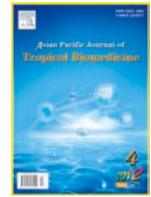




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60025-2 © 2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Antimicrobial resistance of abnormal vaginal discharges microorganisms in Ouagadougou, Burkina Faso

Simplice D Karou^{1,2*}, Florencia Djigma¹, Tani Sagna¹, Christelle Nadembega¹, Moctar Zeba¹, Aboudoulaye Kabre³, Kokou Anani², Djeneba Ouermi¹, Charlemagne Gnoula¹, Virginio Pietra³, Salvatore Pignatelli³, Jacques Simpore^{1,3}

¹Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso

²Ecole Supérieure des Techniques Alimentaires et Biologiques (ESTBA-UL), Université de Lomé, Togo

³Centre Médical Saint Camille de Ouagadougou, Burkina Faso

ARTICLE INFO

Article history:

Received 10 October 2011

Received in revised form 2 November 2011

Accepted 28 November 2011

Available online 28 April 2012

Keywords:

Bacteria

Genital infections

Antibiotics

Mycoplasma

Fungal strain

Antimicrobial resistance

Abnormal vaginal discharge

Vaginal infection

ABSTRACT

Objective: To assess the prevalence of bacterial strains and fungal strains infecting the vaginal tract and test their sensitivity to antibiotics in women attending Saint Camille Medical Centre in Ouagadougou. **Methods:** From January 2008 to December 2009, a total of 2000 vaginal swabs were cultivated for bacterial and fungal identification and isolation. Furthermore, bacterial strains were tested for their susceptibility to several antibiotics used in routine in the centre. **Results:** The results revealed that microbial isolation and identification was attempted for 1536/2000 sample, a positivity rate of 76.80%. *Candida albicans* (48.76%), followed by *Escherichia coli* (16.67%), *Streptococcus agalactiae* (8.14%) and *Staphylococcus aureus* (7.55%) were the major agents of genital tract infections in patients. *Mycoplasma hominis* and *Ureaplasma urealyticum* combined accounted for less than 7%. *Trichomonas vaginalis* was identified in 1.04% cases. The antimicrobial tests revealed that the microorganisms developed resistance to several antibiotics including beta lactams. However, antibiotics such as cefamandol, ciprofloxacin and norfloxacin were still active on these bacteria. **Conclusions:** The results reveal that many sexually active women are infected by one or more microbial pathogens, probably because of the lack of hygiene or the adoption of some risky behaviors, such as not using condoms or having multiple sexual partners. Efforts should be made to address these points in the country.

1. Introduction

Sub-Saharan Africa recorded the highest rates of sexually transmitted infections (STIs) in the world. In some sexually active women, STIs are almost recurring for several reasons, including the anatomy of the female sex, multiple partners and non use of condoms[1]. These infections have a major impact on health, especially in the mother and the baby. Indeed, the infections can cause severe complications such as ascending infections, cancer of the cervix, infertility, spontaneous abortions, premature births and low birth weight[2]. In addition, many studies have shown that ulcerative and non ulcerative STIs significantly increase the transmission of HIV in women[3,4]. For these reasons, culture or at least microscopic examination of vaginal swab was included in prenatal care programs in several countries in

sub-Saharan Africa, in addition to routine HIV test[5].

The Saint Camille Medical Centre in Ouagadougou, Burkina Faso, is one of the main poles of the health of the mother and child in the country. Since 2000, the centre has made the fight against infections affecting pregnant women one of its main goals. Recorded studies to date revealed that many women attending antenatal clinics were co-infected with *Toxoplasma gondii* and viruses including HHV-8, HBV, HCV and HIV[6-8]. More recently, it was found that the *Papillomavirus* took an important part in these infections and that the strains circulating in Burkina Faso were not all covered by vaccines available in the country[9,10].

