Epidemiological profile of microorganisms associated with female infertility at the Mother and Child University Hospital Center of N'Djamena: risk factors and antibiotic resistance

ABSTRACT

Aims: the objective of this work was to determine the resistance phenotypes and the epidemiological profile of microorganisms associated with female infertility at the CHU-ME of N'Djamena.

Material and methods: we conducted a prospective, cross-sectional and analytical study on women admitted for maternity desire at the CHU-ME of N'Djamena and consenting from June 1, 2022 to February 26, 2023. Isolation and identification microorganisms responsible for female infertility were carried out in the laboratories using standard clinical microbiology methods.

Results: Of the 122 patients included in this series due to infertility, 88 (72.13%) tested positive for microbial infection compared to 34 (28%) negative tests (p = 0.01). The average age of infertile women was 34.24 years with extremes ranging from 19 to 45 years. The age groups most affected were 25 to 31 and 32 to 38. Primary infertility was 71.31% and secondary 28.68%. The microorganisms most associated with infertility were *Chlamydia trachomatis* (25.30%), *Mycoplasma hominis* (17.90%), Candida albicans (16.66%) and Staphylococcus aureus (16.04%) *Streptococcus agalactiae* (10 .49%) and *Ureaplasma* spp (8.14%). The risk factors most associated with infertility were advanced age (18.85%), surgical interventions (17.25%) and ovulation disorders (19.39%). The antecedents most associated with infertility were cesarean section 44 (36.07%) followed by miscarriages 29 (23.77).

The sensitivity of bacterial and fungal agents to antibiotics and antifungals was varied. The majority of bacteria were resistant to Cyclins, Betalactams and Macrolides with proportions of 66%, 47.66% and 34% respectively. Strains of *Ureaplasma*spp and *Mycoplasma homonis* develop average resistances of 86.52% and 36.16% respectively against fluoroquinolones.

The fungal strains were sensitive (54.33%) to the azole derivatives and resistant (70%) to the polyenes tested.

Conclusion

The present study made it possible to determine high prevalence of microorganisms and risk factors associated with female infertility. It also highlighted high prevalence of resistance of bacteria to beta-lactams and fluoroquinolones and of Candida albicans to polyenes.

Keywords: female infertility, associated risk factors, microorganism, antibiotic, antifungal, N'Djamena.

1. INTRODUCTION

Defined as the inability to conceive after twelve (12) months of regular, unprotected sexual intercourse, infertility constitutes a public health problem affecting 8 to 12% of couples worldwide [1]. It is of female origin in 30% of cases, male in 20% of cases, mixed in 40% of cases and idiopathic in 10% of cases [1, 2]. It poses a serious problem in Africa due to the stigmatization of couples without children who represent 15 to 30% depending on the region [2, 3]. The average fecundability is estimated at 20-25% per cycle for a couple aged 20 to 30. After 6 months of non-medical attempts, 75% of couples will achieve pregnancy, 90% after 1 year and 97% after 2 years [4]. Infertility is linked to several factors: uterine and tubal factors (fibroma, blocked tube); cervical factors (cervical anomaly); vaginal factors (difficulty retaining sperm (vaginal acidity); age; infectious pathologies; obesity; exposure to certain toxic products (tobacco, cannabis, heroin, cocaine, hallucinogens); endometriosis, stress; voluntary termination of pregnancy (abortion); and cycle disorder. In women, the fundamental causes are often lower genital infections [3] going as far as obstruction of the tubes representing half of the cases aggravated by late consultations making treatment difficult and causing complications [5].Abortions performed in unsafe conditions also constitute one of the main causes of female infertility, causing permanent sterility in most cases [6,7]. According to the WHO, 186 million couples are affected in developing countries [8], the WHO also recognizes infertility due to unsafe abortion (exposing women to salpingitis, endometriosis) and maternal sepsis as the fifth most common disability in low- and middle-income countries [9]. The main microorganisms involved in infertility are: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Mycobacterium tuberculosis*, *Schistosoma hematobium*, *Schistosoma manson*, treponemes, and other common germs.

Other superinfection germs, due to poor hygiene conditions, are also involved, such as streptococci, staphylococci, colibacilli, *Proteus*, and *Bacteroides fragilis*.

In Chad, a study carried out at the Mother and Child University Hospital Center showed that women were responsible in 42.8% of cases, men in 30.3% of cases and the couple in 26.7% of cases. Of 3850 women consulted, 14% were infertile, the cause of which was infectious 42.2%. Management remains complex due to the precariousness of prevention means and the accessibility of diagnostic methods [10].

In recent years, we have witnessed an increase in the incidence of antibiotic resistance in the germs responsible for genitourinary infections, particularly due to the emergence of enterobacteria with extended-spectrum beta-lactamases [11]. So it is necessary to find the right balance between the risks of complications and the increase in resistance to antibiotics without validated consensus [12].

Thus, in order to meet the social needs linked to infertility, this diagnostic work aims to determine the resistance phenotypes and the epidemiological profile of the main microorganisms associated with female infertility at the Mother and Child University Hospital Center of N 'Djamena.

