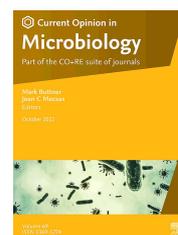




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REVIEW

Bacteriological profile and antibiotic resistance of isolated urogenital tract bacteria at Laboratoire National de Santé Publique de Brazzaville in 2021



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Abstract

Urogenital tract infections (UTIs) are one of the most common infections in men at different stages or phases of their lives. They are increasingly a reason for consultation in hospitals, both inpatient and outpatient. The emergence of antibiotic-resistant bacterial strains is a serious problem and the greatest challenge in public health care. The present study aimed to assess the bacteriological profile and antibiotic resistance of bacteria isolated from the urogenital tract of different patients. A total of 483 positive urine samples and 83 positive semen samples were obtained. Findings revealed that *Staphylococcus aureus* and *E. coli* were the most isolated species, with rates of 31.57%

and 28.94%, respectively. Thus, the analysis of β -lactam resistance phenotypes in the *Enterobacteriaceae* showed 76 strains (28%), presenting positive synergy tests characteristic of ESBL-producing bacteria. This phenotype was found in four (4) bacterial species, including *E. cloacae* (57.4%), *K. pneumoniae* (35.71%), *E. coli* (21.42%), *C. kozeri* (20%), and *K. ozanae* (10%). The carbapenemase phenotype was found in all *Enterobacteriaceae* except *C. freundii*. The penicillinase phenotype frequencies were distributed as follows: *E. coli* (56.34%), *P. mirabilis* (33.33%), *E. cloacae* (25%), *C. kozeri* (30%), *C. freundii* (15%), *P. vulgaris* (13.33%), *K. pneumoniae* (12%), and *K. ozanae* (2%).

The frequency of isolation of bacteria producing low levels of cephalosporinase has been observed at a high rate in *P. mirabilis* (60%) and *E. coli* (33.75%). The low rate was observed in *E. cloacae* (19.73%), *P. vulgaris* (6.66%), *K. pneumoniae* (6%), *K. ozanae* (6%), *C. kozeri* (5%), *E. aerogenes* (2.63%) and *C.*

Key words: ESBL; Penicillinase; Cephalosporinase; Carbapenemase; Bacteria; Brazzaville.

1. Introduction

Urinary tract infections (UTIs) constitute one of the most common infections in men at different stages of their lives. On one hand, sexual activity as well as the misuse of intimate hygiene products that interfere with the normal flora of the woman's vagina are often blamed. On the other hand, the proximity of the anus eases colonization of the reproductive organs and distal parts of the urinary tract by *Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus* species [1]. Pregnancy and the perinatal period are other characteristics of frequent UTIs. The increasing number of cesarean sections and perioperative catheterizations is another risk factor for urinary tract infections [1]. The Enterobacteriaceae are a large family of gram-negative bacteria with about 140 species grouped into 33 genera [2]. These bacteria are found in water, soil, and especially in the digestive tracts of humans and animals [3]. Due to their distinct pathogenicity mechanisms and tropisms, these bacteria are strongly implicated in many infections (community and nosocomial), including urogenital infections [4]. Nowadays, urogenital infections are the second most common infection encountered in hospitals and communities [4]. For anatomical reasons, the female gender is the most affected, with at least 50% of women reporting having had at least one urogenital infection in their

freundii (0%). Then, high level cephalosporinase was only observed in *E. coli* (11.82%), *E. cloacae* (6.58%), *K. pneumoniae* (6%), and *K. ozanae* (6%). Therefore, all the bacterial species isolated showed a high level of bacterial multidrug resistance with rates above 50%.

lifetime [4]. This is due to the relatively small size of the female urethra, which reduces the distance and time for bacterial colonization. Bacteria of the genera *Escherichia*, *Proteus*, and *Klebsiella* are the most isolated in UTIs; however, in the Republic of Congo, there are no published statistics available on this issue. Indeed, this work is the first at the national level. The fight against Enterobacteriaceae is essentially based on antibiotic therapy and strict hygiene measures. With the emergence of numerous antibiotic-resistant strains, it is necessary to produce a new generation of antibiotics that target antigenic determinants that are essential for eradicating infections caused by these bacteria. On the other hand, it is necessary to monitor the epidemiological evolution of these emerging strains in order to control the degree of sensitivity to antibiotics of the bacteria commonly prescribed, particularly in the Republic of Congo. The appearance and emergence of strains that are multi-resistant to antibiotics is a real public health problem [4]. Fluoroquinolones, which were initially the first-line antibiotic family in the fight against these infections, are now being used due to the emergence of strains resistant to this family of antibiotics [5]. There is a wide range of antibiotics used to treat UTIs caused by *E. coli*, *K. pneumoniae*, and *P. mirabilis* [6–9], one of

the three representatives of the Enterobacteriaceae family. More recent studies have shown that Enterobacteriaceae are producers of β -lactamase. This enzyme makes them capable of resisting most antibiotics of the β -lactam family, hence their name "ESBL-producing organisms. Carbapenem is the only antibiotic of choice used to treat these microorganisms [11]. In this study, the epidemiology of community-

acquired urogenital infections in Brazzaville was analyzed over a period of one year. In addition, we were interested in the bacteria isolated from urine and sperm samples. We assessed the susceptibility profiles of isolated Enterobacteriaceae. We then compared the emergence rate of so-called ESBL strains with that of non-ESBL strains isolated at the Laboratoire National de Santé Publique de Brazzaville.

2. Methods

Samples collection

From January to December 2021, a total of 918 urine and sperm samples were collected from outpatients referred to the Laboratoire National de Santé Publique de Brazzaville (Republic of Congo). The samples were collected in sterile urine containers, and analyzed in a period not exceeding 2 hours in order to avoid the overgrowth of bacteria in the urine and sperm.

Urine specimens were collected from male (n = 474) and female (n = 444) outpatients. Sperm specimens were collected from 131

outpatients. Collected specimens were divided based on the age of outpatients into nine different groups: (<10 years, 10-20 years, 20-30 years, 30-40 years, 40-50 years, 50-60 years, 60-70 years, 70-80 years, and 80-90 years. Midstream urine was collected in sterile conditions in order to decrease potential artifact, bacterial, and cellular contamination. All tests were performed in the medical bacteriology department of our laboratory.

Isolation

The various urine and sperm specimen samples were seeded on selective agar media. The following media were used:

- Eosin Methylene Blue Agar (EMB) for the isolation of *Enterobacteriaceae*;
- Ceftrimide Agar for *Pseudomonas sp* isolation;

- BAE Agar for *Enterococcus sp* isolation and Chocolate Agar + Polyvitex for the isolation of *Neisseria gonorrhoeae*;
- Mannitol Salt Agar for the isolation of *Staphylococcus sp*.

Strain identification

An Enterobacter System gallery, Intergral System Stafilococchi and an Integral System Streptococchi gallery comprising

several biochemical reactions were used for the identification of Enterobacteriaceae, Staphylococcus, and Streptococcus strains.