The presence of multiple co-infections would result in the direct transmission of the viruses to newborns. The main reason is that the presence of these pathogens in a patient weakens the placental barrier to let pass one or the other viruses[6,11]. However, no studies have been conducted in the centre to check the incidence of bacterial and fungal strains that can infect the vaginal tract. Therefore, this study was aimed to assess the prevalence of bacterial strains and fungal strains infecting the vaginal tract and to test their sensitivity to antibiotics.

*Corresponding author: Simplicite D Karou, Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), BP 364, Ouagadougou, Burkina Faso.
E-mail: simplicitekarou@hotmail.com

2. Materials and methods

The study population was women aged between 19 and 45 years with mean age of (33.65±5.75). All the women were attending the Laboratory of the Saint Camille of Medical Centre Ouagadougou for vaginal swab (VS) examinations and culture. The study covered the period from January 2008 to December 2009. During the study period, 2 000 samples were cultured. All media used for microbial cultivation and isolation were obtained from Biomerieux. The identification of microorganisms was done using conventional methods adopted in the centre^[12,13]. Confirmation of identity was made with Galleries API 20E (Biomerieux, France) for enterobacteria and Api 20HP (Biomerieux, France) for yeast. Isolation and identification of *Mycoplasma* was performed using the kit *Mycoplasma* system plus of Liofilhem Diagnosis®.

The antimicrobial tests were conducted on agar medium Muller and antimicrobial activities were evaluated by measuring the diameters of inhibition around the disks as previously described by Karou et al^[12,13].

3. Results

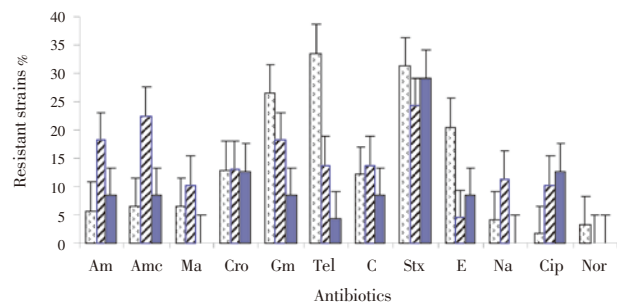
In the present study, among 2 000 cultivated VS samples, 1 536/2 000 were infected with at least one microorganism, a positivity rate of 76.80%. Table 1 showed the prevalence of pathogens isolated. The analysis of the table showed that *Candida albicans* (*C. albicans*), followed by *Escherichia coli* (*E. coli*), *Streptococcus agalactiae* (*S. agalactiae*) and *Staphylococcus aureus* (*S. aureus*) were the major agents of genital tract infections in patients. *Mycoplasma hominis* (*M. hominis*) and *Ureaplasma urealyticum* (*U. urealyticum*) combined accounted for less than 7%. Other bacteria like *Acinetobacter*, *Erwinia*, *Proteus* and *Citrobacter* representing less than 2% of isolated microorganisms were classified as others in the table. *Trichomonas vaginalis* (*T. vaginalis*) was identified in 1.04% cases.

Table 1
Distribution of identified microorganisms in abnormal vaginal discharge [n (%)].

| Microorganisms | Frequency |
|-------------------------------------|-------------|
| <i>C. albicans</i> | 749 (48.76) |
| <i>E. coli</i> | 256 (16.67) |
| <i>S. agalactiae</i> | 125 (8.14) |
| <i>Streptococcus</i> sp. | 7 (0.46) |
| <i>S. aureus</i> | 116 (7.55) |
| <i>Staphylococcus saprophyticus</i> | 24 (1.56) |
| <i>Staphylococcus epidermidis</i> | 6 (0.39) |
| <i>Klebsiella pneumoniae</i> | 43 (2.80) |
| <i>Klebsiella ozenae</i> | 14 (0.91) |
| <i>Klebsiella</i> sp. | 16 (1.04) |
| <i>Enterobacter</i> sp. | 12 (0.78) |
| <i>U. urealyticum</i> | 65 (4.23) |
| <i>M. hominis</i> | 41 (2.67) |
| <i>T. vaginalis</i> | 16 (1.04) |
| <i>Gardenerella vaginalis</i> | 29 (1.89) |
| Others | 17 (1.11) |

Among the analysed samples, coinfections occurred in 295/1 536 positive samples, a frequency of 19.21%. Coinfections of *C. albicans*/bacteria and bacteria/bacteria were the most represented. This accounted for more than 96%. Coinfections of *C. albicans* and bacteria, bacteria and bacteria, bacteria and *T. vaginalis*, *T. vaginalis* and *Candida* were 187 (63.39), 99 (33.56), 5 (1.69), 4 (1.36), respectively.