2. MATERIALS AND METHODS

2.1. Study framework

The study took place in the Gyneco-obstetrics department of the University Hospital Center for Mother and Child (CHU-ME), in the Bacteriology laboratory of the CHU-ME of N'Djamena and in the Bacteriology Unit of the Research, Diagnostic and Scientific Expertise Laboratory (Labo-ReDES) of the Faculty of Human Health Sciences (FSSH), University of N'Djamena.

2.2. Type and period of study

This was a prospective, transversal and analytical study which lasted 6 months from October 2022 to March 2023.

2.3. Study population

2.4. Eligibility criteria

Inclusion criteria

- ✓ Informed consent written and signed by infertile women.
- ✓ Patient with a diagnosis of genital infection and infertility.

Non-inclusion criterion

Refusal of women to participate in the study.

2.5. Sampling

The sampling was exhaustive

The study population consisted of all women who were admitted for maternity desire to the CHU-ME of N'Djamena.

2.5.1. Cervico-vaginal sampling

2.5.1.1. Condition of collection

-no personal hygiene the day before the sample.
-no sexual intercourse for 24 hours.
-suspend antibiotic therapy for at least 48 hours.

2.5.1.2. Sampling equipment

The sampling equipment consisted of:
-sterile, unlubricated speculum;
-sterile swab;
-slide not scratched and well degreased;
-slip, marker or label;
-physiological water in a hemolysis tube.

Regardless of the type of infection, three swabs were used to collect cervical mucus from the upper part of the vagina (in order: endocervix; ectocervix, posterior fornices):
- At the level of the endocervix for culture, research (*N. gonorrhoeae*, *Streptococcus*,*Staphylococcus*, etc.);
- At the level of the ectocervix for GRAM staining;
- At the speculum level: we collected the cervical mucus which was used to produce the fresh state in physiological water for research (*Trichomonas vaginalis*, vaginosis, *Candida*, mycoplasma).

To test for *Chlamydia trachomatis*, a specific swab is used to preferably sample the endocervix (possible posterior fornices) and placed in the recommended transport medium.

The search for *Mycoplasma* required the use of an additional swab (exo-endocervix) and discharging it into the appropriate medium (vial with yellow cap, in the fridge of the sampling room, after bringing the medium to room temperature). The patient had to avoid any intimate hygiene, any local treatment (> 15 days for *Chlamydia*,> 5 days for common germs) as well as any sexual intercourse in the 24 hours preceding the examination. Also avoid sampling during the menstrual period because the flora is modified.

The patient was placed on the gynecological table in the most forward position possible to allow complete flexion of the thighs, which relaxed the peris-vaginal muscles and facilitated placement of the speculum.

The equipment used consisted of single-use gloves, a sterile single-use speculum, and two swabs.

2.6. GRAM coloring

On the endocervix sample: look for intra and extracellular GRAM negative diplococci (Gonococcus).

On the posterior fornicessample, search for and quantify:

• GRAM negative variable intra and extracellular coccobacilli (*Gardenella vaginalis*);
• Intra and extracellular curved GRAM negative bacilli (*Mobiluncus*ssp);

• GRAM positive, + or – long bacilli (Doderlein bacilli);
• Fungal elements (yeasts, mycelial filaments).

2.7. Culture

Poly vitex chocolate agars were used in aerophilic applications., a medium intended for the isolation of demanding bacteria such as *Neisseria* (gonococci) and Streptococci, Staphylococci. This medium is composed of a nutritional base enriched in factor X (hemin) and V (NAD) provided by hemoglobin and poly vitex. It can be used for subculturing bacterial strains to obtain pure cultures.

Muller Hinton agar was a medium intended for performing Gram staining by diffusion. Its composition allows the growth of non-demanding bacteria such as non-fermented gram-negative bacillus enterobacteria, staphylococci and enterococci encountered in pathology while guaranteeing a minimum of interference of the components of the formula on the result of the antibiogram. Its divalent ion concentration is adjusted to ensure better precision in determining the sensitivity of *Pseudomonas* to aminoglycosides and tetracyclin.

Its thymine-thymidine content (element inhibiting the activity of sulphonamides) reduces response phenomena around the discs and allows better determination of diameter.

Sabouraud Chloramphenicol medium was a medium for isolating fungi (mold and yeast).

Most of its germs cause infertility by inducing vulvar and/or vaginal inflammation; ulceration, edema, vesicle, condyloma, at the level of the cervix; obstruction of the tube; cycle disorders responsible for amenorrhea; endometriosis (it causes adhesions, problems with oocyte capture and tubal transport) [13].