Antibiotic susceptibility testing of isolates

Patterns of antimicrobial resistance were studied using the standard Mueller Hinton (Merck, Germany) diffusion susceptibility testing method [12]. EUCAST guidelines were used for this purpose. Antibiotic resistance pattern of the strains isolated from the urine and sperm specimens was assessed against 34 commonly used antibiotic agents, including ampicillin (10 µg), imipenem (30 µg), nalidixic acid (30 µg), cefoxitin (30 µg), cefalexin (30 µg), amikacin (30 µg), kanamycin (30 µg), netilmicin (10 µg), gentamicin (10 µg), tigecycline (15 µg), cefepime (30 µg), ticarcillin (75 µg), piperacillin (30 µg), ceftazidime (10 µg), oxacillin (5 µg), amoxicillin + clavulanic acid (30 µg),

streptomycin (5 µg), cefepime (30 µg), cefotaxim (5 µg), ertapenem (10 µg), cotrimoxazole (30 µg), tetracycline (30 µg), pefloxacin (75 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg), piperacillin (30 µg), ceftriaxone (30 µg), doxycycline (30 µg), azithromycin (15 µg), meropenem (10 µg), lincomycin (15 µg), erythromycin (15 µg), clarithromycin (15 µg), minocycline (30 µg), penicillin (10 µg), doripenem (10 µg) and tobramycin (10 µg) (Oxoid, UK). All of the inoculated plates were incubated aerobically at 37 °C for 18–24 h. The diffusion diameters of the different antibiotics were measured and compared to the reference diameters of EUCAST [13].

Determination of Resistance Phenotypes

The resistance phenotype was determined by reading the antibiogram, which identifies the clinical types (sensitive, resistant, and

intermediate). The resistance phenotype is the group of antibiotics to which the strain is resistant.

Identification of the ESBL-producing isolates

Bacterial suspensions with a concentration of 1.5×10^8 CFU/mL (0.5 McFarland standard) were prepared. The combination disk method was used for detection of ESBL producing *E. coli* strains; these strains were cultured on Muller-Hinton agar plates (Merck, Germany), and then cefotaxim (30µg), cefotaxim/clavulanic acid (30/10 µg), ceftazidime (30 µg), and ceftazidime/clavulanic acid disks (30/10

µg) were placed on each support at a distance of 25 mm between them and 16 mm with the walls of the plates. The plates were incubated for 18–24 h at 37°C. ESBLs producing *E. coli* were detected by an increase of at least 5 mm in the zone of inhibition around the discs containing clavulanic acid compared to those without clavulanic acid.

Statistical analysis

Statistical analysis was done using Excel 2013 software. The chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between

the prevalence of isolated bacterial strains and their antibiotic resistance profiles. A P value <0.05 was considered a statistically significant level.

Ethical Approval and Consent to Participate

As this study involved the analysis of retrospective data from routine records, the need for informed consent was waived, and

data confidentiality was maintained throughout the study.

3. Results

This study included 918 urine samples and 131 sperm samples analyzed in the bacteriology department of the Laboratoire National de Santé Publique de Brazzaville during the period of January to December 2021. Out of these 918 urine samples, 483

showed positive cultures, i.e., a rate of 52.61% with a ratio of approximately 50% for men and women. From the 131 sperm samples analyzed, 82 were positive, a rate of 64.88%. All samples (urine and sperm) tested were of community origin (Figure 1).

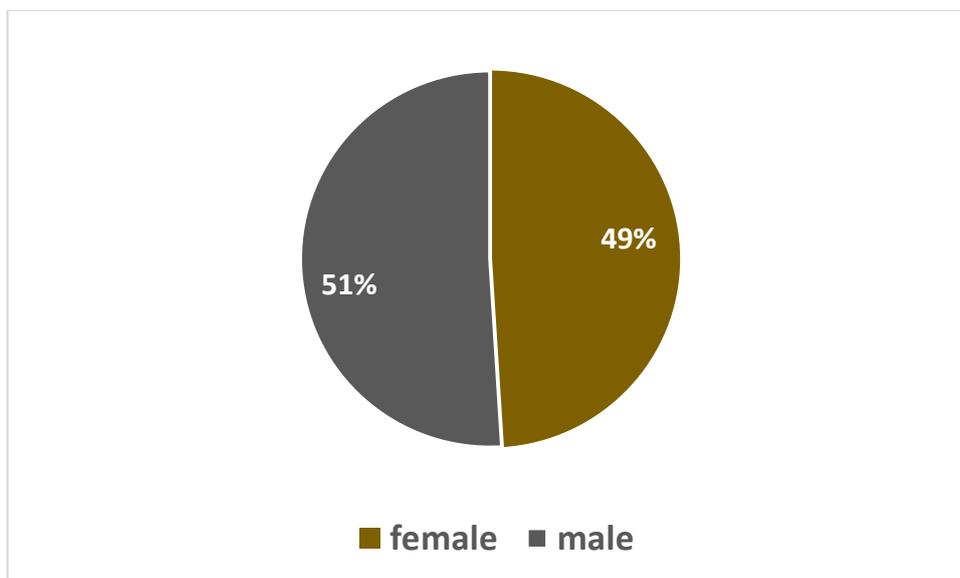


Figure 1: Distribution of urine samples by gender

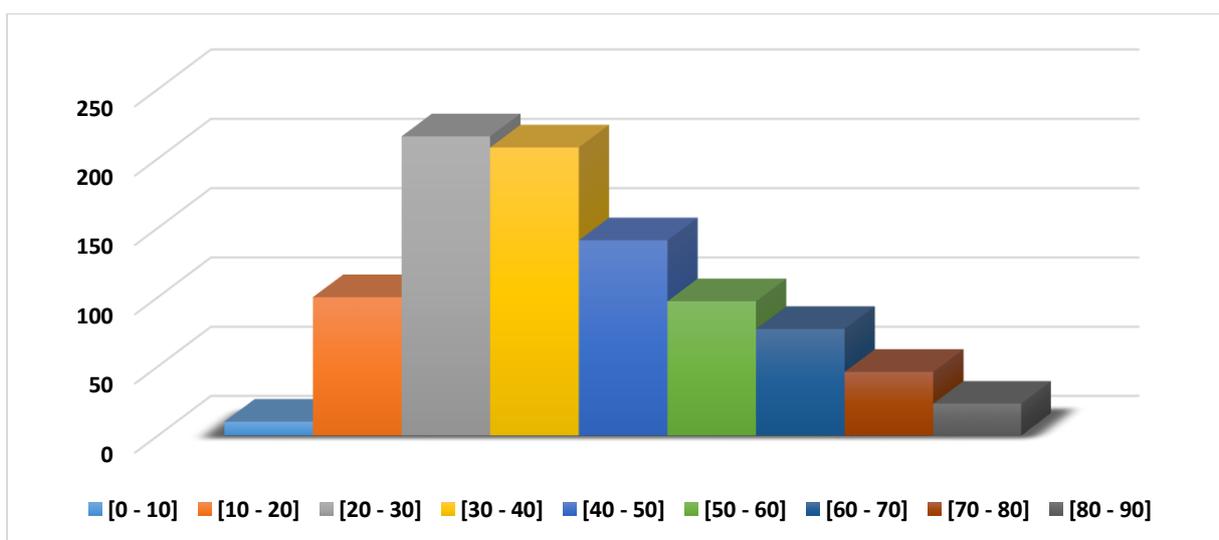


Figure 2: distribution of urine samples by age range.