A total of 12 antibiotics including ampicilline, amoxicilline+ clavulanique acid, tetracycline, chloramphenicol, ciprofloxacine, trimethoprim–sulfametoxazole, ceftriaxone, gentamycin, cefamandole, nalidixic acid, erythromycin and norfloxacine were tested on gram positive cocci (Figure 1). The results revealed that no *Staphylococcus saprophyticus* (*S. saprophyticus*) was resistant to neither norfloxacine nor cefamandole. In the same way, any strain of *S. aureus* did not resist to norfloxacine. The rest of tested antibiotics displayed various resistance rates. The highest resistance rates (>25%) were recorded by *S. agalactiae* with tetracycline trimethoprim–sulfametoxazole and gentamicin. Some strains of this bacteria resisted to all tested antibiotics, however relative low resistant rates (<5%) were recorded with ciprofloxacine, norfloxacine and nalidixic acid. For *S. aureus* the highest resistant rates were recoded with trimethoprim–sulfametoxazole, amoxicillin/clavulanic acid, gentamicin and ampicillin. For the rest of antibiotics, the resistance rate was below 15%. In the case of *S. agalactiae*, high resistance rate was recorded with trimethoprim–sulfametoxazole; the resistance rate remained below 15% for the rest of antibiotics.



□ *S. agalactiae* ▨ *S. aureus* ■ *S. saprophyticus*

Figure 1. Antibiotic resistance patterns within gram positive cocci. Am: ampicillin; Amc: amoxicillin/clavulanic acid; Ma: cefamandole; Cro: ceftriaxone; Gm: gentamicin; Tet: tetracycline; C: chloramphenicol; Stx: trimethoprim–sulfametoxazole; E: erythromycin; Na: nalidixic acid; Cip: ciprofloxacine; Nor: norfloxacine.

The resistance patterns of gram negative bacilli were displayed in Figure 2. A total of 8 antibiotics including ampicillin, amoxicillin/clavulanic acid, cefamandole, trimethoprim–sulfametoxazole, ciprofloxacine, gentamicin, chloramphenicol and ceftriaxone were tested on these bacteria. Very high resistance rates were recorded with beta lactam and trimethoprim–sulfametoxazole. The minimal resistance rate was approximately 30%, recorded with trimethoprim–sulfametoxazole on *Klebsiella ozenae*; while the maximal rate was 72%, recorded with ampicillin on *Klebsiella pneumoniae*. The lowest resistance rates were recorded with cefamandole, gentamicin, ciprofloxacine and ceftriaxone.

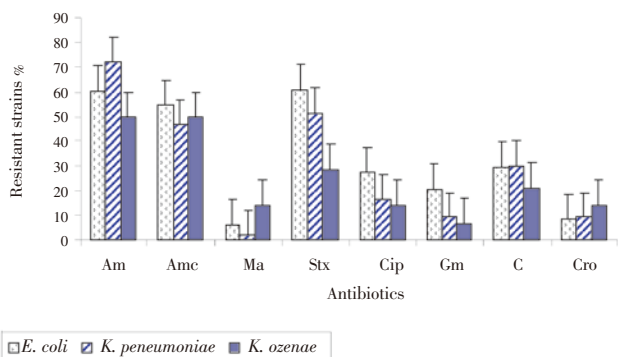


Figure 2. Resistance patterns in gram negative bacilli. Am: ampicillin; Amc: amoxicillin/clavulanic acid; Ma: cefamandole; Stx: trimethoprim–sulfamethoxazole; Cip: ciprofloxacin; Gm: gentamicin; C: chloramphenicol; Cro: ceftriaxone.

