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Cytobacteriological Examination Of Urines And Urinary Tract Infection Seen In The Laboratory Of University Hospital Of Befelatanana

Fidiniana Mamy Randriatsarafara[1], Zafindrasoa Domoina Rakotovao-Ravahatra[2], Miora Koloina Ranaivosoa[3], Andriamiadana Luc Rakotovao[4], Andry Rasamindrakotroka[5].

[1]Fidiniana Mamy Randriatsarafara. Public Health Specialist. Public Health Department of the Faculty of Medicine Antananarivo, Madagascar.

[2]Zafindrasoa Domoina Rakotovao-Ravahatra. Biologist doctor. Laboratory of Joseph Raseta Befelatanana University Hospital Antananarivo, Madagascar.

[3]Miora Koloina Ranaivosoa. Biologist doctor. Laboratory of Biochemistry of Joseph Ravoahangy Andrianavalona University Hospital Antananarivo, Madagascar.

[4]Andriamiadana Luc Rakotovao. Professor in biological hematology. Medical Biology Department of the Faculty of Medicine Antananarivo, Madagascar.

[5]Andry Rasamindrakotroka. Professor of Immunology. Medical Biology Department of the Faculty of Medicine Antananarivo, Madagascar.

Abstract

Background: Urinary tract infections are common in the community and the hospitals. The aims of this study are to describe the socio-demographic characteristics, uropathogen frequencies, and antimicrobial resistance rates of all patients with urinary tract infections.
Methods: This is a prospective and descriptive study of 362 cytobacteriological urine examinations for a period of six months from July to December 2020. This study was carried out in the laboratory of the University Hospital Joseph Raseta Befelatanana Antananarivo in Madagascar.
Results: Among 362 patients, 117 (32.3%) were affected by urinary tract infections. Women (37.1%) were more affected by men (26.3%) (p=0.02). Similarly, patients over 60 years of age (39%)(p=0.04) were the most affected by urinary tract infections. Bacteria were represented by enterobacteria (58.1%), streptococci (8.5%), non-fermenting gram-negative bacilli (3.4%), staphylococci (1.7%) and other microorganisms (28.2%). Concerning the antibiotic resistance, ESBL-producing *Enterobacteriacea* were more resistant to antibiotics than non-ESBL-producing *Enterobacteriaceae* (81.8% versus 60.9% to cotrimoxazole, 72.7% versus 19.6% to ciprofloxacin, 59.1% versus 6.5% to gentamycin and 18.2% versus 10.9% to chloramphenicol). All isolates of non-fermenting gram-negative bacilli were resistant to ticarcillin and gentamycin, 8 (80%) isolates of streptococci to lincomycin and an isolate of staphylococci to penicillin G and tetracycline.
Conclusion: Bacteriological examination of urines and antibiogram were very important to improve the management of patients with urinary tract infections.

Keywords – Urine, Infection, Beta-Lactamase, Antibiotic Resistance.

I. Introduction

A urinary tract infection (UTI) is an infection that affects part of the urinary tract [1]. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known as a kidney infection (pyelonephritis) [2]. Symptoms from a lower urinary tract infection include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder [1]. Symptoms of a kidney infection include fever and flank pain usually in addition to the symptoms of a lower UTI [2]. In the very old and the very young, symptoms may be vague or non-specific [3]. The most common cause of UTI is Escherichia coli, though other bacteria or fungi may sometimes be the cause [4]. In laboratory, cytobacteriological examination of urines is very important to identify the bacteria and to perform the antibiogram. In fact, empirical treatment of UTI should no longer be carried out because multi-resistant bacteria are increasingly numerous today. And only the antibiogram can highlight the sensitivities and resistance of bacteria to different classes of antibiotics. The aims of this study are to describe the socio-demographic characteristics, uropathogen frequencies, and antimicrobial resistance rates of all patients with UTIs.

