as bacterial vaginosis (BV). BV is the result of a disruption of the vaginal ecosystem, i.e., the sudden replacement of Lactobacilli by anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*,

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Ureaplasma urealyticum, *Mycoplasma hominis*, etc. It is the most common cause of vaginal discharge in women of reproductive age, accounting for approximately 30% of all causes [3].

Bacterial vaginosis (BV) is the most common form of vulvovaginitis [4]. At any given time, they affect up to one in three women worldwide [3]. The etiology of this dysbiosis remains unknown, but its repercussions are significant, including obstetrical complications, an increased risk of sexually transmitted infections and urogenital infections. Its diagnosis is based on Amsel's clinical criteria and/or a Gram stain based on the Nugent score [2]. A characteristic symptom is the abundant secretion of white to grayish malodorous discharge [5].

Although behavioral factors such as smoking, stress or hygienic errors are recognized causes of recurrence, other leads are beginning to be explored such as the role of sexual transmission, the resistance of certain bacteria to imidazole drugs or the lack of efficacy of conventional treatments on the dysbiosis itself [6].

This present study was conducted to determine the prevalence of vaginal infection, establish microbial epidemiology of vaginal infections and to study the susceptibility of strains isolated to antibiotics in the region of Marrakech, Morocco.

2. Material and methods

2.1. Type, location and period of study

This is a retrospective descriptive study, covering a period of 9 years, from January 15, 2013 to October 1, 2022, conducted at the Bacteriology Department of the Military Hospital Avicenne in Marrakech.

2.2. Patients

Were included all the patients followed on an outpatient basis or hospitalized and having benefited from a vaginal sampling for cytobacteriological study.

2.3. Data collection

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

2.4. Vaginal swab

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 intercourse.

On inspection, the macroscopic appearance is noted, i.e. the presence of leucorrhoea, its color, odour 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.

2.5. Microbiological analysis

2.5.1. Isolation and identification of bacteria

Direct examination after Gram staining provided information on the morphology of the bacteria, their grouping and their color affinity. 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 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 detection of resistance phenotypes was completed by the conventional method of disc diffusion in agar medium. The reading and interpretation criteria are those of the French Association of Microbiology (CASFM/EUCAST 2022)

2.5.2. Antibiotic susceptibility testing

For each strain, susceptibility was determined by automated susceptibility testing (BD Phoenix i1000) in liquid medium, or by standard susceptibility testing by swabbing using the Mueller-Hinton agar diffusion method.

2.5.3. Detection of multidrug-resistant bacteria (MDRB)

In our study, the search for MDRB concerned:

- Meticillin-resistant *Staphylococcus aureus* (MRSA).
- MRSA resistant to glycopeptides.
- Glycopeptide-resistant *Enterococcus faecium*.
- Enterobacteriaceae resistant to third-generation cephalosporins (C3G) through production of extended-spectrum beta-lactamase (ESBL) or cephalosporinase.
- Carbapenem-resistant Enterobacteriaceae.
- *Pseudomonas aeruginosa* resistant to ceftazidime and/or carbapenems.
- *Acinetobacter baumannii* multi-resistant to betalactam.

2.6. Detection of ESBL character

2.6.1. Synergy test

The search for the ESBL phenotype is performed on the antibiogram by placing the Cefotaxime (30µg) and Ceftazidime (30µg) discs at a distance of 20-30 mm (center to center) from an amoxicillin/clavulanic acid (20/10 µg) disc.

2.6.2. Combined disc method

This method consists of placing on a Mueller-Hinton agar previously inoculated with a bacterial suspension adjusted to 0.5 Mac Farland, 2 pairs of antibiotics; a cefotaxime disk facing a cefotaxime/clavulanic acid disk at a distance of 25 mm (center to center), and a ceftazidime disk facing a ceftazidime/clavulanic acid disk (same distance). An increase ≥ 5 mm in the inhibition diameter of the clavulanic acid-containing discs compared to those without clavulanic acid is in favor of the presence of an ESBL.

2.7. Cloxacillin test

Cloxacillin, a cephalosporinase inhibitor, is incorporated into Mueller-Hinton agar. A disk containing ticarcillin/clavulanic acid is placed in the center and 20 mm from it are placed the cefotaxime and ceftazidime disks. ESBL producing strains show synergy between the ceftazidime and/or cefotaxime discs and the ticarcillin/clavulanic acid disc.

2.8. Detection of methicillin resistance

To detect methicillin resistance, the method performed consists of placing a cefoxitin disc (30µg), on Mueller-Hinton agar seeded with a heavy inoculum (107UFC\ml) and incubated at 37 °C. The reading is taken after 48 hours of incubation. *Staphylococcus aureus* characterized by cefoxitin MICs >4 mg/L are resistant to methicillin.

