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Prevalence and Characterization of Bacterial and Yeast Vaginal Infections in a Public Health Institution of Ouagadougou, Burkina Faso

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Abstract

Vaginal infections are the most prevalent women's health problem. This study carried out from November 2018 to May 2019 aimed to determine the prevalence and the antimicrobial susceptibility of bacteria and yeasts implicated of vaginal infections in Ouagadougou, Burkina Faso. A total of 43 samples were collected from patients consulting at the National Public Health Laboratory of Ouagadougou. Then, microscopic observations and culture on specific media of women's vaginal discharge were used to identify the incriminated microorganism. The Candida albicans species was identified as 45.95% with high resistance to amphotericin B (93.75%) and flucytosine (100%). Staphylococcus aureus was identified at 21.62% with total resistance to penicillin G (100%), amoxicillin (100%), and trimethoprim-sulfamethoxazole (100%). This study raises concerns about the therapeutic management of vaginal infections.

Keywords: Burkina faso; Vaginal infections; *Staphylococcus aureus; Candida albicans;* Antimicrobials

Abbreviations

Ph: Potential of Hydrogen; RTIs: Reproductive Tract Infections; NPHL: National Public Health Laboratory; HIV: Human Immunodeficiency Virus.

Introduction

Vaginitis is an inflammation of the vagina in which bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis are the most vaginitides [1,2]. The healthy vaginal tract of reproductiveaged women is colonized by normal microbiota dominated by lactobacilli, which protect against pathogenic bacterial species when present in sufficient numbers [3]. However, the invasion of the normal vaginal flora by a range of microorganisms leads to vaginal infections [4]. Thus, nearly 5-10 million females seek gynecologic advice for vaginitis every year worldwide [5].

Bacterial vaginosis is a polymicrobial syndrome in which there is a change in the complex balance of microflora in the vagina [6]. It is characterized by an increase in the vaginal pH, a reduction in lactobacilli, predominantly hydrogen peroxide producing species, and an increase in facultative and anaerobic bacteria in number and/or type [7]. Besides, women with vaginal infections caused by resistant bacteria are at increased risk of poor clinical outcome and mortality [8].

Out of yeasts, especially *C. albicans* is a commensal of digestive and genito-urinary tract of both healthy people [9] but can also act as opportunistic pathogen of skin and mucosae in receptive animals and in immunocompromised patients [10]. The recent increase in candiduria has been attributed to several factors, such as the length of hospital stay, indiscriminate use of antimicrobial agents, high frequency of use of invasive devices, the patient's degree of susceptibility [11-15]. Vaginal colonization can also be a risk to nosocomial candiduria [16].

Generally, Reproductive Tract Infections (RTIs) are important causes of illness among women of reproductive age worldwide and especially in developing countries [17]. Nevertheless, public health research give very little attention to RTIs [18] meaning that little is known about the characteristics of bacteria and yeasts isolated from these infections. There is insufficient data from Burkina Faso, with regards to prevalence and characteristics of bacteria and yeasts associated responsible of vaginal infections. Only a study reported that vaginal infection was due to *C. albicans* (48%), *Gardnerella vaginalis* (2.13%) and other endogenous bacteria (28%) [19]. Therefore, this study was conducted to determine the prevalence of bacteria and yeasts associated with vaginal infections and their susceptibility to antimicrobials.

Materials and Methods

Study design, area and population

From November 2018 to May 2019, a cross-sectional study was conducted among 43 women seeking healthcare in National Public Health Laboratory (NPHL) of Ouagadougou (Burkina Faso). Women were excluded from participation if they were menstruating, pregnant, or if they had received antimicrobial therapies in the preceding week or vaginal douching within the previous 24 hr.

Swabbing

The patient was placed in dorsal position and specimens were collected from vaginal walls with two cotton-tipped swabs. The vagina of each woman was examined for the characteristics of vaginal discharge (color, consistency and odour).

Microbial isolation and identification

The microbial isolation and identification were immediately performed on the collected samples at the Bacteriology-virology Department of the NPHL using the conventional methods. In brief the process included microscopic examination and the growth on selective culture media.

Bacterial vaginosis was diagnosed using any three of the four criteria recommended by Amsel et al. [20] presence of homogenous white-grey vaginal discharge; presence of clue cells (>20% of epithelial cells with clue cells) on wet mount; a fishy amine odor of the vaginal discharge before and after addition of 10% KOH (positive whiff test) and a vaginal pH>4.5. A direct smear was performed by transferring the vaginal fluid present on the swab to a glass slide. Then, vaginal smears were heatfixed and Gram stained [21]. If gram-negative bacilli, the oxidase and the API 20E tests were performed to identify the species. Otherwise, if gram-positive cocci, the catalase test was then carried out to differentiate Staphylococcus from Streptococcus. The suspected colonies were therefore reseeded on mannitol salt, Dnase agar and used to perform a Coagulase test for S. aureus identification. The identification of Streptococcus was based primarily on the nature of hemolysis around colonies isolated on cooked blood agar and isovitalex. Group B Streptococcus has beta hemolysis, which appeared as a fairly large (3-4 mm) clear area around the colonies.