The samples analyzed during this study were taken from patients whose predominant age group was between 20 and

40 years old. The mean age was 33 years, and the extremes were 11 months and 88 years (Figure 2).

Frequency of bacterial isolation

Overall, ten (10) bacterial genera were isolated in this study and *Staphylococcus* was the most prevalent genus with a frequency of 42.37%. Specifically, the isolation of germs from the 483 positive urine samples showed ten (10) different bacterial genera distributed as follows: *Staphylococcus* (34.78%), *Escherichia* (22.74%), *Enterobacter* (17.39%), *Klebsiella* (8.69%), *Enterococcus* (6.35%), *Proteus* (4.68%), *Citrobacter* (3.01%), *Pseudomonas* (1.0%), *Streptococcus* (0.67%) and *Neisseria* (0.67%) (Figure 3a). *E. coli* was the most isolated species of bacteria belonging to the Enterobacteriaceae family (Figure 3b).

Similarly, out of the 81 positive sperm samples collected during this period, eight (8) different bacterial genera were identified and distributed as follows: *Staphylococcus* (70.37%), *Escherichia* (22.47%), *Enterobacter* (2.47%), *Klebsiella* (2.47%), *Enterococcus* (1.23%), *Proteus* (1.23%), *Citrobacter* (1.23%), *Streptococcus* (1.23%) (Figure 3a). The findings obtained after identification

revealed the presence of several species in varying frequencies. Generally, *S. aureus* was the most isolated bacterium during this study (Figure 3b). However, as regards Enterobacteriaceae, *Escherichia coli* species with a rate of 28.94% was the most isolated species followed by *E. cloacae* (14.74%). The species *P. vulgaris* with a rate of 1.32% was the least isolated species of this family. Other bacteria of this family were isolated, such as: *Klebsiella pneumoniae* (7.89%), *K. ozanae* (5.26%), *E. aerogenes* (5.26%), *C. kozeri* (3.16%), *P. mirabilis* (2.63%) and *C. freundii* (2.11%) (Figure 3b).

Therefore, concerning the genus *Staphylococcus*, *S. aureus* was the most isolated bacterium with 31.57%, followed by *S. saprophiticus* (10.79%) and *S. epidermidis* (5.26%). Other bacteria of the genus *Streptococcus agalactiae* (1.57%), *Enterococcus faecalis* (5.26%), *Pseudomonas aeruginosa* (0.79%), and *N. gonorrhoeae* (at 0.53%) were isolated at different frequencies (Figure 3b).

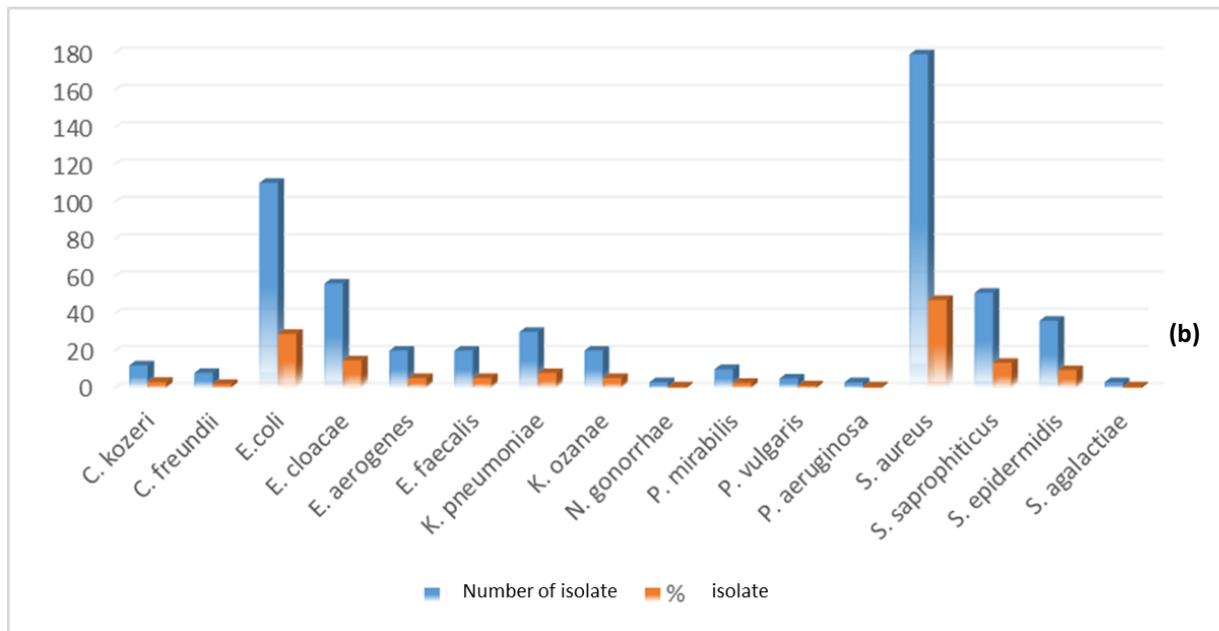
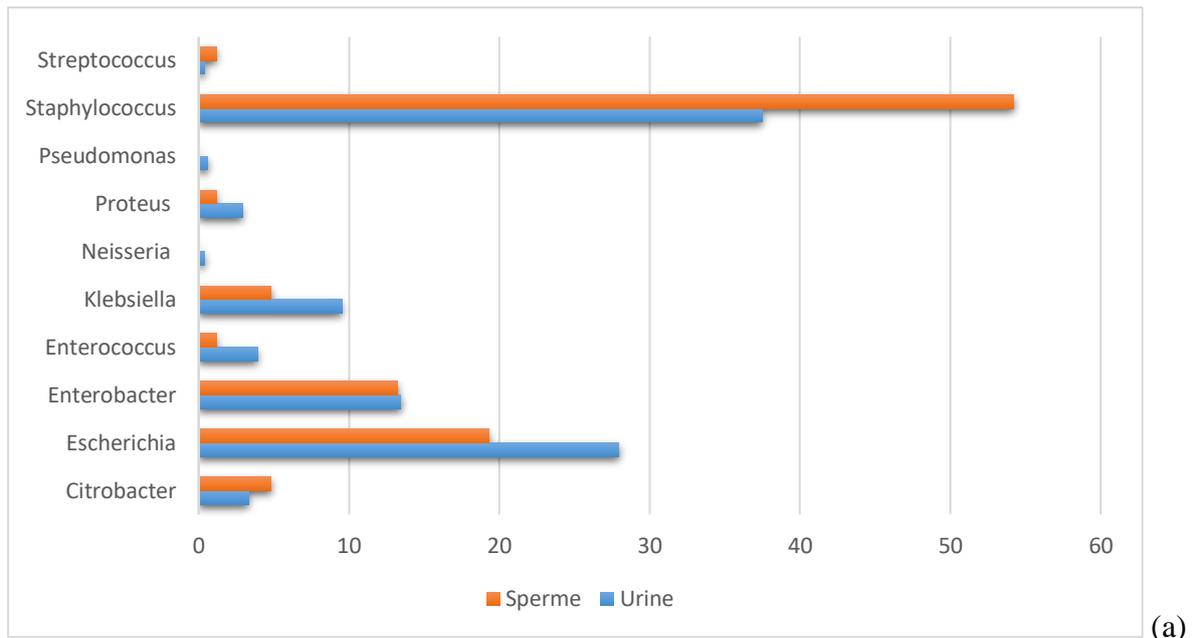