2.8. Antibiogram [13, 14].

The antibiogram was carried out according to the KIRBY BAUER method. It is a method based on the diffusion of paper disks impregnated with antibiotic on agar medium (preferably Mueller-Hinton). The previously dry antibiotic disks, once placed on the agar, absorb a sufficient quantity of water to dissolve the antibiotic which thus gradually diffuses into the medium following the physical laws of molecular diffusion) through a gel. In this method there is a direct correlation between the Minimum Inhibitory Concentration (MIC) and the diffusion zone. From a pure culture made from the vaginal sample. It is carried out as follows:

✓ Preparation of the inoculum: From a pure and fresh culture taken on agar medium, prepare a suspension with an opacity equivalent to the 0.5 Mac Farland standard;

✓ Inoculation of the plates: By swabbing, inoculate the colony in the agar. Immerse a sterile, non-toxic swab in standardized inoculum, squeeze it gently on the walls of the tube containing the suspension; then pass it 2 to 3 times over the entire surface of the agar medium in order to obtain homogeneous seeding and confluent colonies;

✓ Drying the boxes: Allow the boxes to dry for 10 minutes before placing the discs;

✓ Arrangements of the antibiotic disks: Place the disks using a dispenser or pliers, pressing them lightly, and place them at least 15 mm from the periphery of the box so that the areas of inhibition do not overlap. A concentration gradient of the antibiotic is thus formed around each disc;

✓ Incubation: Incubate the agar plates at 37°C for 24 hours;

✓ Reading the diameter of the inhibition zones: No growth appears when the antibiotic is present at inhibitory concentrations and is sensitive to the strain. It is then possible to measure, using a caliper, the diameter of the inhibition zone which is directly proportional to the minimum inhibitory concentrations;

✓ Interpretation: After measuring the inhibition zone translated by a clear zone around the antibiotic; we deduce that the larger the diameter of the zone, the more sensitive the antibiotic.

2.9. ***Chlamydia*** **test**

It is an immunochromatographic test which was used for the qualitative detection of *Chlamydia*. To carry out the test, a smear was placed in a reaction vessel with extraction solution A. After 2 minutes, extraction solution B was added and then 3 drops of the sample solution (approximately 150 µL) are applied to the test.

The test membrane was coated with antigen-specific, monoclonal antibodies at the test line (T) as well as with goat anti-mouse antibodies at the control line (C). During the test, any *Chlamydia* antigens present in the extracted antigen solution reacted with Chlamydia antibodies. Thanks to capillary forces, the mixture reacted with the *Chlamydia* antibodies on the membrane and generated a pink line at the test zone (T). The presence of a pink line at the test area indicated a positive result while the absence of a pink line indicated a negative result. As a procedural control, a pink line will always appear at the control area (C) indicating that the test is working.

2.10. Detection of *Mycoplasma* and *Ureaplasma*

The MYCOPLASMA IST 3 gallery (BioMérieux, Marcy l'Etoile, France) allowed the identification and enumeration of *Mycoplasma hominis* and *Ureaplasma* spp. Additionally, 6 antibiotics could be tested. Five (levofloxacin, moxifloxacin, tetracycline, erythromycin and telithromycin) were tested against *Ureaplasma* and four (clindamycin, levofloxacin, moxifloxacin and tetracycline) were tested against *Mycoplasma hominis*.

The MYCOPLASMA IST 3 gallery is a system for the identification of *Mycoplasma hominis* and *Ureaplasma*spp using standardized and miniaturized biochemical tests, in microtubules in dehydrated form. The principle is based on the inoculum of microtubules with a suspension of urine samples and or any other sample: cervicovaginal (PCV), urethral sample (PU) and sperm which rehydrates the media. Incubation was done at 37°C for 48 hours. during which biochemical reactions take place (decarboxylation, fermentation, deamination) which result in spontaneous colored products revealed by the addition of the reagents.

The biochemical reactions and antibiogram was done using the API-MYCOPLASMA IST 3 positive control (red coloring: (≥104 -106)). The positive control provided the identification of a large number of profiles obtained on API-MYCOPLASMA IST 3, which gave great reliability to the interpretation of the results.

2.10.1. Interpretation of the results of the MYCOPLASMA IST 3 gallery

Table 1: interpretation of the results from the MYCOPLASMA IST 3 gallery

Parameter	Positive control	Coloration		**Result**
Bacteria		Positive	Negative	
Ureaplasma spp	red	red	yellow	positive
Mycoplasma hominis	red	red	yellow	positive
Antibiogram	Positive control	Coloration		**Result**
	red	yellow	yellow	sensitive
	red	red	red	resistant
	red	yellow	red	intermediate

NB: the interpretation of the results of the antibiogram is double staining except telithromycin

2.11. Written and signed informed consent

Madam,

We would like to take your cervicovaginal sample (PCV), this is a procedure usually done to look for the microorganisms responsible for infertility. Infertility in women constitutes a public health problem and can occur in all age groups and is most often

associated with pathogenic microorganisms responsible for several complications that are often difficult to resolve.

The sample will not cause any risk to your health, it will be used to identify the pathogen(s) responsible for your health problem. Under no circumstances will other examinations be carried out without your consent. The results obtained will be made available to you and will undoubtedly allow you to better understand the cause of your infertility.

You will be informed of any change in the purpose of the research on the samples and you will be able to object.

Madam, your participation is essential for the completion of this study which will allow us to contribute to improving your care.