Identification of *Candida* was possible by using the blast test. 0.5 ml to 1 ml of serum was distributed in a hemolysis tube; the strain to be tested was taken from chloramphenicol sabouraud agar and inoculated with a loop to obtain a suspension of slight opacity. The suspension thus obtained and standardized to Mac Farland 0.5 was incubated at 37° C for 2-3 hours. A drop of this suspension was observed under an optical microscope. The presence of a germ tube with no constriction at its base indicated that the strain identified was C. albicans. The identification of *C. albicans* was also made on a chromagar medium specific to Candida. The culture of *C. albicans* was positive on this medium if the color of the colonies obtained was blue.

Antimicrobials susceptibility testing

Antimicrobial susceptibility tests were performed as recommended by the French Society for Microbiology. The following antibiotics were used: gentamycin (10 μ g), tobramycin, penicillin G, amoxicillin (30 μ g), trimethoprim-sulfametoxazole (25 μ g), kanamycin, lyncomycin, oxacillin (5 μ g), vancomycin, fosfomycin, ceftriaxone (30 μ g); erythromycin (15 μ g), imipenem (10 μ g). The antifungal discs tested were fluconazole (100 mg), econazole (10 mg), clotrimazole (50 mg), ketoconazole (10 mg), miconazole (10 mg), amphotericin B (20 mg), nystatin (100 IU).

Ethical consideration

The study was explained to the participants and informed verbal and written consent was obtained from them. Ethical clearance for the study was obtained from the Ethics Committee of Burkina Faso and the NPHL authorities. Approval Deliberation N $^{\circ}$ 2009-30 was issued on 17 July 2009.

Analysis

Data collected were analyzed using the IBM Statistical Package for Social Sciences version 20 (SPSS Chicago, IL, USA). Categorical variables were presented in frequency and percentages with test of significance done using Chi square while continuous variables were expressed in mean and standard deviation with significance test done using student test. Level of significance was set at p value<0.05.

Results

Frequency of vaginal infections

Of 43 patients, 31 (72%) were diagnosed positive for vaginal infections. Of 43 women, the majority of women (49%) were aged between 20 and 29 years (**Table 1**).

Age (years)	< 20	[20 ; 29]	[30 ; 39]	[40 ; 49]	≥ 50	All ages
Numbe r of infecte d patient s (%)	2 (6)	18 (58)	8 (26)	3 (10)	0 (0)	31 (100)

Table 1: Prevalence of vaginal infections by age group.

Microbiological profile of vaginal swabs

Among the positive cultures, 56% were single infection and 16% were dual infection (**Figure 1**).



Figure 1: Proportion of infected vaginal swabs.

The most isolated being microorganisms were *C. albicans, S. aureus, Gardnerella vaginalis* and *E. coli* (**Table 2**).

Type of microorganism	Microorganisms	Number of isolates (%)	
Bacteria	Escherichia coli	03 (8)	
	Gardnerella vaginalis	03 (8)	
	Staphylococcus aureus	08 (22)	
	Group B Streptococcus	02 (5)	
	Klebsiella pneumoniae	01 (3)	
Yeast	Candida spp	03 (8)	
	Candida albicans	17 (46)	
Total	All	37 (100)	

 Table 2: Distribution of identified microorganisms.

Antibiotics and antifungal susceptibility

Staphylococcus aureus was the most frequently isolated bacterium from vaginal specimens and had 100% resistance rate to penicillin G, amoxicillin and trimethoprim-sulfamethoxazole (Figure 2). Isolates of *Candida (C. albicans and Candida spp.)* were mainly resistant to flucytosin, amphotericin B and fluconazol (Figure 3). Contrariwise, molecules such as tobramycin, gentamycin and lyncomycin have shown good efficacy against *S. aureus*; as well as antifungals such as econazole, nystatin, clotrimazole and miconazole against *C. albicans*.



Figure 2: Antibiotics resistance of *Staphylococcus aureus* isolates.



Figure 3: Antifungals resistance of Yeasts isolates.

Discussion

Our results showed high prevalence of vaginal infections in the 20-29 age group which is comparable to the values reported by [19,22] in Burkina Faso and Nigeria respectively. Indeed, studies have shown a link between age and vaginal infections [23,24]. The predominance of vaginitis in this group of age can be explained by the fact that these women were sexually active [19] but also by the increase in gestational hormonal activity [25].