II. Materials And Methods

This is a prospective and descriptive study of 362 cytobacteriological urine examinations for a period of six months from July to December 2020. This study was carried out in the laboratory of the University Hospital Joseph Raseta Befelatanana Antananarivo in Madagascar. This study includes all urinary samples of patients who have applied for cytobacteriological examination. This study excludes any non-compliant urinary samples such as samples contained in a transport medium other than the laboratory vial (sterile red cap vial). In the laboratory, the urinary samples were cultured in ordinary agar called Uriselect® and incubated in the oven at 37 ° C for 24 hours. After the culture, cytological examination was performed with a kova slide (brand Glassitic® Slide 10 with Grids) to count the leukocytes, erythrocytes, epithelial cells, yeast, crystals, casts parasites… Afterwards, a microscope examination is performed after gram staining to identify bacteria and other microorganisms (yeast, parasite…). After 24 hours, the positivity criteria UTI are the presence of a single type or two types of bacterial colonies ≥ 10^5 CFUs (colony-forming units) per mL and the number of leukocytes ≥ 104/mL [5]. Then, the bacterial colonies are identified by the various bacterial identification tests available in the laboratory. Finally, the antibiogram corresponding to the identified bacterial species is carried out. Resistance to antibiotics was determined by the Mueller / Hinton agar diffusion method, according to the recommendations of the "comité de l'antibiogramme de la société française de Microbiologie" (Société Française de Microbiologie, 2019) [6]. The dependent variable is constituted by the positivity of the microbiological culture. The independent variables consist of the gender, the age, the result of bacteriological culture and the results of antibiogram. The antibiotics tested are represented by penicillins (penicillin G, amoxicillin, ticarcillin), amoxicillin-clavulanic Acid (AMC), 3rd generation cephalosporins (3CG)(ceftazidime, cefotaxime, cefixime), penemes (imipenem), aminoglycosides (gentamycin, tobramycin, amikacin), quinolones (nalidixic acid, ciprofloxacin, levofloxacin), macrolides (erythromycin), lincosamides (lincomycin), cyclins (tetracyclin) , sulfonamides (trimethoprim- sulfamethoxazole : cotrimoxazole) and amphenicols (chloramphenicol). This study was authorized by the director of establishment of the University Hospital of Befelatanana and the Department Head of the laboratory before its implementation. This study respected the notion of anonymity and confidentiality. The data entry and processing was performed on the software Epi-info 3.5.2. The comparison of percentages used the Chi square tests. The statistical significance threshold used was p = 0.05.

III. Results

3.1. Results of the bacteriological urine examinations

Among 362 patients, 117 (32.3%) were affected by UTIs (figure 1).

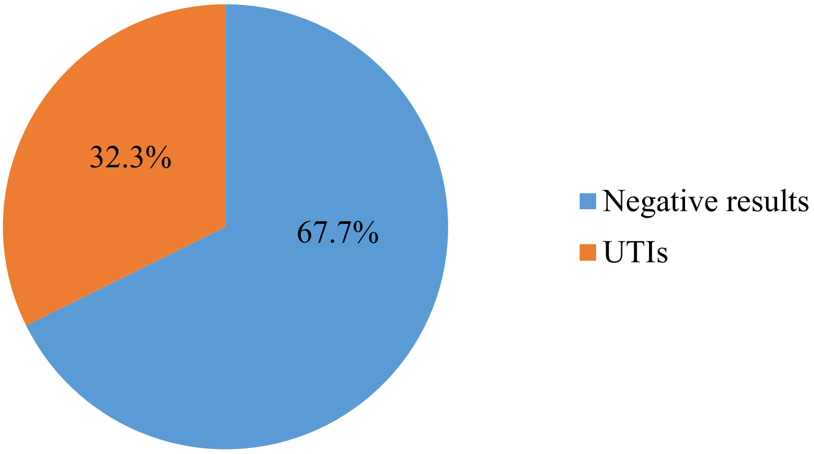

Figure 1: The positivity rate of cytobacteriological urine examinations

Bacteria were represented by enterobacteria (58.1%), streptococci (8.5%), non-fermenting gram-negative bacilli (NFGNB) (3.4%), staphylococci (1.7%) and other microorganisms (28.2%)(figure 2).