Patient signature and telephone number

2.12. Data analysis

The data are entered and analyzed with Microsoft Word and Excel, the chi-square test was used for the correlation between variables with a significance rate of 5%.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Geographical location of the study area

The cervicovaginal (PCV) samples were taken from women in the Gyneco-obstetrics and prenatal consultation departments of the CHU-ME. The CHU-ME is located in the Gardolé district in the third Municipal District of N'Djamena and covers an area of 15,000 m².

The PCV analyzes were carried out in the Bacteriology laboratories of the CHU-ME of N'Djamena and at the Bacteriology Unit of the Research, Diagnostic and Scientific Expertise Laboratory (Labo-ReDES) of the Faculty of Sciences of the Human Health (FSSH), University of N'Djamena (figure 1).

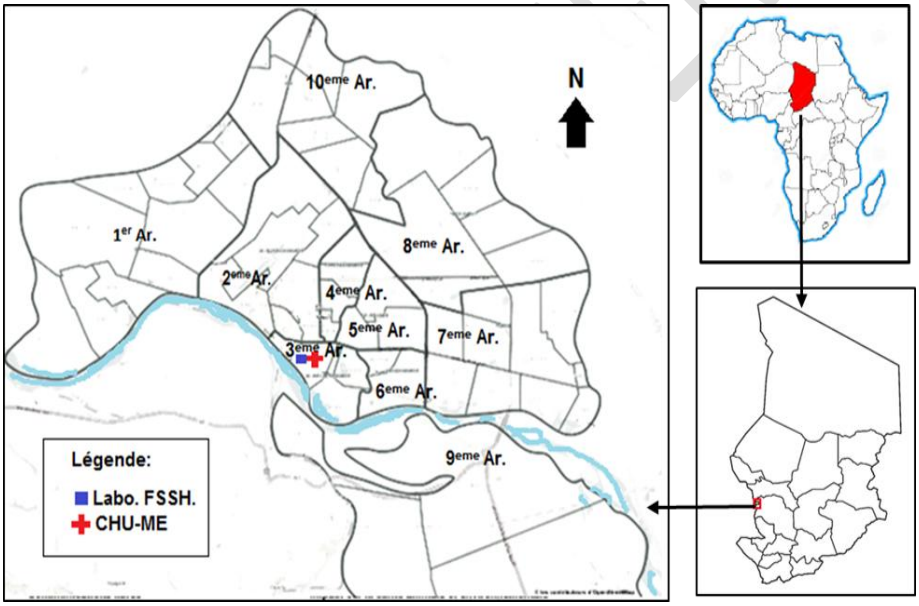

Figure 1: Presentation of the CHU-ME study area

3.1.2. Distribution of patients according to age group

Depending on the age group, the most represented patients were 25 to 31 years old in 44 (36.06%) of the cases.

Table 2: Distribution of patients according to age group

Age (year)	Effective	Percentage (%)

18-24	20	1739
25-31	44	36.06
32-38	39	31.96
39-45	19	15.57
Total	122	100

3.1.3. Distribution of patients according to origin

Of 122 women who participated in our study, 117 (95.90%) came from N'Djamena compared to 5 (4.09%) from the provinces (ρ = 0.01), a significant difference in the predominance of female patients. coming from N'Djamena).

3.1.4. Distribution of patients according to profession

The majority of women admitted for maternity desire and consenting were housewives 70 (57.38%) followed by traders 31 (25.41%).

Table 3: distribution of patients according to profession

Profession	**Effective**	**Percentage (%)**
Household	70	57.38
Shopkeeper	31	25.41
Pupil/student	12	9.84
Civil servant	9	7.38
Total	122	100

3.1.5. Distribution of patients according to the space between marriage and the first consultation

52 (42.62%) of women were consulted between 37 and 48 months after marriage.

Table 4: distribution of patients according to the space between marriage and the first consultation

Space between marriage and first consultation	**Effective**	**Percentage (%)**
12 months	9	7.38
13 to 24 months	14	11.48
25 to 36 months	20	16.39
37 to 48 months	52	42.62
49 to 60 months	24	19.67
61to 72 months	3	24.6
Total	122	100

3.1.6. Distribution of patients according to history

44 (36.07%) women had a history of cesarean section followed by 29 (23.77%) who had miscarriages.

Table 5: distribution of patients according to history

History	**Effective**	**Percentage (%)**
Caesarean section	44	36.07
Miscarriage	29	23.77
OvarianCyst	9	7.38
Ovulation disorder	12	9.84

Obesity	5	4.10
Abortion	23	18.85
Total	122	100

3.1.7. Distribution of patients according to the appearance of leukorrhea

Regarding the appearance of leucorrhea, 66 (54.10%) women had abundant whitish leucorrhoea compared to 56 (45.90%) and 56 (45.90%) had scanty leucorrhea.

3**.1.8. Microbial etiology associated with patient infertility**

Of the 122 women included in this study series for a cause of infertility, 88 (72.13%) tested positive for microbial infection and 34 (27.87%) tested negative (ρ = 0.01), a significant difference in favor of positive detection tests).