2.9. Resistance to carbapenems

Any strain of enterobacteria with decreased susceptibility to ertapenem (MIC ≥0.5 mg/L or inhibition diameter <25 mm; 10 µg discs) by agar diffusion test was considered a suspect carbapenemase-producing enterobacteria . To improve the sensitivity of detection of carbapenemase production, 2 different carbapenems: imipenem and ertapenem were tested.

2.9.1. At the request of the prescriber

Cervical swabbing was performed following the prescriber's request for *Chlamydiae trachomatis* and Mycoplasma testing. The sample was taken using two sterile cotton swabs at the endocervix. The swab is turned several times before being removed.

2.9.2. Diagnosis of Chlamydia trachomatis (CT)

In the laboratory, identification of CT is done by Direct Immunofluorescence Technique (DIF). This technique allows the specific marking of elementary CT bodies by the use of monoclonal antibodies.

At the laboratory level, we used "Chlamydia direct IF", a kit that allows the detection, by DIF, of Chlamydia in urogenital samples using 2 monoclonal antibodies, one directed against the antigen of the genus Chlamydia and the other directed against an antigen specific to the species trachomatis. These antibodies are conjugated with fluorescein. The CT diagnosis was performed according to the kit recommendations.

2.9.3. Diagnosis of Mycoplasma

Genital Mycoplasma infections do not have specific clinical features to identify them. Culture on adapted media (commercialized kits) with quantification is the method of choice for *Mycoplasma hominis* and *Ureaplasma spp*. At the laboratory level, we used "Mycoplasma IST 2", which is a complete kit for the diagnosis of genital mycoplasmas. It allows the culture, identification, indicative count and antibiotic susceptibility testing of *Mycoplasma hominis* and *Ureaplasma spp*. The diagnosis of *Mycoplasma hominis* and *Ureaplasma spp* was performed following the recommendations of the kit.

3. Results

3.1. Swabs

The number of vaginal swabs performed in our study over a 9-year period (2013-2022) was 693, of which 131 cases of vaginal infections were diagnosed, or 19%.

3.2. Wet mount examination

We noted the presence of *Trichomonas vaginalis* in 15 specimens or 2.1%.

3.3. Direct examination

The direct examination after Gram staining showed the presence in 33.4% of clue cells evoking *Gardnerella vaginalis* ; Gram positive Coccis were present in 22.1%, yeasts 17%, Gram negative bacilli in 5% with absence of germs in 22.5% of the samples taken.

3.4. Microbial culture

The culture was non-significant in 562 of the cases (81%), monomicrobial in 112 cases (16.2%), and bimicrobial in 19 cases (2.8%).

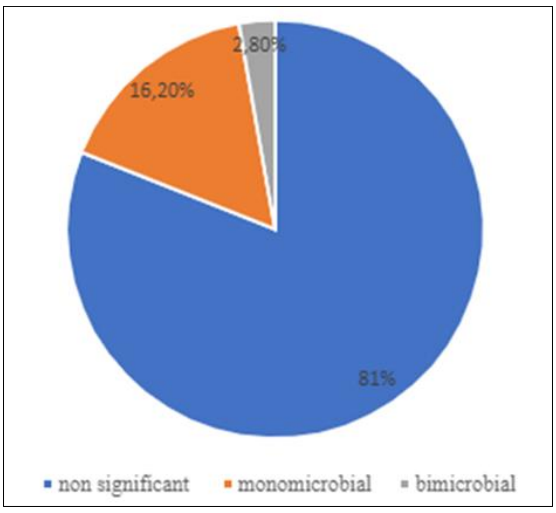

Figure 1 Different results of microbial culture

3.5. Microbiological profile

The number of positive cultures was 20% with 177 germs isolated, spread over 19 different species. The rates of isolation of Gram-positive Coccis (GPC) and Gram-negative bacilli (GNB) were 38% and 15% respectively.

The distribution by families showed the predominance of *Gardnerella vaginalis* which represented 35% of the isolates, followed by Streptococci (34%) and Yeasts (18%) and then Enterobacteriaceae which occupied the fourth place with a rate of (13%).

The distribution by species showed the predominance of clues cells evoking *Gardnerella vaginalis* which represented 35% of isolates, followed by *Streptococcus agalactiae* at 28%, *Candida albicans* at 10%, *Escherichia coli* by a rate of 3%.