Figure 3 displayed the resistance patterns of *Mycoplasma* isolated in the study. According to the figure, *M. hominis* strains were more resistant than the strains of *U. urealyticum*. The highest resistance rates (>40%) were recorded with erythromycin, clarithromycin, azithromycin and tetracycline. For the rest of antibiotics, the resistance rates remained under 40% for both *M. hominis* and *U. urealyticum*.

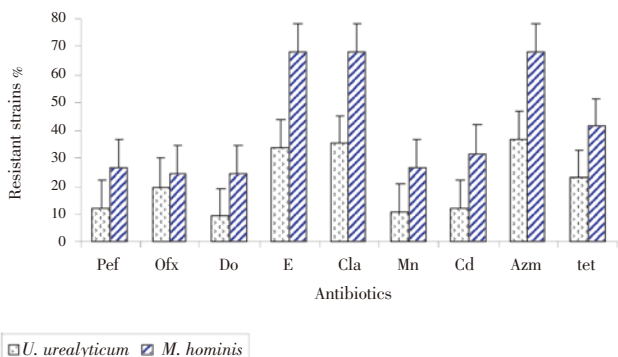


Figure 3. Resistance patterns of *Mycoplasma*. Pef: pefloxacin; Ofx: ofloxacin; Do: doxycycline; E: erythromycin; Cla: clarithromycin; Mn: Minocycline; Cd: clindamycin; Azm: azithromycin; Tet: tetracycline.

4. Discussion

This study was undertaken to assess the prevalence of microorganisms isolated from vaginal secretions in Saint Camille Medical Centre. Our results showed that *C. albicans* was the most isolated microorganism in vaginal secretions of the study population. Previous studies showed that this yeast is the most isolated microorganism in female genital tract[1,14]. *C. albicans* has been by far regarded as commensal, but nowadays with the HIV infection, which weakens the immune system, this microorganism can contribute to the emergence of sexually transmitted diseases. Enterobacteria were the first family of bacteria isolated. Among these bacteria *E. coli* accounted for more than 75% of Enterobacteriaceae. *S. agalactiae*, also known

as group B *Streptococcus* (GBS) is the main microbial agent responsible for serious infections in newborns[15]. In Burkina Faso few studies have been done on the genus, in particular *Streptococcus pneumoniae* but no prevalence study has been done on the GBS[16,17]. In this study, GBS is ranked third among the microorganisms responsible for vaginosis, with prevalence around 10%. This rate is very high referring to the study of Cissé *et al* who found a recovery rate of 2.5%[18]. This may be because, in their study these authors used in addition to vaginal secretion, bronchial secretions and urine. In Burkina Faso, the responsibility of GBS is fairly known. Surely, the bacterium represents the third leading cause of bacterial meningitis in neonates after pneumococci and Enterobacteriaceae and the second leading cause of neonatal sepsis in sub-Saharan Africa. The majority of these infections have been associated with significant morbidity and mortality[18].