Of the 162 microbial agents isolated and identified, 135 (83.33%) were bacteria and 27 (16.66%) fungal agents (*Candida albicans*).

Of the 135 bacterial agents isolated, 41 (30.37%) *Chlamydia trachomatis* detected by immunochromatographic tests, 18 (13.33%) *Mycoplasma hominis* and 11 (8.14%) *Ureaplasma*spp were identified by biochemical tests (API-MYCOPLASMA IST 3.) with antibiogram and 65 (48.14%) bacteria and 27 (16.66%) *Candida albicans* were isolated by culture on agar media.

Among the 162 bacterial and fungal agents isolated, 26 (16.04%) cases of *Chlamydia trachomatis/Candida albicans* coinfection were identified, 14 (8.64%) cases of Chlamydia trachomatis/*Mycoplasma homonis* coinfection, 14 (2%) cases of *Ureaplasma*spp/Escherichia coli co-infection and 1 (%) case of *Chlamydia trachomatis/Mycoplasma hominis/Candida albicans* co-infection.

Table 6: distribution of isolated germs

microorganism	**Effective**	**Percentage (%)**
Staphylococcus aureus	26	16.04
Escherichia coli	19	11.72
Mycoplasma hominis	18	13.33
*Ureaplasma*spp	11	8.14
Chlamydia trachomatis	41	25.30
Neisseria gonorrhoeae	3	1.85
Candida albicans	27	16.66
Streptococcus agalactiae	17	10.49
Total	162	100

3.1.9. Distribution of patients according to type of infertility

The primary type of infertility was predominant with 87 (71.31%) followed by 35 (28.68%) secondary.

3.1.10. Distribution of patients according to the cause of infertility

Advanced age was the most represented cause 23 (18.85%) followed by surgical intervention 21 (17.21%) and ovulation disorder 20 (16.39%).

Table 7: distribution of patients according to the cause of infertility

Cause of infertility	**Effective**	**Percentage (%)**
advancedage	23	18.85
Infection	16	13.11
Ovulation disorder	20	16.39

Salpingitis	6	4.92
Endometriosis	17	13.93
Surgical intervention	21	17.21
Idiopathic	19	15.57
Total	122	100

3.1.11. Distribution of patients according to cycle

Out of 122 women sampled, 97 (79.50%) had a regular cycle compared to 25 (20.49%) irregular cycles.

3.1.12. Distribution of patients according to parity

The most dominant parity was observed in nulliparous women 78 (63.93%).

Table 8: distribution of patients according to parity

Parity	**Effective**	**Percentage**
Nulliparous	78	63.93
Primiparous	23	18.85
Multiparous	21	17.21
Total	122	100

3.1.13. *Ureaplasma*spp antibiogram result

It appears from the analysis of this table that *Ureaplasma*sppdeveloped an average resistance of 86.52% against fluoroquinolones, 32% for macrolides and a resistance of 36.36% for tetracycline (table 9).

Table 9: Result of the antibiogram for 11 *Ureaplasma*spp

	Sensitive		**Intermediate**		**Resistant**	
Antibiotic	Effective	Percentage	Effective	Percentage	Effective	Percentage
Lévofloxacin	1	9.09	0	0.00	10	91
Moxifloxacin	2	18.18	0	0.00	9	82
Tétracyclin	6	54.54	1	9.09	4	36.36
Erythromycin	7	63.63	0	0.00	4	36.36
télithromycin	8	73	0	0.00	3	27.27

3.1.14. Result of antibiogram for 18 *Mycoplasma homonis*

It appears from the analysis of this table that *Mycoplasma homonis*developed an average resistance of 36.16% against fluoroquinolones, a resistance of 39% for tetracycline 28%, and resistance for lincosamides and (table 10).

Table 10: Result of the antibiogram for the 18 *Mycoplasma homonis*

	Sensitive		**Intermediate**		**Resistant**	
Antibiotic	Effective	Percentage	Effective	Percentage	Effective	Percentage
Lévofloxacin	11	61.11	0	0.00	7	39
Moxifloxacin	12	66.66	0	0.00	6	33.33
Tétracyclin	10	55.55	1	5.55	7	39
Clindamycin	13	72.22	0	0.00	5	28

3.1.15. Evaluation of the effectiveness of 65 bacterial agents and 27 *Candida albicans* isolated with antibiotics and antifungals tested

The ever-increasing frequency of fungal and bacterial infections associated with female infertility and the rapid emergence of strains resistant to most antifungals and antibiotics, now require sensitivity tests to antibiotics and antifungals used in therapy, to each isolated strain of bacteria or tissue mycoses, systematic or generalized. Generally speaking, the isolated *Candida albicans* were sensitive in the average order of 48.25%, and 51.75% resistant to the antifungals tested.

Candida albicans were more sensitive by an average of 54.33% and 44.66% resistant to the azole derivatives tested. On the other hand, they were much more resistant, averaging around 70%, and sensitive (30%) to the polyenes tested (table 11).