Figure 3: frequency of isolation of strains: **(a):** according to the pathological products; **(b)** according to the overall number of the species isolated from urine and sperm.

Antibiotic susceptibility of enterobacterial strains isolated from urine and spermatozoa **Betalactam resistance**

Concerning the β -lactam antibiotics tested, high resistance to imipenem was observed with *C. kozeri* (70%), *P. mirabilis* (66.66%), and *E. coli* (63.75%). However, *K. pneumoniae* species showed a low rate

of resistance to imipenem of about 8%. Ticarcillin resistance was 15.71% and 16% for *E. coli* and *K. pneumoniae*, respectively. It was also shown in this study that resistance to Cefoxitin was variable and

significantly different between the bacterial species isolated and distributed as follows: *E. cloacae* (16.7%), *P. mirabilis* (13.33%),

Quinolone and fluoroquinolone resistance

Concerning the overall resistance to quinolones, this study reveals that it is variable. Generally, *Enterobacter aerogenes* was the most resistant strain to quinolones, with a rate of 68.33%. Resistance to

Resistance to aminoglycosides

The aminoglycoside molecules showed very good activity on all studied strains. However, resistance rates of 60 and 30% were observed in *E. aerogenes* with

Resistance to cyclins

The tetracycline molecules tested showed very good activity in all strains tested. The overall resistance of the cyclins was very low and variable depending on the species. The highest resistance was observed in *P. mirabilis* and *C. kozeri*, at around 5%. *E.*

Resistance to macrolide drugs

The bacteria of the *Enterobacteriaceae* family studied, showed a very high sensitivity to the antibiotics of this family

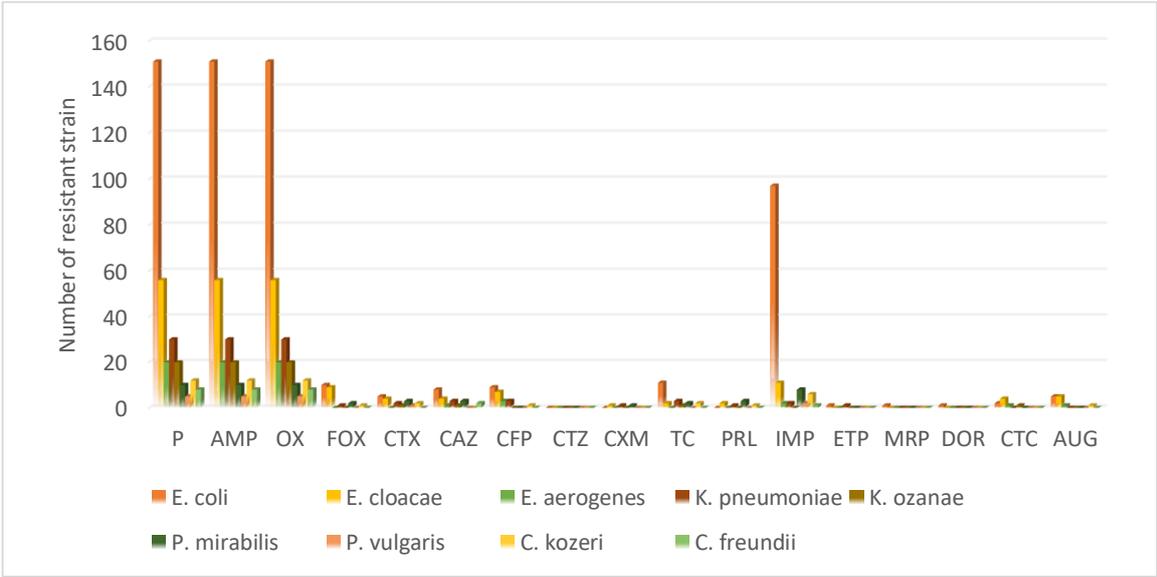
C. kozeri (14.28%), *E. coli* (6.25%), and *K. pneumoniae* (3.33%) (Figure 4a).

Norfloxacin and Pefloxacin was 10.87% and 20% with *E. cloacae* and *E. coli* strains, respectively. In contrast, all *Proteus* strains were sensitive to this family of antibiotics (Figure 4b).

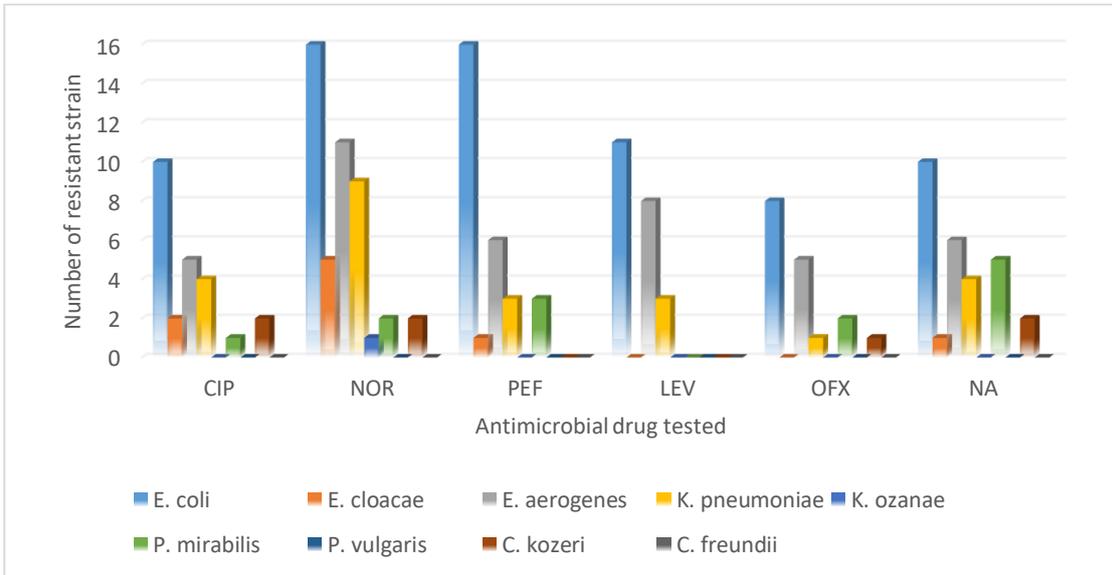
tobramycin and amikacin, respectively. Figure 4c illustrates the number of aminoglycoside resistant strains observed during this period.

coli (2.81%); *E. cloacae* (1.97%); *P. vulgaris* (1.66%); *E. aerogenes* (0.32%); *K. pneumoniae* (2.5%); and *K. ozanae* (0.5%). *C. freundii* showed no resistance to cyclins (Figure 4d).