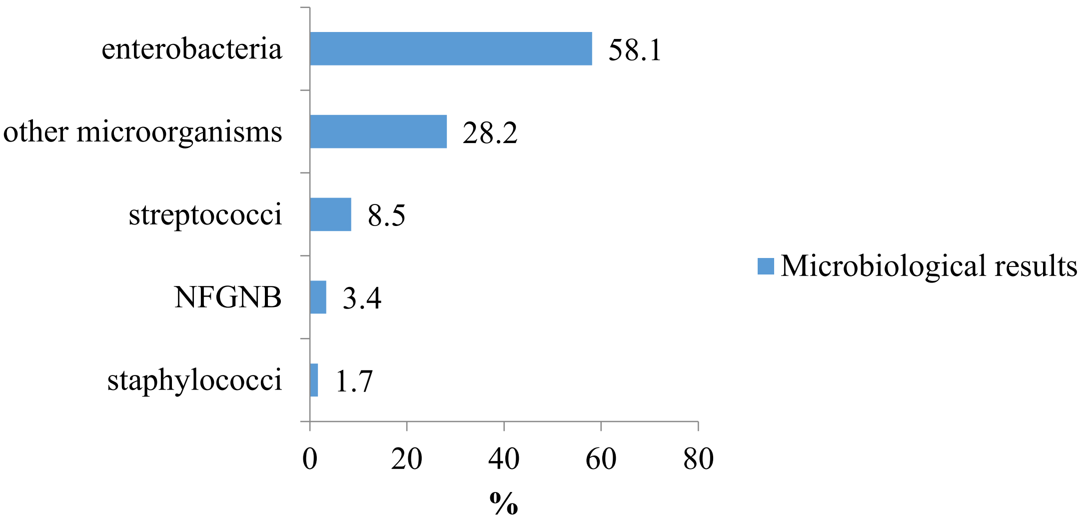

Figure 2: Microbiological results of cytobacteriological urine examinations

Enterobacteria were represented by *Escherichia coli* (60%), *Klebsiella penumoniae* (20.2%), *Proteus mirabilis* (4.6%) and othes enterobacteria (15.2%). 70% of streptococci were represented by *Enterococcus spp*. And NFGNB were represented by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

3.2. Socio-demographic factors associated with UTI

Women (37.1%) were more affected by men (26.3%) (p=0.02). Similarly, patients over 60 years of age (39%)(p=0.04) were the most affected by UTIs (Table 1).

Table 1: Socio-demographic factors associated with UTI

	Negative results (N=245)		UTIs (N=117)		Total	p-value
	n	%	n	%		
Age (years)						
< 20	51	63.0	30	37.0	81	0.04
20 - 60	130	73.9	46	26.1	176	
> 60	64	61.0	41	39.0	105	
Gender						
Female	127	62.9	75	37.1	202	0.02
Male	118	73.8	42	26.3	160	

3.3. Results of antibiogram

Concerning the antibiotic resistance, Extended-Spectrum Beta-Lactamases (ESBLs) - producing *Enterobacteriaceae* were more resistant to antibiotics than non-ESBL –producing *Enterobacteriaceae* (81.8% versus 60.9% to cotrimoxazole, 72.7% versus 19.6% to ciprofloxacin, 59.1% versus 6.5% to gentamycin and 18.2% versus 10.9% to chloramphenicol)(figure 3).

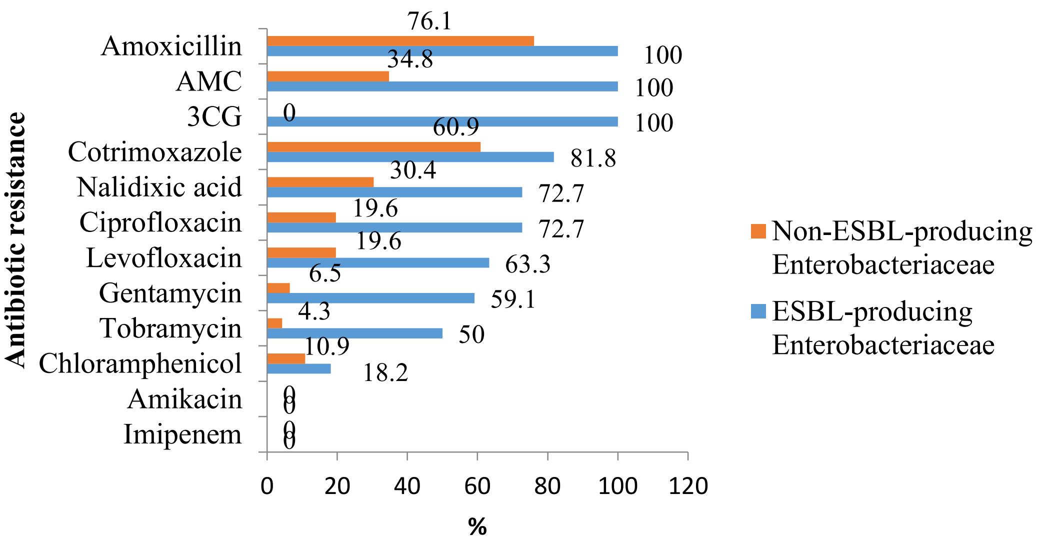

Figure 3: Antibiotic resistance of enterobacteria

All isolates of non-fermenting gram-negative bacilli were resistant to ticarcillin and gentamycin (figure 4).