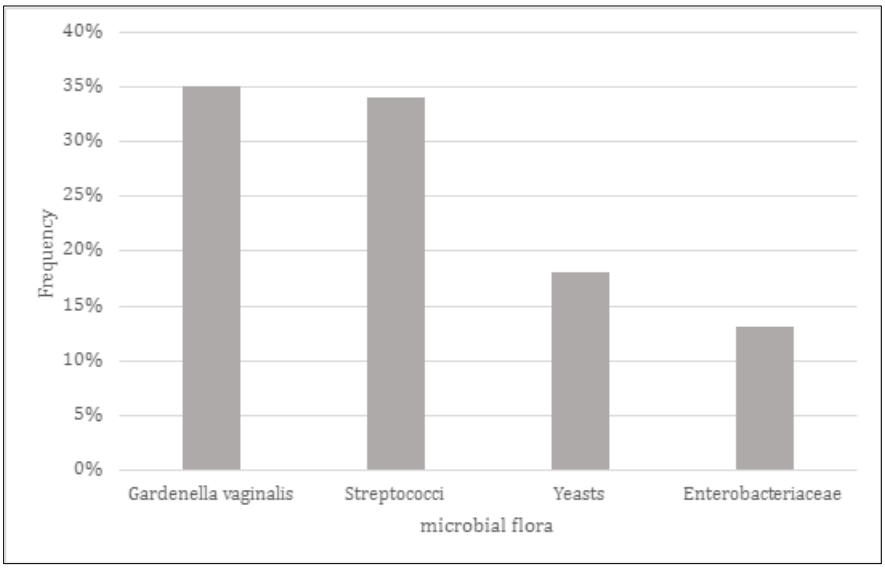

Figure 2 Distribution of microbial flora by families

3.6. Bacterial susceptibility to different antibiotics

In our work, we studied the sensitivity of the most frequently isolated bacteria.

3.6.1. Streptococci

The rate of *Streptococcus agalactiae (B)* was 28%, and had a sensitivity rate of 100% to moxifloxacin, oxacillin and ceftriaxone. The combination of amoxicillin, ampicillin, levofloxacin, linezolid, teicoplanin, vancomycin, Penicillin G and pristinamycin showed good activity on Streptococcus B isolates, while high rates of resistance of isolates were noted for tetracycline (92%), cotrimoxazole and erythromycin (14%)

3.6.2. Enterobacteriaceae

Enterobacteriaceae isolates accounted for 13%, and showed a high rate of resistance to amoxicillin, ampicillin combination (68.18%), nitrofurantoin (45.45%) and to cotrimoxazole (18.18%). Aztreonam, imipenem, amikacin, norfloxacin, ceftazidime, ciprofloxacin, ertapenem, fosfomycin, ceftriaxone were the most effective antibiotics on enterobacterial isolates.

3.7. Multidrug-resistant bacteria

We isolated 14 multidrug-resistant bacteria, representing 7% of isolates. Enterobacteriaceae resistant to third generation cephalosporins (C3G) were predominant representing 100% of the MDRBs and 82% of the enterobacteriaceae.

ESBL-producing Enterobacteriaceae represented 71.4% of the MDRB, including 5 strains of *Escherichia coli* and *Klebsiella pneumoniae* (35.8%), and 2 strains of *Proteus mirabilis* (14.2%). Two *Enterobacter cloacae* strains producing cephalosporinases were isolated representing 14.2 % of the MDRB.

No strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated. No strains of *Acinetobacter baumannii* multi-resistant to betalactam were isolated. No glycopeptide-resistant *Enterococcus faecium* or ceftazidime and/or carbapenem-resistant *Pseudomonas aeruginosa* were isolated.

4. Discussion

In our study, we worked on 693 samples taken over a 9 years period between 2013 and 2022, of which 131 were positive.

4.1. Microbiological profile

BV can occur at any reproductive age (between 15 and 44 years). Its prevalence rates vary considerably between geographic regions of the world, within countries, and even within populations, depending on ethnicity and socioeconomic status. Although its exact prevalence remains difficult to determine, BV ranges from 4% to 75%, depending on the population studied [3].

The prevalence of BV is estimated to be intermediate in the United States (29%) and low in Europe, with a maximum (>20%) in Poland and Norway. In Africa, the estimated prevalence tends to be high. However, the prevalence of BV is lower in West Africa (6-8% in Burkina Faso) than in Southern and Eastern Africa: 32.5% in Zimbabwe, 37% in Kenya, 38% in Botswana and 68.3% in Mozambique [3].

In our study, vaginitis represented 19% of cases, results similar to those of our study were reported in Nigeria by Afolabi et al. with a rate of 26% [7], a rate of 27.2% reported by Abdul-Aziz et al. in Yemen [8] and by Koanga et al. with a rate of 28% in Cameroon [9].

In our study, cultures were monomicrobial in 16.2% of cases and polymicrobial in 2.8% of cases. Similar results were reported in Cameron by Koanga et al. with a rate of 24.66% for monomicrobial cultures and 2% for polymicrobial cultures [9]. On the other hand, in Ghaddar et al. the culture was polymicrobial in 25% [10] and in 21% of cases in the study of Shawaky et al. [11].