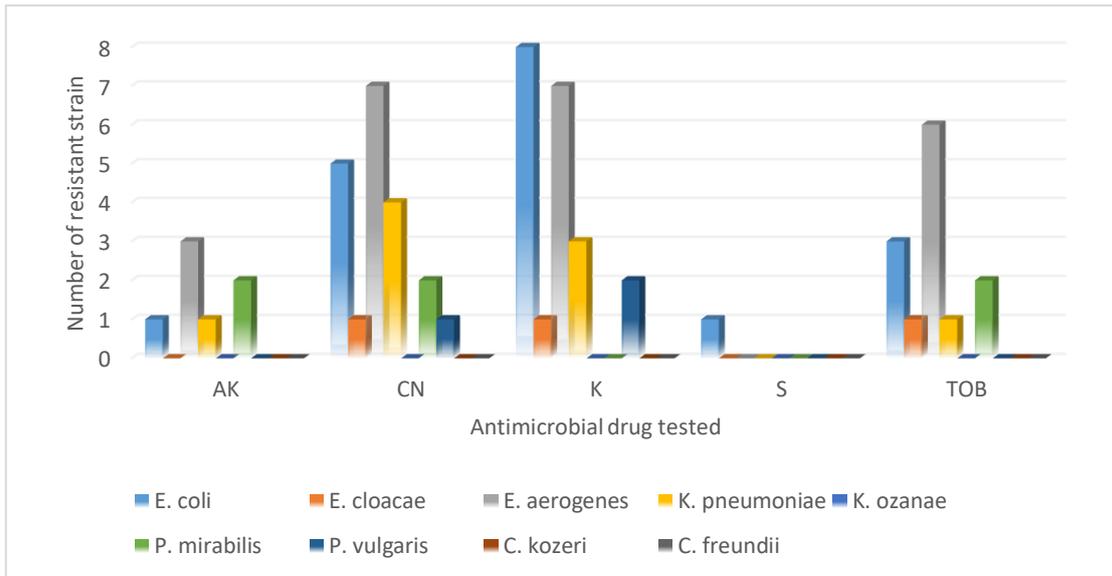
(Figure 4e); therefore, no significant resistance was observed in any of the isolated species regarding macrolide drugs.



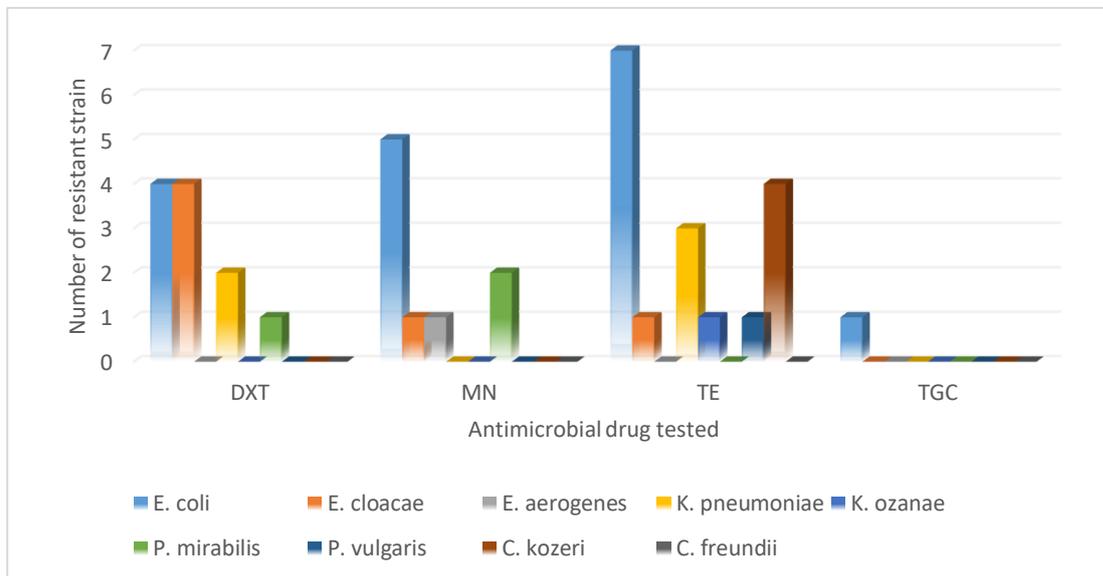
(a)



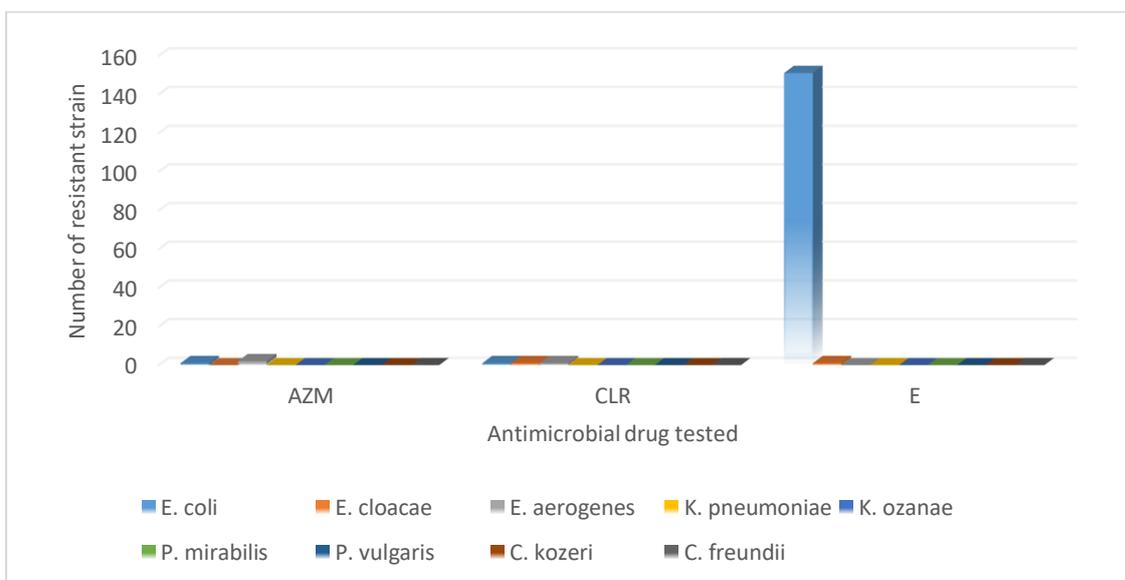
(b)