Susceptibility testing of microorganisms showed the presence of multiple resistances to certain antibiotics. In fact, these results reflect the real situation of antibiotic resistance in the town. It should be noted that our study population is composed mainly of urban residents who can pay for microbiological cultivation and who have the habit of using antibiotics. It is the first case study on the susceptibility of *Mycoplasma* to antibiotics in the country. Our results showed that these bacteria mainly *M. hominis* have developed multiple resistances. Several previous studies have already been conducted on the resistance of other bacteria to antibiotics[19,20]. Overall resistance rates recorded in this study were lower than those registered with the microorganisms isolated from urinary tract infections in the centre[12]. Indeed, rates of up to 80% for Enterobacteriaceae and about 50% resistance for gram-positive cocci were previously recorded in the centre with beta lactam antibiotics. The low rates can be explained by the fact that microorganisms from urinary tracts were always more resistant than those isolated from the genital tract. Indeed, most of antibiotics are eliminated with urine; the microbes in urinary tracts have probably developed resistance against these antibiotics[21]. Another hypothesis that may explain the low rate of resistance is that a general decline has occurred in the centre since 2006. Indeed, to address the problem of resistance to penicillin in the late 90s, the Ministry of Health conducted a reformulation of the therapeutic lines with the restriction to a bare minimum of antibiotics such as ampicillin, amoxicillin, amoxicillin/clavulanic acid and cefazolin. Amoxicillin for example is the first line antibiotic used in the treatment of acute respiratory infections (ARIs) in infants. It is no longer prescribed for urinary tracts infections, where it is replaced by ciprofloxacin. Amoxicillin/clavulanic acid continues to be used only in the treatment of ARIs. In contrast, erythromycin has been increasingly used in recent years, particularly among pregnant and lactating women, as part of the syndromic approach to STIs. The drugs pressure lowering, by the changes in protocol may have led the decline in resistance for such antibiotics[13].

It appears from this study that some antibiotics such as norfloxacin, ciprofloxacin and cefamandole are the antibiotics of choice against gram positive cocci whilst cefamandole, gentamycin, ceftriaxone and ciprofloxacin should be used against gram negative bacilli isolated from female genital tract; however, the use of these antibiotics should be done carefully to avoid the rapid emergence of resistance.

Antimicrobial resistance of microorganisms is a quite dynamic phenomenon. This highlights the need for current prevalence and susceptibility data for institutions or geographic areas. Our results showed that many women are infected with one or more microbes. The presence of these microbial pathogens in the genital tract may be due to lack of hygiene rules or the adoption of some risky behaviors, such as not using condoms or having multiple sexual partners. Efforts should be made to include corporal hygiene rules in antennal counseling programs in the country.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors gratefully thank Mr. Charles Dabire, Oscar Zoungrana and Mrs Fatou Nana for their technical assistance.