The microorganisms' growth and identification was attempted for 72% samples in accordance with 64% and 76.8% previously reported in Ouagadougou [19,26] and is higher than 15.6% reported in Ethiopia [27]. Our study revealed 56% of single infection versus 16% of dual infection in agreement with the previous study conducted in Ouagadougou which reported and 48% of single infection versus 16% of dual infection [19]. In contrast, Bohbot et al. [28] found 60.9% of single infection and 8.9% of dual infection in their study on the etiological diversity of vaginitis in France.

Our results also showed that *C. albicans* was the most frequently associated with vaginitis among the identified pathogens (46% of the cases). This can be explained by factors leading to an ecological disorder of the vaginal environment i.e.

diet, hygiene, inadequate contraception, diabetes, HIV or medication (broad-spectrum antibiotics) [29]. Interestingly, several studies have made the same observation previously in Burkina Faso with 48.76% [26] and 48% [19] as prevalence. However, our prevalence seemed to be higher than that of Sanou et al. [30] who found 19.3% in Ouagadougou (Burkina Faso). Other African countries also showed a low prevalence of vaginal *C. albicans* infection. Thus a prevalence of 10% among 17-21 years was reported in South Africa [31] while 3.2% and 7.3% were reported in Ethiopia in women under age 20 and women 20 to 29 years respectively [32].

As reported by Nadembega et al. [19], no Trichomonas vaginalis infection was detected in our study, although the previous prevalence of Trichomonas vaginalis infection was 1.04% in Ouagadougou [26] and 6% in South Africa [31]. The normal flora already contains microorganisms such as Lactobacillus without any mention of infection. However, the presence of a single yeast or Trichomonas leads to infection, hence the possibility of single and dual infections. Additionally, Karou et al. [26] identified other microorganisms such as Staphylococcus epidermis, Staphylococcus saprophyticus, Streptococcus agalactiae, Klebsiella pneumoniae, Klebsiella ozenae, Enterobacter sp., Ureaplasma urealyticum and Mycoplasma hominis. As suggested by Oduyebo et al. [33], occasional colonization of the vagina by digestive bacteria may explain the presence of these different bacteria in the vagina. On the other hand, the imbalance of the vaginal bacterial flora can also cause the development of commensal bacteria like enterobacteria [19].

Susceptibility testing of *Staphylococcus aureus* showed the presence of multiple resistances to certain antibiotics. Likely, studies conducted in Ethiopia and in Morocco showed resistance rates to amoxicillin, penicillin A/G and trimethoprim-sulfamethoxazole [27,34] meaning that these antibiotics remain less active on gram-positive bacteria. This resistance can be explained by the uncontrolled use of these drugs [35]. Penicillin resistance may be due to the acquisition of a plasma penicillinase, a penicillin-degrading enzyme [36]. *Staphylococcus aureus* is resistant to penicillin G because of the beta-lactamases produced [37].

In the present study, clotrimazol, econazol, ketoconazol, miconazol and nystatin have proven very effective against C. albicans and Candida spp strains isolated while flucytosin, amphotericin B and fluconazol exhibit fair resistance. These results corroborate those of Anane et al. who found the same resistance concerning Candida in their study on risk factors for vulvovaginal Candidiasis in Ethiopia [38]. Resistance to fluconazole may be related to target modification due to gene mutation, target overexpression, efflux phenomenon, alteration of the ergosterol synthesis pathway by inhibition of 14-alpha demethylase [39]. The mechanism of resistance to amphotericin B involves a reduction in ergosterol content in the cell membrane. However, as far C. albicans, C. glabrata, Candida rugosa, Candida lusitaniae and Candida tropicalis are concerned, resistance to amphotericin B can also be acquired [40]. Flucytosine, on the other hand, inhibits protein and DNA synthesis, and its resistance may be due to a defect in

intracellular penetration or transformation into the active molecule 5 fluorouracil [39]. It should not be used as monotherapy but in combination with another antifungal agent, most commonly amphotericin B [41].

Antibiotic and antifungal treatment of genital infections is not always effective, and complications persist due to microbial resistance, side effects, and recurrent infections [42].

Conclusion

The results showed that *Candida albicans* and *Staphylococcus aureus* were the most involved in vaginal infections. *C. albicans* was resistant to amphotericin B, flucytosine and fluconazole while *S. aureus* was 100% resistant to penicillin G, amoxicillin and trimethoprim-sulfamethoxazole. Therefore, a future investigation over a larger sampling area should make it possible to better identify the risk factors associated with genital infections in Burkina Faso as well as a molecular characterization of pathogenic species. At least, the implementation of community programs for the early detection of these infections is necessary for an effective control of them.

This study enrolled women attending only the National Laboratory for Public Health of Ouagadougou Burkina Faso. Future study should cover other hospitals of Ouagadougou city and across country to determine entire country wise prevalence.

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Competing Interests

The authors declare that they have no conflicts of interest.

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