Most of the isolated bacterial strains were resistant to Cyclins (tetracycline), Betalactams (oxacillin, ceftriaxone, Ceftazidime) and Macrolides (erythromycin) in the average proportions of 66%, 47.66% and 34% respectively. On the other hand, the bacterial strains were sensitive to Carbapenems (Imipenem), Aminoglycosides (gentamicin) and Fluoroquinolones (Ciprofloxacin) of the order of 69%, 69% and 63% respectively.

Table 11: Evaluation of the effectiveness of the antibiotics and antifungals tested

Microorganism	Antibiotic																	Antifungal							
Fungal agent	Nb	OXA		CRO		IMP		GNM		TET		ERY		CIP		CAZ		FLZ		NYS		CLZ		ECZ	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Candida albicans	27																	17	10	8	19	7	20	20	7
Total (%)	27																	17 (63)	10 (37)	8 (30)	19 (70)	7 (26)	20 (74)	20 (74)	7 (26)
Bacterial agent																									
Staphylocoque aureus	26	11	15	12	14	20	6	15	11	10	16	16	10	18	8	13	13								
Escherichia coli	19	NR		13	6	11	8	16	3	7	12	NR		11	8	9	10								
Neisseria gonorrhoeae	3	1	2	1	2	2	1	2	1	1	2	1	2	0	3	0	3								
Streptococcus agalactiae	17	9	8	11	6	12	5	12	5	4	13	7	10	12	5	6	11								
Total (%)	65	21 (46)	25 (54)	37 (57)	28 (43)	45 (69)	20 (31)	45 (69)	20 (31)	22 (34)	43 (66)	24 (37)	22 (34)	41 (63)	24 (37)	28 (43)	37 (57)								

R=resistant + intermediate; S= sensitive. Oxacillin (OXA): R <15, 15≤ I ≤ S, s≥ 21. Ceftriaxone (CRO): R<15, 15≤ I≤ 21, s> 21. Impenem (IMP): R< 17, 17≤ I ≤24, s >24. Gentamicin (GNM): R<14, 14 ≤I≤16, s>16. Tetracycline (TET): R<17, 17≤I≤19, S>19. Erythromycin (ERY): R<23, 23 ≤ I ≤24, s>24. Ciprofloxacin (CIP): R<19, 19≤I≤22, s>22; Ceftazidime (CAZ): R<15, 15≤I≤21, s>21; FLZ= Fluconazole; NYS=Nystatin; CLZ =Clotrimazole; ECZ=Econazole; NR= not required.

3.2. DISCUSSION AND COMMENTS

Out of 3699 consultations made to the gyneco-obstetrics department of the CHU-ME of N'Djamena ranging from June 1 to February 30, 2023, i.e. 9 months; 542 had consulted for maternity desire, of which 122 met our inclusion criterion. During the study period we reported a frequency of 14.65% of infertile couples. This result is within the range that other African authors have reported varying from 12 to 23%. [15, 16, 17, 18]. This prevalence in our regions could be explained by the high frequency of sexually transmitted infections, the lack of education; socio-economic level; which does not allow adequate management of these infections.

The average age of the patients was 28.9 years. The prevalence of microorganisms associated with female infertility was 72.13%. The average age of the patients was 34.24 years with an extreme of 18 to 45 years. This result is close to that found by Neerja in India in 2014 which reported 35 years [19] and is also close to that found by CNGOF in France in 2015 which reported 34.2 years among women experiencing pregnancy loss [20]. It is close also that of Ikechebelu in Nigeria which finds an age range between 26 and 30 years [21]. After 35 years, most studies have shown a significant drop in fertility. Women's fertility reaches its maximum between 20 and 30 years and thereafter gradually declines until menopause. Regarding the type of infertility, our study reported that 71.31% of patients had primary type infertility and 28.68% secondary type. This result is similar to that of Bukar in Niger in 2011 which found 72.69% and 27.30% respectively [22].

From the point of view of profession, women housewives were more numerous, i.e. a percentage of 57.38%. This result is comparable in terms of predominance to that of Coulibaly in Mali in 2009 who reported that 20% of patients who were affected by infertility had a secondary or higher level of education [23].

Regarding risk factors; we found, among other things, surgical interventions on the abdominal regions; endometriosis with respective percentages of 17.21% and 13.93%; the percentage of surgical intervention is close to that of Mai and Demmouche in Algeria in 2014 who found 24.61% [24]. On the other hand, endometriosis represented 13.93% compared to (5.95%), from Bukar to Niger in 2011 in 2011 [22].

Regarding isolated germs, we found that *Chlamydia trachomatis* was more representative with a proportion of 25.30%. This result is superimposable to that of Sogodogo (2014) in Mali which found 25.8% of cases of infection but rather *Ureaplasma urealyticum* in women suffering from infertility[25]. This difference could be explained by the refusal of several women to take the sample.