References

- [1] Nwadioha S, Egesie JO, Emejuo H, Iheanacho E. A study of female genital swabs in a Nigerian Tertiary Hospital. *Asian Pac J Trop Med* 2010; **3**: 577–579.
- [2] Ekabua JE, Agan TU, Iklaki CU, Ekanew EI. Adjuncts to case assessment of vaginal discharge syndrome in pregnant women. *Asian Pac J Trop Med* 2010; **3**: 63–65.
- [3] Simpore J, Ilboudo D, Karou D, Pietra V, Granato M, Esposito M, et al. Prevalence of HHV-8 infections associated with HIV, HBV and HCV in pregnant women in Burkina Faso. *J Med Sci* 2006a; **6**: 93–98.
- [4] Simpore J, Savadogo A, Ilboudo D, Nadambega MC, Esposito M, Yara J, et al. Seroprevalence and coinfection of *Toxoplasma gondii*, HBV and HCV among HIV-negative and HIV infected pregnant women in Burkina Faso. *J Med Virol* 2006b; **78**: 730–733.
- [5] Shujatullah F, Khan HM, Khatoun R, Rabbani T, Malik A. An evaluation of OSOM BV blue test in the diagnosis of bacterial vaginosis. *Asian Pac J Trop Med* 2010; **3**: 574–576.
- [6] Simpore J, Pietra V, Pignatelli S, Karou D, Nadembega WMC, Ilboudo D, et al. Effective program against mother-to-child transmission of HIV at saint Camille Medical Centre in Burkina Faso. *J Med Virol* 2007; **79**: 873–879.
- [7] Ilboudo D, Karou D, Nadembega WMC, Aly Savadogo A, Ouermi D, Pignatelli S, et al. Prevalence of human herpes virus-8 and hepatitis B virus among HIV seropositive pregnant women enrolled in the mother-to-child HIV transmission prevention program at Saint Camille Medical Centre in Burkina Faso. *Pak J Biol Sci* 2007; **10**: 2831–2837.
- [8] Ouermi D, Simpore J, Belem AMG, Sanou DS, Karou D, Ilboudo D, et al. Co-infection of *Toxoplasma gondii* with HBV in HIV infected and uninfected pregnant women in Burkina Faso. *Pak J Biol Sci* 2009; **12**: 1188–1193.
- [9] Sagna T, Djigma F, Zeba M, Bisseye C, Karou DS, Ouermi D, et al. Human papillomavirus prevalence and genital co-infections in HIV-seropositive women in Ouagadougou (Burkina Faso). *Pak J Biol Sci* 2010; **13**: 951–955.
- [10] Djigma FW, Ouédraogo C, Karou DS, Sagna T, Bisseye C, Zeba M, et al. Prevalence and genotype characterization of human papillomaviruses among HIV-seropositive in Ouagadougou, Burkina Faso. *Acta Trop* 2011; **117**: 202–206.
- [11] Ilboudo D, Simpore J, Sanou DS, Karou D, Sia DJ, Ouermi D, et al. Mother-to-child HIV and HHV8 transmission in neonates at Saint Camille Medical Centre in Burkina Faso. *Pak J Biol Sci* 2009; **12**: 908–913.
- [12] Karou DS, Ilboudo DP, Nadembega WMC, Ameyapoh Y, Ouermi D, Pignatelli S, et al. Antibiotic resistance in urinary tract bacteria in Ouagadougou. *Pak J Biol Sci* 2009; **19**: 712–716.
- [13] Karou SD, Nadembega MCW, Zeba B, Ilboudo DP, Ouermi D, Pignatelli S, et al. Evolution of antibiotic resistance of *Staphylococcus aureus* in the Saint Camille Medical Centre in Ouagadougou, Burkina Faso. *Med Trop (Mars)* 2010; **70**: 241–244.
- [14] Boisivon A, Berard H, Nandeuil A, Cheron M, Lafon J, Fabayre C. Diagnosis of vaginitis in general practice: compared clinical and bacterial data. *Med Mal Infect* 2003; **33**: 202–205.
- [15] Lefebvre N, Forestier E, Mohseni-Zadeh M, Remy V, Lesens O, Kuhnert C, et al. Invasive *Streptococcus agalactiae* infections in non-pregnant adults. *Med Mal Infect* 2007; **37**: 796–801.
- [16] Traore Y, Tameklo TA, Njanpop-Lafourcade BM, Lourd M, Yaro S, Niamba D, et al. Incidence of seasonality, age distribution and mortality of pneumococcal meningitis in Burkina Faso and Togo. *Clin Infect Dis* 2009; **2**: S181–S189.
- [17] Bere LC, Simpore J, Karou DS, Zeba B, Bere AP, Bannerman E, et al. Antimicrobial resistance and serotype distribution of *Streptococcus pneumoniae* strains causing childhood infection in Burkina Faso. *Pak J Biol Sci* 2009; **12**: 1282–1286.
- [18] Cissé MF, Camara B, Diaw CF, Ba M. Serotypes and antibiotypes of *Streptococcus agalactiae* strains isolated in Dakar. *Med Mal Infect* 2003; **33**: 318–322.
- [19] Bonfiglio G, Simpore J, Pignatelli S, Musumeci S, Solinas ML. Epidemiology of bacterial resistance in gastro-intestinal pathogens in a tropical area. *Int J Antimicrob Agents* 2002; **20**: 387–389.
- [20] Simpore J, Zeba B, Karou D, Ilboudo D, Pignatelli S, Nacoulma OG, et al. Epidemiology of antibiotic resistance in Burkina Faso. *Asian Pac J Trop Med* 2008; **1**: 1–6.
- [21] Elhamzaoui S, Benouda A, Allali F, Abouqual R, Elouennass M. Antibiotic susceptibility of *Staphylococcus aureus* strains isolated in two university hospitals in Rabat, Morocco. *Med Mal Infect* 2009; **39**: 891–895.