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International Journal of Current Research
Vol. 9, Issue, 12, pp.63028-63032, December, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ECOLOGY OF MICROBIAL GERMS OF THE UROGENITAL TRACT IN WOMEN IN SOUTHEAST GABON

[1,*]Ndong Atome Guy Roger, [1]Ngoua Meye Misso Rick-Leonid, [1]Sima Obiang Cédric and [2]Onanga Richard

[1]Laboratory of Research in Biochemistry (LAREBIO), University of Sciences and Technology of Masuku, P. O. Box 913 Franceville, Gabon
[2]International Medical Research Center of Franceville (CIRMF), P.O Box 769 Franceville Gabon

ARTICLE INFO

Article History:
Received 25th September, 2017
Received in revised form
14th October, 2017
Accepted 10th November, 2017
Published online 31st December, 2017

Key words:
Vaginitis,
Urogenital Pathology,
co-Infection, Resistance.

ABSTRACT

Many studies showed that the presence of the germs, not diagnosed earlier were responsible for urogenital pathologies. This study was carried out in order to determine the frequency of certain germs of the urogenital tract of women. Several germs were identified as *Klebsiella spp* (17%), *Pseudomonas* (6%), *Escherichia coli* which is the majority germ 77% in the urine, fungi of the genus Candida especially *Candida albicans* (94%), Mycoplasma analysis has revealed the following rates; *Mycoplasma hominis* (36%), *Ureaplasma urealyticum* (44%) and also cases of co-infection *Ureaplasma urealyticum* and *Mycoplasma hominis*. The overall prevalence of urogenital infections was 32.31% with predominance almost -égale between urinary tract infection (47.67%) and vaginitis (46%). Among the series of tests performed urinary infections detected by urine culture was the most frequent to 48.57% in the age group of 26 to 34 years. Analysis of microbial infections by age showed that Mycoplasma gradually increased from age 15 and beyond 36 years from 77% to 79.06%. Other minor infections were detected such as *pseudomonas* in 5.71 cases, and *Chlamidia trachomatis* 0.38%. The increasing resistance occurs against the usual antibiotics. Durable solutions need to be found to fight against all these germs implied in sexually transmissible diseases.

Citation: Ndong Atome Guy Roger, Ngoua Meye Misso Rick-Leonid, Sima Obiang Cédric, Onanga Richard and Nkogue Mba Dieudonné. 2017. "Ecology of microbial germs of the urogenital tract in women in Southeast Gabon", *International Journal of Current Research*, 9, (12), 63028-63032.

INTRODUCTION

The female genital tract, like other superficial mucosa, constitutes a specific ecosystem. Indeed, polymorphic microbial flora lining the mucous membranes of the genital tract and plays a significant role in occurred of the urogenital infections among women (Xia *et al*., 2004, Yan *et al*., 2009). The high prevalence of sexually transmitted infections (STIs) is a public health problem in developing countries. This is accentuated since the advent of the pandemic of HIV infection because they share the same transmission path (sexually) (Djima *et al*., 2008). A particular interest was consequently brought to these STI including *Chlamidia trachomatis*, *Candida albicans* and *gonorrhea*, infection with *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Mycoplasma genitalium*. Along with this dominant flora, many species can be classified into three groups according to their ecological origin, we can mention: the bacterial flora of usual carriage (*Lactobacilli*), the digestive flora often colonizing the vagina, including enterococci, staphylococci, and oropharyngeal flora. Symbiotic coexistence is thus established between the different components of this microbiota with each other and with their environment (Cardieux *et al*., 2002). Moreover this fragile balance can be broken thus causing disorders within this microscopic ecosystem, there are various factors modifying the balance of this flora such as age, puberty, duration of the menstrual cycle, pregnancy, infections , birth control methods, frequency of intercourse, number of sexual partners, and various practices such as the vaginal toilets (Cardieux *et al*., 2002). Therefore some microorganisms to commensal origin, acquire pathogenicity and cause various infections. The composition of the vaginal flora reflects the physiological state of the urogenital tract. Therefore to establish microbiological profile of this genital tract may help to prevent possible infections that could occur if this vaginal environment is to be changed. These changes causing infections and inflammation are one of the main causes of serious reproductive morbidity including infertility and ectopic pregnancy (OMS, 2008). Thus an accurate understanding of the composition of the vaginal ecology is important for understanding the aetiology of these