Our study revealed that the isolation rates of GPC and GNB were 38% and 15% respectively, the most frequently isolated species was *Gardnerella vaginalis* with a rate of 35%, with the second ranking for *Streptococcus agalactiae* by an isolation rate of 20%, and 17% for Candida. In Gabon, Bignoumba et al. found a rate of 41.6% of Gram-positive bacteria, while the rate of isolation of GNB was 17.4%, while the most frequently isolated species was *Streptococcus B* [10]. Yalew et al. found a rate of 68.1% of Gram-positive bacteria, of which coagulase-negative staphylococci and *Staphylococcus aureus* were the most predominant bacteria [11]. 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 rate of isolation of *Gardnerella vaginalis* was 46.7% [6].

Among the GNB, we noted the predominance of Enterobacteriaceae representing 12.99% of isolates, *Escherichia coli* was the most frequently isolated species with a rate of 5%. These results are close to those reported by Bohbot et al. where enterobacteria were isolated in 11% of cases [6], and those of Bignoumba et al. where the most frequently isolated enterobacteria were *Klebsiella spp*. at 11.6% and *Escherichia coli* at 5.8% [10].

In our study, TV infection represented a rate of 2.1% close to that described by Tibaldi et al. where trichomoniasis represents 1.6% [12] and by konadu et al. with a rate of 1.4% [13]. As well as bigoumba et al. where the prevalence of TV was 2.1% [10]. No case of gonococcus was identified because the woman are an asymptomatic carrier and because of the fragility of the germ. We observed a 12.5 % rate of chlamydia similar to the 13% rate described in the United States by Morri et al [14]. The rate of *Ureaplasma urealyticum* and *Mycoplasma hominis* was respectively 7.9% and 2%, results of 16.9% for *Ureaplasma urealyticum* and 1.7% for *Mycoplasma hominis* were reported by Adane et al [15].

4.2. Bacterial resistance

In our study, Gram-positive bacteria expressed a high rate of resistance to tetracycline (92%). Moxifloxacin, clindamycin, vancomycin, erythromycin were the most active antibiotics. Clindamycin was active on all Gram-positive bacteria, and erythromycin was active on 84.72% of Streptococci which is in line with the study of Wondemagegn et al. where clindamycin and erythromycin were the most active on Gram-positive bacteria [16].

In the study of Yalew et al. the highest rate of resistance was observed against penicillin (100.0%) in staphylococci, while 86.7% of them were sensitive to ciprofloxacin [11].

Concerning gram-negative bacteria, the strains of enterobacteria isolated in our study expressed a high rate of resistance to the combination amoxicillin-ampicillin at 58%. Amikacin and imipenem were the most active antibiotics with a sensitivity rate of 86.36% and 72% on isolates. However, Yalew et al. observed that the resistance rate of enterobacteria varied from 0.0% for ciprofloxacin and chloramphenicol to 100.0% against amoxicillin/clavulanate [11].

In the present study, we isolated 14 MDRB representing 7% of the isolates, on the other hand the study of Sbiti et al. reported 12.2% of MDRB [17]. Enterobacteria resistant to third generation cephalosporins (C3G) were predominant representing 100% of MDRB and 82% of enterobacteria. ESBL-producing Enterobacteriaceae represented 5% of the isolates and 71.4% of the MDRB, including 5 strains of *Escherichia coli* and *Klebsiella pneumoniae*, i.e. 35.8%, and 2 strains of *Proteus mirabilis*, i.e. 14.2%. Comparable to the results of our study, 95.8 % of the MDRB identified were strains of *Escherichia coli* and *Klebsiella spp* according to Sbiti et al. Two strains of *Enterobacter cloacae* producing cephalosporinases were isolated representing 14.2 % of the MDRB [17].

5. Conclusion

Our study highlighted the great etiological diversity of female genital infections with a predominance of bacterial vaginosis due mainly to *Gardnerella vaginalis* but also a high frequency of bacterial vaginitis represented essentially by *Streptococcus agalactiae, Escherichia coli and Klebsiella pneumoniae.*

The high rates of resistance found in our study confirm the current trend in our region of the emergence of new bacterial resistances, and therefore the difficulty of treatment, which could lead in the long term to a therapeutic impasse.

Compliance with ethical standards

Acknowledgments

Authors thank women who participated in the study.

Disclosure of conflict of interest

The authors declare that they have no ties of interest.

Statement of ethical approval

This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Avicenne Military Hospital of Marrakech, Morocco.

Statement of informed consent

Informed written consent was obtained from each participant after a clear explanation of the study objectives.

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