(c)



(d)



(e)

Figure 4: Antibiotic resistance profile of *Enterobacteriaceae* isolated **(a):** Resistance profile to beta-lactam antibiotics; **(b):** Resistance profile to quinolone antibiotics; **(c):** Resistance profile to aminoglycoside

Legend: **AT:** Aztreonam; **AML:** Amoxicillin; **FOX:** Cefoxitin; **FOS:** Fosfomycine, **FEP:** Cefepime, **AK:** Amikacin; **TGC:** Tigecycline; **CN:** Gentamicin; **CIP:** Ciprofloxacin. **NOR:** Norfloxacin **ETP:** Ertapenem; **NA:** Acide Nalidixique; **PRL:** Piperacillin; **AUG:** Amoxicilline + Acide Clavulanique; **CAZ:** Ceftazidime; **IMI:** Imipenem; **CTX:**

antibiotics; **(d):** Resistance profile to cyclin antibiotics; **(e):** Resistance profile to macrolide antibiotics. Natural resistance to antibiotics has been reported in the documents.

Cefotaxime; **CFP:** Cefepime; **LEV:** Levofloxacin; **TC:** Ticarcilline; **TE:** Tetracycline; **TGC:** Tigecycline; **NET:** Netilmicin; **TOB:** Tobramycin; **K:** Kanamycin; **S:** Streptomycin; **CLR:** Clarithromycine; **PEF:** Pefloxacin; **OX:** Oxacillin; **P:** Penicillin; **E:** Erythromycin; **AZM:** Azithromycin; **OFX:** Ofloxacin; **AMP:** Ampicillin.

Susceptibility to antibiotics of non-*Enterobacteriaceae* isolated from urine and spermatoc fluids

Five (5) isolated non-*Enterobacteriaceae* groups were also tested for antibiotic susceptibility.

Resistance to beta-lactams

Betalactam resistance by species isolated is distributed as follows: *S. aureus* (7.4%), *S. epidermidis* (2.35%), *S. saprophiticus* **Enterococcus**

The *E. faecalis* strains isolated showed about 70% resistance to penicillin, Ampicillin and Oxacillin. Amoxicillin +

(2.8%), *P. aeruginosa* (5.88%), *E. faecalis* (18.24%), *S. agalactiae* (21.57%), and *N. gonorrhoeae* (26.47%) (Figure 5a).

Clavulanic acid was the most active antibiotic for these strains with a resistance rate of 10%.

Staphylococcus

The resistance conferred to the isolated species of *Staphylococcus* was distributed as follows: *S. aureus* (7.4%), *S. saprophiticus* (2.8%), and *S. epidermidis* (2.35%). Resistance of *S. aureus* to ampicillin and Oxacillin was 4.16% and 8.33%, respectively. In this research, only

Streptococcus:

The different isolated strains of *S. agalactiae* were all 100% resistant to Penicillin, Ampicillin and Oxacillin. A

8.33% of the strains were of the Meti R phenotype (oxacillin resistance): *S. aureus* Meti R (MRSA). Cefoxitin resistance was also a key marker for the detection of MRSA. These bacteria were isolated at a rate of 10.56% (Figure 6c) of all *Staphylococcus* isolated during this period.

resistance of 66.66% was observed with Cefepim and no resistance observed with the other antibiotics tested during this study.

Other bacteria: *P. aeruginosa* et *N. gonorrhoeae*

Resistance to Penicillin, Ampicillin and Oxacillin was 66.66% in *N. gonorrhoeae*.

No resistance was observed for these drugs in *P. aeruginosa*; also, no resistance to Amoxicillin + Clavulanic Acid was

observed in *N. gonorrhoeae* compared to 33.33% in *P. aeruginosa* during this study.

Resistance to quinolones and fluoroquinolones

The overall resistance to this family of antibiotics was less than 20% for all strains tested.

Enterococcus

The *E. faecalis* strains isolated in this study showed an overall resistance of 11.66%; resistance to Norfloxacin was 25%.

Staphylococcus

Resistance to the antibiotics of this family showed low rates of resistance for *S. saprophyticus* (2.85%), *S. epidermidis* (3%) and *S. aureus* (19.16%). Among the tested molecules, Norfloxacin was the only one to show a significant resistance rate of 49.16%. The *Staphylococci* tested were all resistant to Nalidixic Acid, due to their natural resistance to this molecule.

Streptococcus

S. agalactiae showed no resistance to Norfloxacin, Ciprofloxacin and Nalidixic acid. However, 33.33% resistance was observed against Pefloxacin and Levofloxacin.

Other bacteria: P. aeruginosa et N. gonorrhoeae

Resistance to Ciprofloxacin, Norfloxacin, Ofloxacin and Levofloxacin was 33.33% for *P. aeruginosa*. No resistance was observed against Pefloxacin and Nalidixic acid. For *N. gonorrhoeae* isolates, no resistance was observed against Nalidixic acid, Ciprofloxacin, Ofloxacin and Pefloxacin. Resistance of 33.33% was observed against Norfloxacin and Levofloxacin.

Resistance to aminoglycosides

No significant resistance was observed in *S. agalactiae*, *S. epidermidis*, *S. saprophyticus* and *P. aeruginosa*. In contrast, the overall resistance to aminoglycosides for *E. faecalis* and *S. aureus* was 5% and 10.83% respectively (Figure 5c).

Resistance to Cyclins

The strains tested with this family of antibiotics showed a very high sensitivity and no resistance was observed for *S. agalactiae* and *N. gonorrhoeae* (Figure 5d).

Enterococcus

The overall resistance to this group of antibacterial was very low, around 2.5%. Specifically, of all the molecules tested, only Doxycycline showed a very low antibacterial activity of 10%.