Corresponding author: **Ndong Atome Guy Roger,**
Laboratory of Research in Biochemistry (LAREBIO), University of Sciences and Technology of Masuku, P. O. Box 913 Franceville, Gabon.

diseases (Xia *et al*., 2004). The aim of this study was to establish the infective germs profile of urogenital tract of women in the Southeast region of Gabon and to determine the prevalence of pathogens.

MATERIALS AND METHODS

Ethical Clearance

The sampling was done in agreement with patients after informed consent. All the information collected has been treated in strict respect of the anonymity and confidentiality of the persons. And the study has obtained approval from the National Ethics Committee.

Area and study population

A study on the bacterial ecology of the urogenital tract causing sexually transmitted infections was conducted in the International Medical Research Center (CIRMF) from January to October 2015. The inclusion criteria allowed to enroll all women with intense sexual activity who presented at this time to the CIRMF with or without symptoms of the urogenital sphere and aged 15 to 45 years. Were excluded women menstruating and menopausal women. Recommendations before vaginal swabs required no toilet during12 hours preceding the taking and sexual abstinence during the three days preceding the taking.

Sampling from vaginal tract

The speculum was introduced into the genital tract to widen it by 4 to 5 cm. Cells and vaginal secretions were collected from the mucous membranes using a swab. Then a maximum casualty was collected at the cervix with another swab. Both swabs were sent to the laboratory for microbiological analyzes. The physical and chemical tests performed on the samples consisted in determining the physicochemical parameters of the sample such as the appearance of the sample which consisted in a visual assessment, the measurement of the pH using a pH paper and the observation of different colors and carrying out the test with potassium hydroxide (KOH) which allowed the detection of *Gardnerella vaginalis* with a deposit KOH liquid secretions, the characterization of the odor allowed to confirm or not the Vaginosis *Gardnerella* infectious state.

Search for *Neisseria gonorrhoeae*

This analysis was done mainly on cultures; the swab was introduced at the level of the external orifice of cervix with the valves of the speculum, when the secretion is a little important. At least 30 seconds after contact, the swab was slowly removed and spread on the culture medium.

Searching for Candidiasis

Isolation was done by sterile sowing on Sabouraud glucose-agar plates (2%) containing antibacterial antibiotics and vitamins. The addition of cycloheximide (Actidione), was used to inhibit the growth of possible contaminating fungi. The media and seeded were kept for at least one week at 37°C. If positive, the development of microorganisms appeared in two to four days (sometimes longer, especially when the patient was already receiving antifungal), whitish colonies, creamy, thick, shiny suggested the presence of fungi. Incubation was done at 37°C, in an aerobic atmosphere for 24-48 hours. Identification of germs was considered according the color of the colonies: *Candida albicans* and *Candida spp* producing blue and greenish colonies. Differentiation was made by the test of filamentation which was to introduce the colonies in human serum and incubated at 37°C for 2 hours. The drop of the serum was examined between slide and coverslip. Only *Candida albicans* filamentation gave a positive test.

Cytobacteriological tests of urine

This test consisted in observing under microscope 10µL of urine (it is the direct observation) in order to seek the presence of the leucocytes, red blood cells and potential microorganisms, and a culture was performed on suitable media to identify the microorganism responsible for the infection and perform an antibiogram. The first step was of macroscopic analysis; it allowed describing the appearance and the color of the urine to be analyzed. The urine is normally clear, yellow citrus. The urine received in the laboratory, however, may have other aspects that may be turbid, haematic, purulent, lactescentes or faecaloid. It was followed by culturing where 10µL was deposited after the urine sample seeded in sterile conditions by streaks exhaustion technical tightened the middle CLED (Cystine Lactose Electrolyte Deficient); the other EMB media (Eosin Methylene Blue),and BCP (BromoCrézol Purple) were seeded by the dials technique.