Generally speaking, *Candida albicans* was sensitive in the average order of 54.35% and resistant to 44.66% to the azole derivatives tested. On the other hand, they were much more resistant, averaging around 70%, and sensitive (30%) to the polyenes tested. The high sensitivity to azole derivatives of Candida albicans to antifungals could be explained by the fact that these antifungals are not part of the drugs sold everywhere to the public in our country. More than half of *Candida albicans* were resistant (70%) to Nystatin. This could be explained by the misuse of nystatin without laboratory evidence (Table 8). Furthermore, polyenes such as Nystatin and Amphotericin B were inactive according to Somo in 2014 [26]. An isolated strain of *Candida albicans* was resistant to these two antifungals tested (Table 11). This observed resistance phenotype could also be explained by the expression of mutated forms of this fungal agent.

Most of our isolated bacterial strains (Table 11 were resistant to Cyclins, Betalactams (oxacillin, ceftriaxone, Ceftazidime) and Macrolides (erythromycin) of the average proportions of 66%, 47.66% and 34% respectively. This increase in resistance would be due to self-medication, poor prescription, uncontrolled use of antibiotics in agriculture and livestock. These results corroborate the results of the work of Bessimbaye et al (2020) on

bacterial and fungal infections in people living with HIV who obtained a high sensitivity of the average order of 65% to the azole derivatives tested and a high resistance of the average order of 74.5% to the polyenes tested [27] (table 11). On the other hand, the bacterial strains isolated by Bessimbaye et al (2020) were resistant to Cyclins (tetracycline), Betalactams and Sulfonamides (clotrimazole) of the middle order at proportions of 46%, 47% and 68% respectively. This increase in resistance to bacterial strains could be explained by self-medication, use of antibiotics in agriculture, livestock breeding, incorrect prescription.

Compared to mycoplasmas and ureaplasmas, the antibiotic resistance of *Mycoplasma homonis* was determined using the MYCOPLASMA IST-3 kit (BioMérieux, Marcy l'Etoile, France). It varied between 82 and 91% for fluoroquinolones, between 27.27 and 36.36% for macrolides and a resistance rate of 36.36% for tetracycline (table 9). On the other hand, the antibiotic resistance of *Ureaplasma*spp was also determined using the MYCOPLASMA IST-3 kit (BioMérieux, Marcy l'Etoile, France); but, it varied between 33.33 and 39% for fluoroquinolones, 28% for lincosamides and a resistance rate of 39% for tetracycline (table 10). The results of this study corroborate with those of previous work carried out in France, China and Mali [28, 29, 30].

4. CONCLUSION

The present study made it possible to determine a high prevalence of the microorganisms most frequently associated with female infertility (*Chlamydia trachomatis*, *Mycoplasma hominis* and *Candida albicans*). The other risk factors most associated with female infertility are history (cesarean sections, miscarriages, surgical interventions).

This study also made it possible to determine high prevalence of resistance of bacteria to beta-lactams (oxacillin, ceftriaxone, Ceftazidime) and of Candida albicans to polyenes (Nystatin). Bacterial strains are more sensitive to Carbapenems (Imipenem), Aminoglycosides (gentamicin), Fluoroquinolones (ciprofloxacin) and *Candida albicans* are more sensitive to azole derivatives (fluconazole, Econazole).

In view of these results, we recommend carrying out expanded studies to determine the causes of risk factors associated with female infertility at the national level.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Hedon B, Ayel JP., Fauconnier A, Barrière P, Belaisch J, Crecy M et al. Recommendations for clinical practice December 2010: management of infertile couples. J Gynecol Obst et Biol Reprod. 2010 ;39 :1-342.

2. Fiadjoe MK, Adjenou V, Kolani JC, EgahKK. CNGOF recommendations for clinical practice. Tubal infertility in Africa. CNGOF. 2012: 641-656. https://cngof.fr/app/pdf

3. Touré Ecra A, Brou Kadjo-Morokro M. Management of the infertile couple/rationalization of the approach. J Human reproduction and hormones.2012 ; 25 (3-4) :2-12.

4. Leridon H. Does the human species have a fertility problem? population and societies. INED. 2010;471: 1-4.

5. Okafor CO, Okafor CI, Okpala OC, Umeh E. The pattern of hysterosalpingographic findings in women being investigated for infertility in Nnewi, Nigeria. Niger J Clin Pract .2010;13 (3):264-267.

6. OMS BM. Rapport Mondial sur le Handicap [Internet]. 2011. Availablefrom: file:///C:/Users/USER/Desktop/BUREAU%20OLD%20COMPUTER/Infertility/Rapport%20mondial%20sur%20le%20handicap_fre.pdf

7. Fatou M, Toure I D. Developing approaches to understand, characterize and address infertility and its consequences for individuals and families in sub-Saharan Africa: The case of Senegal. 2019; 1-21.

8. Brochard M. Normes reproductives, infertilité et nouvelles technologies de reproduction au Sénégal. [France] : Universités Paris-Descartes ; 2014 : 423p.

9. Maya N. Mascarenhas, Seth R. Flaxman, Cravates Boerma, Sheryl Vanderpoel, Gretchen A. Stevens. Tendances nationales, régionales et mondiales de la prévalence de l'infertilité depuis 1990 : une analyse systématique de 277 enquêtes sur la santé. 2012 Dec ; Availablefrom: https://doi.org/10.1371/journal.pmed.1001356.