Staphylococcus:

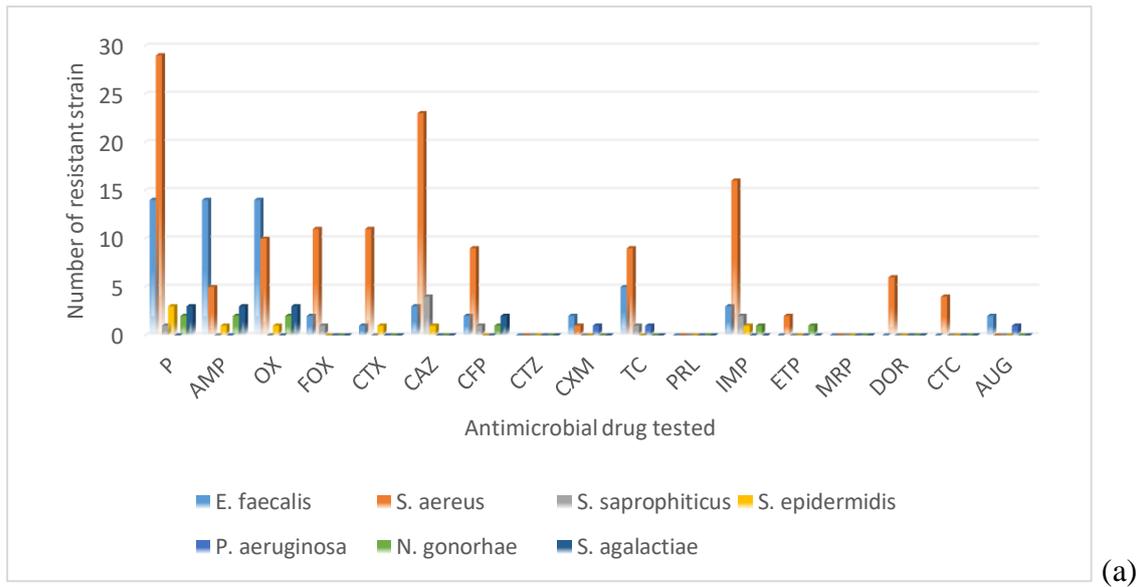
The molecules tested showed very weak antibacterial activity against the bacteria of the group: *S. saprophyticus* (1.19%), *S. epidermidis* (2.5%), and *S. aureus* (3.54%).

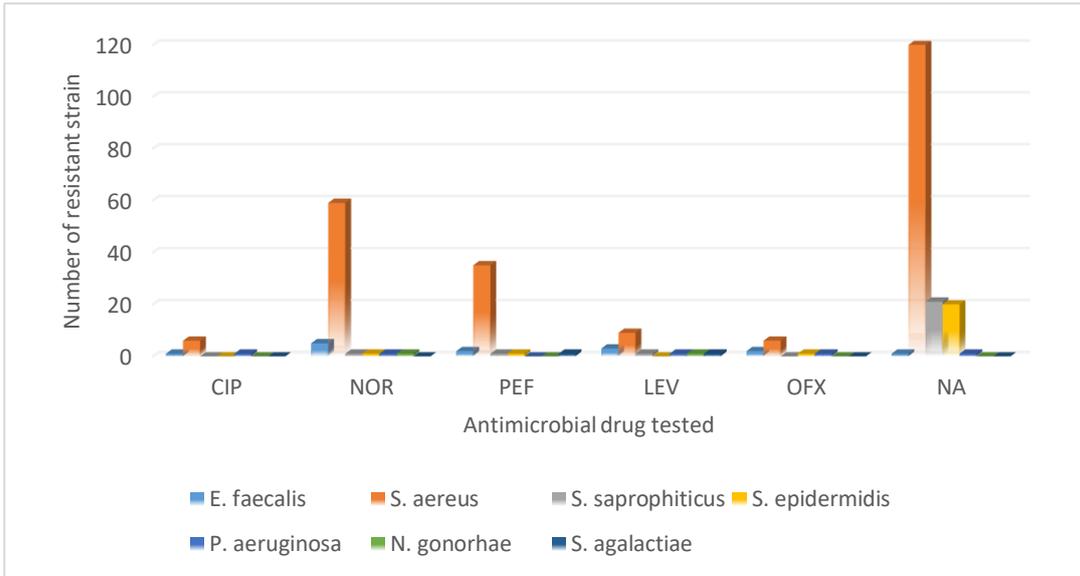
Other bacteria: *P. aeruginosa*

Of all the molecules tested, only Doxycycline showed an antibacterial activity of 33.33% against these germs.

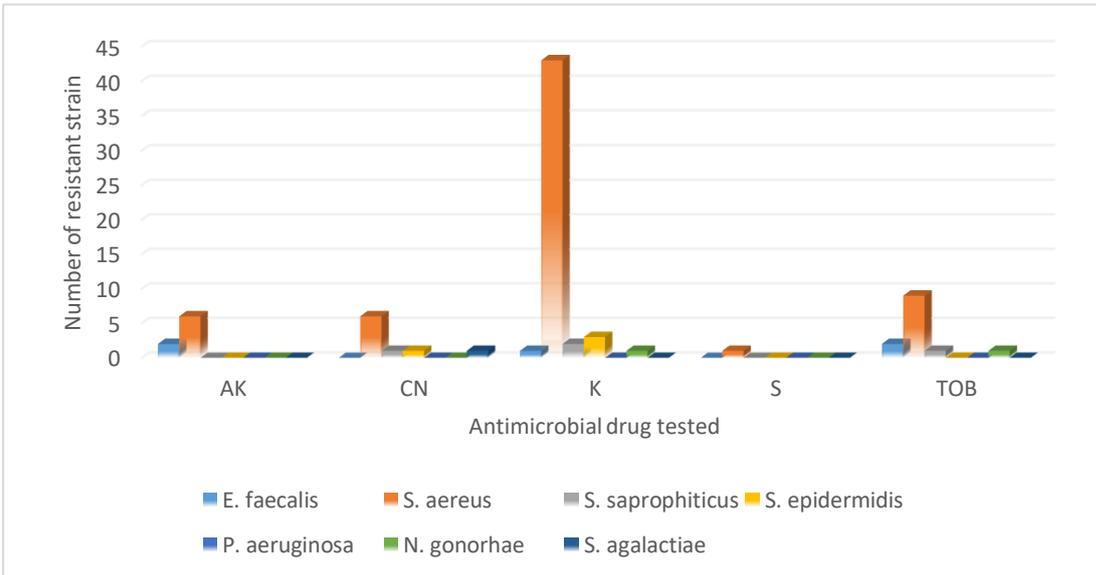
Resistance to macrolide drugs

No resistance to this family of antibiotics was observed in *S. agalactiae*, *S. saprohiticus*, *P. aeruginosa* and *N. gonorrhoeae*. Only *S. aureus* showed a variable and relatively significant resistance profile to Erythromycin and Azithromycin of 32.5% and 8.33% respectively (Figure 5e).