Biochemical tests with the urinary strip

A strip with reactive zones was immersed in the urine sample for the detection of various parameters such as leucocytes, nitrites, pH, proteins, glucose, ketones, urobilinogen, bilirubin, erythrocytes (or blood) and density. The presence of these parameters made it possible to suspect a urinary tract infection that may be caused by a microorganism, in particular the diagnosis of enterobacteria such as *Escherichia coli* which was confirmed by the positivity of nitrite in the urine specimen.

Cytological test

This test was carried on a slide Kovacs, 10µL collected urine was filed and observed with an optical microscope, first at low magnification × 10 for labeling the different elements and the higher magnification × 40 for identification. This test made it possible to observe among others crystals, the presence and mobility of germs, cells of the urinary tree (urethra, renal), epithelial cells of vaginal origin. It also allowed the quantification of leukocytes and red blood cells. Meanwhile 5ml of urine were taken and placed in a Falcon tube, followed by centrifugation for 5 minutes at 3900 rpm.The pellet was saved for direct examination between slide and coverslip in magnification x 40.

Mycoplasma research

This search was done from the first jet urine, 10 mL were taken and introduced into the Falcon tube followed by centrifugation for 5 minutes at 3900 rpm; the supernatant was removed and the pellet was kept which is added a diluent (Mycoplasma R1), and then homogenized. 3mL of this mixture was used and introduced into the flask containing the reagent (Mycoplasma R2) and then homogenized again the mixture.

55 .µL of the mixture were introduced into each of the wells of the Mycoplasma Ist2 Kit (BIO-MERIEUX, France). For the Mycoplasma R2 reagent (BIO-RAD, France), each vial contained 3.1 ml of a stable nutrient broth needed for sample preparation. It ensured the screw-selectivity of main Gram (+) bacteria and Gram (-) and allowed the recovery of the Mycoplasma R2 reagent (BIO-RAD, France). In parallel for Mycoplasma R1 (BIO-RAD, France), each vial contained 1 mL of Urea - Arginine broth in lyophilized form. After recovery of the Mycoplasma with 3 mL of Mycoplasma R1, the composition was in conformity with the following formula composition (Mycoplasma R1 + Mycoplasma R2).

RESULTS

The overall population depending on the number of requiered tests were 1727patientes, all received and registered at the CIRMF. Mapping established the most requested type of test with cytobacteriological test of urines (CTU), cell culture on vaginal swabs taking (CC) and research of Mycoplasma to 47.54%, 32.65%, and 19.80% respectively (Table 1). The overall prevalence of infections was 32.31% (n = 558), Urinary tract infections had a rate of 6.27% (n = 35), genital tract infections had a rate of 46% (n = 257) and Mycoplasma infections 47.67% (n = 266) of cases (Table 2).

urealyticum 43.60% (n = 116) of cases. Among the infections of the urogenital tract, the urinary infections seemed to be predominant in the patients aged 26 to 34 years that was 48.57% vs 40.07% for vaginitis and 32.70% for the Mycoplasmes respectively (Table 3). The data obtained by specific test showed that Mycoplasma represent the main infection in all age groups of patients. It appears in this study that the prevalence of infection with Mycoplasma gradually increased from 77% to nearly 79% by the age of 15 years to 35 years and over (Table 4).