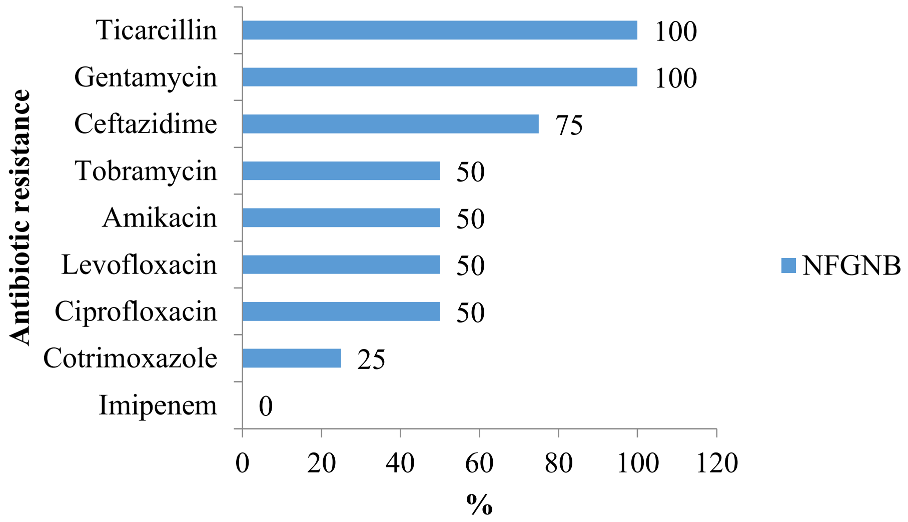

Figure 4: Antibiotic resistance of NFGNB

8 (80%) isolates of streptococci were resistant to lincomycin (figure 5).

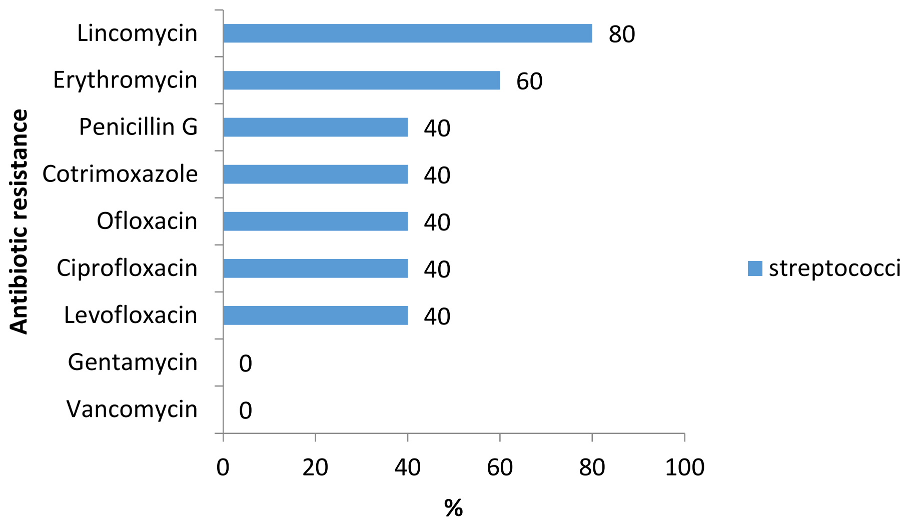

Figure 5: Antibiotic resistance of streptococci

And an isolate (1/2) of staphylococci was resistant to penicillin G and tetracycline.

IV. Discussion

4.1. Results of the bacteriological urine examinations

UTIs are among the most frequent infectious diseases affecting humans, and represent an important public health problem with a substantial economic burden. Our study found 32.3% UTI prevalence among patients who performed a cytobacteriological examination of urines. Odoki and al also found the same result in Uganda (32.2% UTI prevalence) [7]. UTI may be asymptomatic, acute, chronic, and complicated or uncomplicated, and the clinical manifestations of UTIs depend on the portion of the urinary tract involved, the etiologic organisms, the severity of the infection, and the patient's ability to mount an immune response to it. Both asymptomatic and symptomatic UTIs pose a serious threat to public health care, hence reducing the quality of life and resulting into work absenteeism [7]. Thus, the cytobacteriological examination of urines is very important to early diagnosis UTI for ovoid complications. This examination should be performed as soon as a urinary tract infection is suspected.