10. 10. WHO (World Health Organization). World Disability Report. 2011; 1-28.

11. Foumsou L, DamtheouS, Gabkika BM, Dangar D, Djongali S, Hissein A, Prevalence and profile of couples treated for couple infertility at the Mother and Child Hospital of N'Djamena. KisMed .2018 ;8 (1) :314-318.

12. Leridon H. Does the human species have a fertility problem? Population & Societies. 2010; 471:1-4.

13. Bonnet R, Caron F, Cavallo J D, Chardon H, Chidiac C, Courvalin P, Drugeon H, Dubreuil L, Jarlier V, Jehl F. Antibiogram Committee of the French Society of Microbiology (CA-SFM). Recommendations.2013;19: 133- 142.

14. Wayne P A. National Committee for clinical laboratory standards (NCCLS), methods for dilution antimicrobial susceptibility tests for bacterial that grow aerobically, 6thedn. Approved standard. 2015 ; 35 (2) : M07-A10. 15p.

15. Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. Int J Womens Health. 2011; 3:25-35.

16. Schmitz T, Senat M-V, Sentilhes L, Azria E, Deneux-Tharaux C, Huchon C, Bourd N, Fritel X, Fauconnier A. CNGOF recommendations for clinical practice: methodological review of the development process / Gynecology Obstetrics Fertility &Senology. Recommendations. 2020 ; 48 (2020) : 3-1

17. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev. 2010 ;31(5):702-55. doi: 10.1210/er.2009-0041. Epub 2010 Jun 23. PMID: 20573783.

18. Nana PN, Wandji JC, Fomulu JN, Mbu RE, Leke RJI et Woubinwou MJ. Psycho-Social Aspects Among Infertile Patients at the Main Maternity Unit of the Central Hospital of Yaoundé, Cameroon. Clinics in Mother and Child Health. 2011; 8 (2011): 1-5.

19. Neerja Jain K. Role of laporoscopy-hysteroscopy in cases of infertility with pregnancy outcome. J Indian Med Assoc. 2014;112 (2):85-6, 88. PMID: 25935960.

20. CNGOF (Collège national des gynécologues et obstétriciens français). Recommandations pour la pratique clinique. Les pertes de grossesse 2014. 2015. ; 621-642. www.ibrahimaidibe.com.

21. Ikechebelu JI, Mbamara SU. Should laparoscopy and dye test be a first-line evaluation for infertile women in south-east Nigeria? Niger J Med. 2011; 20 (4):462-465.

22. Bukar M, Mustapha Z, Takai UI, Tahir A. Hysterosalpingographic findings in infertile women: a seven-year review. Niger J Clin Pract. 2011;14(2):168-170.

23. Coulibaly HB**.** Place of the post-coital test and the spermogram in the assessment of marital infertility at the National Institute of Public Health Research (INSP). 2019. www.keneya.net/fmpos/theses/2009/ med/pdf/09M407.pdf

24. Mai AH, Demmouche A. The prevalence of fetal macrosomia at the specialized gynecology and obstetrics hospital of Sidi Bel Abbès (western Algeria). Journal of Nutrition and Food Sciences. 2014; 4 (3) :272

25. Sogodogo E. mise en place de la surveillance des résistances aux antibiotiques des bactéries responsables d'infections sexuellement transmissibles dans le laboratoire Rodolphe Mérieux de Bamako. [Thèse]. 2014 : 149p.

26. Somo R. Sensitivity to antifungals of Candida spp isolated from digestive candidiasis in HIV-positive subjects in Yaoundé-Cameroon. Health Sci. Dis. 2014; 15(3): 1-6.

27. Bessimbaye N,Via Issakou B, Mbanga D, Valere TFP, Barro N**.** Biochemical Profile and Resistance of Bacterial and Fungal Agents Isolated from Diarrhea in People Living with the Human Immunodeficiency Virus, N'djamena. European Journal of Biomedical and Pharmaceutical Sciences. 2020;3 (7): 56-67.

28. Skiljevic D, Mirkov D, Vukicevic J. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasmaurealyticum*in genital samples collected over 6years at a Serbian University Hospital. Indian J Dermatol VenereolLeprol.2016 ;82 :37-41.

29. Jingjuan Song, Xuanlan Wu, Yingying Kong, Hong Jin, Ting Yang, Xinyou Xie and Jun Zhang. Prevalence and antibiotics resistance of *Ureaplasma* species and *Mycoplasma hominis* in Hangzhou, China, from 2013 to 2019.Frontiers in Microbiology. 2022 ; (Tome 13-2022) : 01-08.

30. Guindo I, Ag Baraïka M, Sangaré MS, Sima M, Ongoiba S, Dao S5and Bougoudogo F. Antibiotic resistance profile of *Mycoplasma hominis* and *Ureaplasma urealyticum* identified in women in Bamako, Mali.Rev Mali Infect Microbiol. 2022; 17 (1): 32-37.