DISCUSSION

This study was conducted from January to October 2015 on 1727 patients who came to the Medical Service of International Medical Research Center. The objective was to identify the germs responsible for infections of the urogenital tract of women in the South-East region of Gabon. In the study population, the overall prevalence of urogenital and sexually transmitted infections was of 32.31%. The germs found are either pathogenic or commensal, possibly associated with pathological manifestations (Alemu *et al*., 2012). This result reveals the infection rate of patients with symptoms and those coming for consultation with a test card for screening for sexually transmitted infections.

Table 1. Analysis Tests by Infection

Test Types	CTU		CC		Mycoplasma		Total tests
	n	%	n	%	n	%	
Number of tests by infection	821	47.54	564	32.65	342	19.80	1727

Table 2. Prevalence of infections by test

Variables	CTU		CC		Mycoplasma		Total Positive samples
	n	%	n	%	n	%	N
Number of Positive tests	35	6.27	257	46	266	47.67	558

Table 3. Profile of infections by age group

Variables	Urinary infections			Vaginitis			Mycoplasma		
	n	(+)	%	n	(+)	%	n	(+)	%
15 – 25	349	6	17.15	195	85	33.09	100	77	28.94
26 – 34	199	17	48.57	183	103	40.07	113	87	32.70
≥ 35	273	12	34.28	186	69	26.84	129	102	38.36
Total	821	35	100	564	257	100	342	266	100

Table 4. Distribution of infections by age group

Age group	15-25 ans			26-34 ans			≥ 35 ans		
	n	tests +	%	n	tests +	%	n	tests +	%
Urinary infection	349	6	1,71	199	17	8,54	273	12	4
Vaginitis	195	85	43,58	183	103	56,28	186	69	37
Mycoplasma	100	77	77	113	87	76,99	129	102	79,06

The results revealed after various tests that in the case of cytobacterial test of urine (CTU), different infections are mainly due to three seeds namely *Escherichia coli* in 77.14% of cases (n = 27), *Klebsiella spp* in 17.14% of cases (n = 6) and *pseudomonas* in 5.71% of cases (n = 2). Analysis of cell culture after vaginal swabs taking highlights *Candida albicans*, *Candida spp* at 93.77% (n = 241), 6.22% (n = 16) and *Chlamidia. trachomatis* 0.38% (n = 1) respectively. As for Mycoplasma research, 36.46% of *Mycoplasma hominis* (n = 97) were detected and co-infected with *Ureaplasma uréalyticum* at 19.92% (n = 53) and finally *Ureaplasma*

Among the vaginitis, the presence of *Candida albicans* in the normal way is a commensal organism in the genital tract (Alemu *et al*., 2012), that has been found in high level at 93.77%, causing vaginal yeast infections. In tropical countries women are often victims of Candida vulvovaginitis, with increasing frequency. About 75% of women experience at least one episode of candidiasis in their lives. Indeed, in some African countries, Nigeria, for example, an unusual prevalence of *Candida albicans* has been reported, up to 68%, but the causes are unknown (Zhao *et al*., 2009). A study conducted in a hospital in New Guinea found that of 206 examined pregnant

women, 23% were positive for *Candida albicans* infection (Klufo *et al*., 1995). The *Candida Albicans* rate reported in this study is higher than the studies in West Africa (Meda *et al*., 1997, Okonkwo *et al*., 2010). A study in Cameroon showed a frequency of 55.40%, far lower than that of the present study (Vroumsia *et al*., 2013). Vulvovaginal candidiasis with a high rate and when they are not asymptomatic classically presents with vulvar pruritus and characteristic vaginal discharge. These signs may be associated with dysuria, dyspareunia, vaginal dryness or vulvar burns (Zhao *et al*., 2009). Paradoxically, *Chlamidia trachomatis* germs showed a low threshold with only an infection rate of 0.38%. In Gabon, for decades, the most incriminated germs in sexually transmitted infections and the most prevalent have been Chlamidia infections at 60% in a South-east region, with irreversible consequences leading to secondary sterility in young couples (Bertherat *et al*., 1998). This low rate would most probably be the result of patient awareness as a result of intensive awareness-raising campaigns and antibiotic-based curative treatments, even though resistance is now being developed by these bacteria. According cytobacterial test of urine analyzes showed a high rate of *Escherichia coli* in 77.14% of cases, in fact the presence of this germ can be the result of contamination due to the proximity of anal and urinary tract in women. The strains of *E. coli* are the cause of enterobacterial infections, in a study conducted in China in 2009, *E. coli* is present at 59% in an infection of the urinary tract in women (Zhao *et al*., 2009). Moreover, in patients in gestation period one study found that *Escherichia coli* was predominant to 47% in the same urinary tract (Alemu *et al*., 2012). The colonization of the uro-genital tract in women and the environmental change and high concentration promotes pathogenicity leading to urinary tract infections.