Concerning the uropathogens, enterobacteria were the most frequent in this study with a predominance of isolates of *Escherichia coli*. Other studies also found the same results [8-4].

This study also found isolates of other enterobacteria (*Klebsiella penumoniae*, *Proteus mirabilis*…), streptococci (*Enterococcus spp, Streptococcus spp*…), NFGNB (*Pseudomonas aeruginosa, Acinetobacter baumannii*), staphylococci (*Staphylococcus aureus*, non aureus staphylococci) and other microorganisms. According to a study carried out by Odoki et al in Uganda, *Escherichia coli* was the most prevalent bacterial uropathogen with 36/86 (41.9%) followed by *Staphylococcus aureus* 27/86 (31.4%), *Klebsiella pneumoniae* 10/86 (11.6%), *Klebsiella oxytoca* 6/86 (7.0%), *Proteus mirabilis* 3/86 (3.5%), *Enterococcus faecalis* 3/86 (3.5%), and *Proteus vulgaris* 1/86 (1.2%) [7]. Similarly, Al-Badr et al found that *Proteus mirabilis*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, and *Klebsiella pneumonia can cause UTIs [8].* The presence of these microorganisms in the urine depends on the patient's condition or disease. In fact, in diabetic patients, *Klebsiella* and group B *streptococcus* infections are more common. *Pseudomonas* infections are more common in chronically-catheterised patients [8]. Thus, the cytobacteriological examination of urines is very important to identify these different species responsible for UTI because the antibiotic treatment depends on the bacteria identified. Thanks to this examination, the patient will be well treated and complications will be avoided.

4.2. Socio-demographic factors associated with UTI

In this study, women were more affected by UTIs than men with a significant difference. Other studies found the same results [8, 9]. According to a study carried out by Al-Badr et al, UTIs are one of the most frequent clinical bacterial infections in women, accounting for nearly 25% of all infections. Around 50–60% of women will experience a UTI in their lifetime. The estimated number of UTIs per person per year is 0.5 in young females. Recurrences usually occur within three months of the original infection, and 80% of recurrent UTIs are reinfections. The incidence of UTI increases with age and sexual activity. Post-menopausal women have higher rates of UTIs because of pelvic prolapse, lack of oestrogen, loss of *lactobacilli* in the vaginal flora, increased periurethral colonisation by *Escherichia coli*, and a higher incidence of medical illnesses such as diabetes mellitus. The microorganism that causes recurrent UTIs is similar, in most cases, to the sporadic infection. Most uropathogens from the rectal *flora* ascend to the bladder after colonising the periurethral area and urethra [8].

Concerning the age, patients over 60 years of age were the most affected by UTI in this study. Other studies found the same results [7, 10]. In fact, the prevalence of UTI has been shown to increase with age. Anatomic and functional theories to explain this phenomenon include bladder dysfunction, pelvicorgan prolapse, urinary and fecal incontinence, and changes in estrogen status [10]. Similarly, there are other risk factors for UTI. Several factors such as race, circumcision, HIV, diabetes, urinary catheter, genitourinary tract abnormalities, pregnancy, infants, and hospitalization status bear significant risk for recurrent UTIs [7]. In fact, the study carried out by Odoki et al has demonstrated that age ≤19 years, female gender, married individuals, genitourinary tract abnormalities, diabetes, hospitalization, indwelling catheter <6 days, and indwelling catheter >6 days had statistically significant relationships with UTI. Screening for UTI in hospitalized patients, female gender, married individuals, genitourinary tract abnormalities, indwelling catheter, and diabetics should be adopted [7]. In short, the cytobacteriological examination of urines must be carried out systematically in any patient hospitalized with or without risk factor after a few days of hospitalization.

4.3. Results of antibiogram

Concerning the antibiotic resistance, this study found ESBL-producing *Enterobacteriaceae*. Due to the high empiric use of antibiotics for the treatment of UTI, antibacterial resistance of *Enterobacteriaceae*, specifically the main uropathogens *Escherichia coli* and *Klebsiella* pneumoniae, has significantly increased worldwide. The increased prevalence of multi-drug-resistant (MDR) *Enterobacteriaceae*, limiting available treatment options for infections caused by these organisms, and the lack of new antibiotics provide good rationale for using older antibiotics, such as fosfomycin, that have been shown to retain some activity against MDR bacteria [11]. The diagnosis of these β-lactamases is of clinical and epidemiological interest. Over the past 10 years, several methods have been developed aiming to rapidly detect these emerging enzymes, thus preventing their rapid spread [12]. Excessive uses of third-generation cephalosporins, which are commonly used as an empiric and definitive therapy in hospitals, are expected to trigger the emergence and spread of ESBL producers [13].