Search for Mycoplasma is a review that allowed the detection of a commensal organism of the genital tract, potentially pathogenic (Mycoplasma hominis) and a pathogen Ureaplasma urealyticum, their quantification allowed a diagnosis in the case a urinary infection. According to different tests, results showed cases of infection with *Mycoplasma hominis* with a rate of 36.46%, *Ureaplasma urealyticum* infection to 43.60% but also cases of co-infection with *Mycoplasma hominis* and *Ureaplasma urealyticum* at 19.92%. Search for Mycoplasma showed that these bacteria were predominant among all diagnosed urinary tract infections. Indeed according to the stratification of the study population by age Mycoplasma infection is higher than all other infections, gradually from 77% at age 15 to 79.06% beyond 36 years. However, rates of Mycoplasma in microbiological analysis showed that 26.7% women were carriers of Mycoplasma, and the age group of 25-33 years had the highest prevalence. These results show that infection with Mycoplasma is not related to age unlike Chlamydia. While vaginal tract infection affected particularly the age group of 26 to 34 years up 56.28% and then dropped to 37% after 35 years. It is therefore a sample of urban women of childbearing age, which explains the higher number of women under 42 and over 25 years of age who are the most infected. The *Ureaplasma urealyticum* is a pathogen that, when present in the genital tract is necessarily a source of infection of the lower genital tract that can lead to infertility by promoting later infection of the upper genital tract in the case of late detection. Indeed *Ureaplasma urealyticum* has been implicated in the occurrence of infertility and habitual abortion in Africa (Klufo *et al*., 1995). But *Mycoplasma hominis* when it is present at high levels (10^4) is also responsible for infection of the lower genital tract leading to the same consequences. In addition, a study in Burkina Faso showed that the combination of Mycoplasma hominis and *Ureaplasma urealyticum* at 7.5% could be a source of infertility (Djigma *et al*., 2008). Some germs have not been identified as *Trichomonas vaginalis, Gardnerella vaginalis* and *Neisseria gonorrhoeae* that are pathogens causing genital infections probably because of their rarity or absence in the samples analyzed.

Conclusion

The bacterial profile of the female urogenital tract in the southeastern region of Gabon, allowed us to identify the microbial flora present and to determine the prevalence of urinary and genital tract infection women. The germs circulating in the sexually active female population would be in order of importance the *Mycoplasma* and *Candida albicans*. Indeed, knowledge of the quality of this ecology is important because it can be used for the establishment of a monitoring process to better fight against low urogenital infections that are the initial process high infections urogenital tract. One of the causes of infertility which is a major public health problem in young people in Africa comes from to the lack of appropriated governmental policy. Alternative treatment options reside in the identification of plants with antibacterial and antifungal properties and able to eradicate resistant strains and / or multi resistant to current pharmaceutics drugs in order to improve the care of patients particularly those exposed to HIV, and secondly to reduce the phenomenon of primary and secondary infertility.

Acknowledgment

The authors sincerely acknowledged Dr Nkoghe Dieudonné PhD, MD who initiate this research and ensured a through and accurate supervision. The authors also thank the International Medical Research Center of Franceville (CIRMF) for the procedure of enrolment of patients and Medical diagnosis.

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