In this study, ESBL-producing *Enterobacteriaceae* were more resistant to antibiotics than non-ESBL-producing *Enterobacteriaceae*. In addition to their resistance to majority of the β-lactam agents, the ESBL producing isolates were also more resistant to other classes of antibiotics such as cotrimoxazole, gentamycin, ciprofloxacin and chloramphenicol, than the non ESBL producing isolates. Indeed, ESBL-producing *Enterobacteriaceae* are frequently found in hospital and are responsible for several nosocomial infections [14]. These antibiotic resistant bacteria had emerged under the pressure of frequent use and misuse of certain antibiotics in the hospitals. The fluoroquinolone (ciprofloxacin) use and misuse, which is also frequent, has been identified as a risk factor for other infections caused by ESBL-producing *E. coli*, probably as a result of co-selection [15]. Otherwise, the empiric use of 3rd and 4th generation cephalosporins should be curtailed, as cephalosporin use was associated with an increased risk of ESBLs production. The empiric use of cephalosporins associated with use of cotrimoxazole increase the MDR of these ESBL-producing *Enterobacteriaceae*. Indeed, cotrimoxazole is used frequently by the Malagasy population and it is sold in small grocery stores. The self-medication reinforces this high resistance [16].

In view of their excellent invitro activity, carbapenems should be the initial empiric choice for serious life threatening UTIs caused by ESBLs-producing *Enterobacteriaceae*, with prompt de-escalation when culture and susceptibility results become available [16]. Indeed, no strain of ESBLs was resistant to carbapenems in this study, in particular, to imipenem. Similarly, all enterobacteria isolates tested in this study were susceptible to amikacin. Another study in Douala also found that imipenem (1.3% of resistance) and amikacin (12.9% of resistance) are the most effective antibiotics against enterobacteria [17]. These data suggest that penems (imipenem, meropenem) and aminoglycosides especially amikacin could be an effective choice in ESBLs-producing *Enterobacteriaceae*. The other aminoglycosides like gentamicin and tobramycin are less effective because they are used frequently in the hospital resulting in the antibiotic resistance of enterobacteria [16].

Concerning the NFGNB (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*), all isolates were resistant to ticarcillin and gentamycin. Indeed, these bacteria have been isolated from soil, aquatic environment, and hospital environments such as anesthesia equipment, sinks, intravenous fluids, and even distilled water. Their survival in different hospital settings facilitated them to transfer from patient to patient through fomites or the hands of medical staff causing hospital acquired infections [18]. So, these NFGNB were resistant to ticarcillin and gentamycin because these antibiotics are frequently used in hospitals. Similarly, another study found 60% resistance to gentamycin of *Acinetobacter baumannii* in hospital [19]. Otherwise, penicillin is used frequently by the Malagasy population and it is sold in small grocery stores like cotrimoxazole. This situation may explain the high resistance of NFGNBs and other bacteria to penicilin, in particular ticarcillin.

Concerning the isolates of streptococci, 80% were resistant to lincomycin. In fact, the majority of streptococci isolates were represented by *Enterococcus spp*. In literature, 72% to 99% of *Enterococcus* species were resistant to lincomycin [20]. According to the study carried out by Cho et al, lnu(B) was the only gene detected for lincomycin resistance. However, Only a small portion of the lincomycin-resistant isolates (18.4%) were positive for the gene, suggesting that there may be mechanisms for lincomycin resistance among enterococci other than lnu(B) [21].

Concerning the isolates of staphylococci, an isolate was resistant to penicillin G and tetracycline. As before, these classes of antibiotics are used frequently by the population and are sold in small grocery stores. These situations increase the resistance of staphylococci to these antibiotics.

V. Conclusions

Women and patients over 60 years of age were the most affected by UTI. A cytobacteriological examination of urines should be performed as a first line for a woman or an elderly subject with urinary signs associated or not with a fever. Similarly, this examination should be performed for hospitalized patients. Otherwise, the ESBL –producing *Enterobacteriaceae* responsible for UTI were more resistant to antibiotics than non-ESBL-producing *Enterobacteriaceae*. In brief, cytobacteriological examination of urines and antibiogram were very important to improve the management of patients with UTI.

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