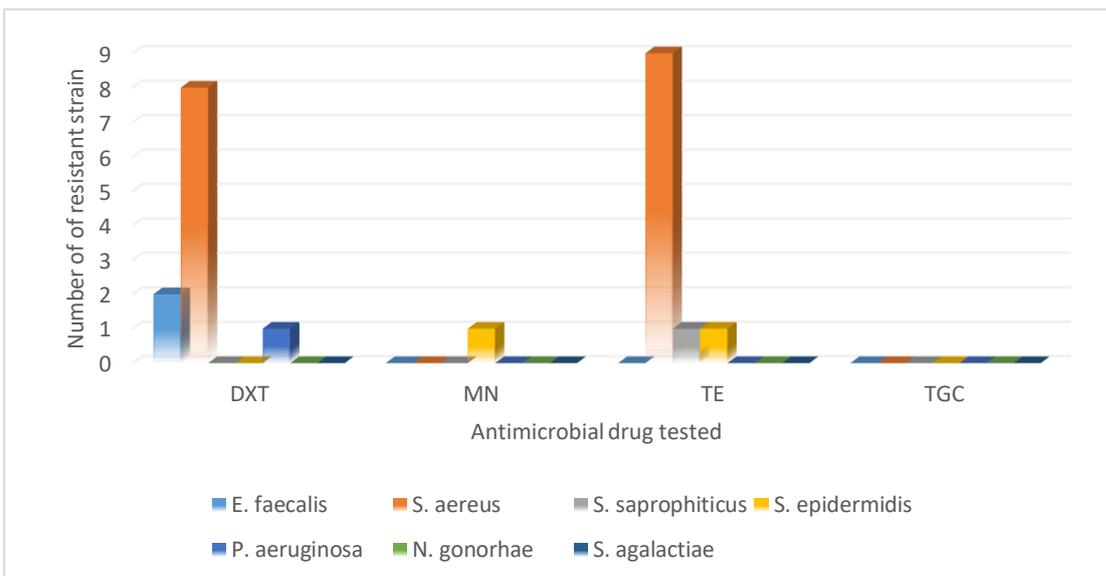




(b)



(c)



(d)

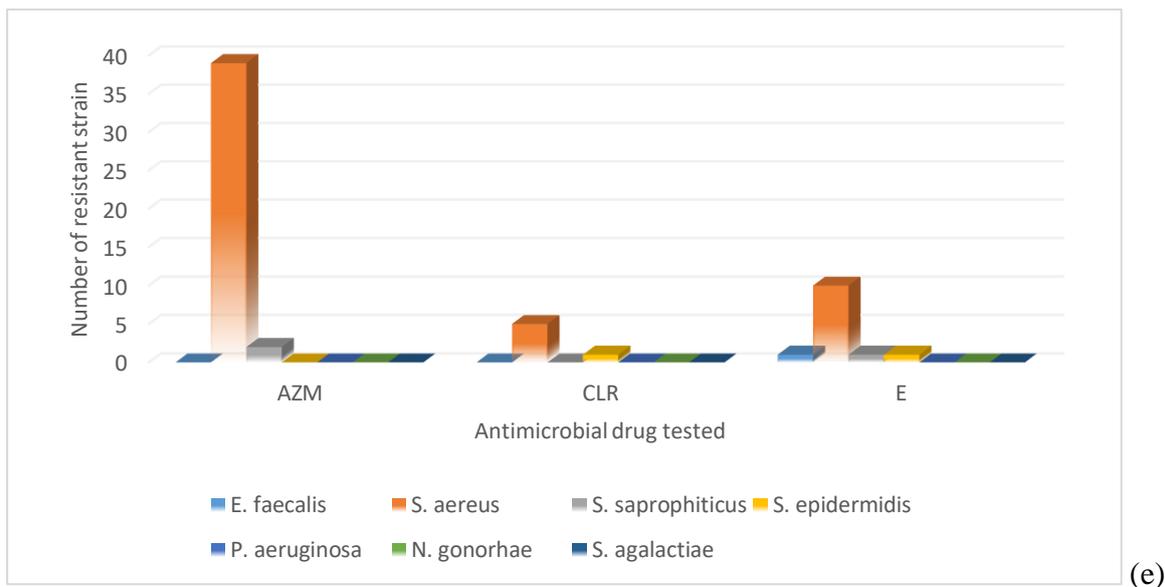


Figure 5: Antibiotic resistance profile of strains isolated from the urogenital tract. **(a):** Resistance pattern to beta-lactam antibiotics; **(b):** Resistance profile to quinolone antibiotics; **(c):** Resistance pattern to aminoglycoside antibiotics; **(d):** Resistance profile to cyclin antibiotics; **(e):** Resistance profile to macrolide antibiotics.

Legend: **AT** : Aztreonam ; **AML** : Amoxicillin ; **FOX** : Cefoxitin ; **FOS** : Fosfomycin, **FEP** : Cefepim, **AK** : Amikacin ; **TGC** : Tigecycline ; **CN** : Gentamicin ; **CIP** : Ciprofloxacin. **NOR** : Norfloxacin **ETP** : Ertapenem ; **NA** : Acid Nalidixic ; **PRL** : Piperacillin ; **AUG** : Amoxicillin + Acid Clavulaniqu ; **CAZ** : Ceftazidime ; **IMI** : Imipenem ; **CTX** : Cefotaxim ; **CFP** : Cefepim ; **LEV** : Levofloxacin ; **TC** : Ticarcillin ; **TE** : Tetracycline ; **TGC** : Tigecycline ; **NET** : Netilmicin ; **TOB** : Tobramycin ; **K** : Kanamycin ; **S** : Streptomycin ; **CLR** : Clarithromycin ; **PEF** : Pefloxacin ; **OX** : Oxacillin ; **P** : Penicillin ; **E** : Erythromycin ; **AZM** : Azithromycin ; **OFX** : Ofloxacin; **AMP** : Ampicillin.

Antibiotic resistance phenotypes in isolated *Enterobacteriaceae*

β -lactam resistance phenotypes

The analysis of β -lactam resistance phenotypes of the *Enterobacteriaceae* showed 76 strains or 28% presenting positive synergy tests (Figure 6a) characteristic of ESBL-producing bacteria. This phenotype was found in four (4) bacterial species including: *E. cloacae* (57.4%), *K. pneumoniae* (35.71%), *E. coli* (21.42%), *C. kozeri* (20%) and *K. ozanae* (10%) (Figure 6b).

The Carbapenemase phenotype was found in all *Enterobacteriaceae* except *C. freundii*, with specific frequencies distributed as follows: *E. coli* (63.75%), *P. mirabilis* (66.66%), *E. cloacae* (22.36%), *P. vulgaris* (13.33%), *K. pneumoniae*

(10%), *K. ozanae* (4%) and *E. aerogenes* (1.32%) (Figure 6c). The Penicillinase (PASE phenotype) frequencies was distributed as follows: *E. coli* (56.34%), *P. mirabilis* (33.33%), *E. cloacae* (25%), *C. kozeri* (30%), *C. freundii* (15%), *P. vulgaris* (13.33%), *K. pneumoniae* (12%) and *K. ozanae* (2%) (Figure 6c). The frequency of isolation of bacteria producing low level Cephalosporinase (CASE LL) has been observed at high rate in *P. mirabilis* (60%) and *E. coli* (33.75%). The low rate was observed in *E. cloacae* (19.73%), *P. vulgaris* (6.66%), *K. pneumoniae* (6%), *K. ozanae* (6%), *C. kozeri* (5%), *E. aerogenes* (2.63%) and *C. freundii* (0%). Then, high

level Cephalosporinase (CASE HL) was only observed in *E. coli* (11.82%), *E.*

cloacae (6.58%), *K. pneumoniae* (6%) and *K. ozanae* (6%) (Figure 6c).

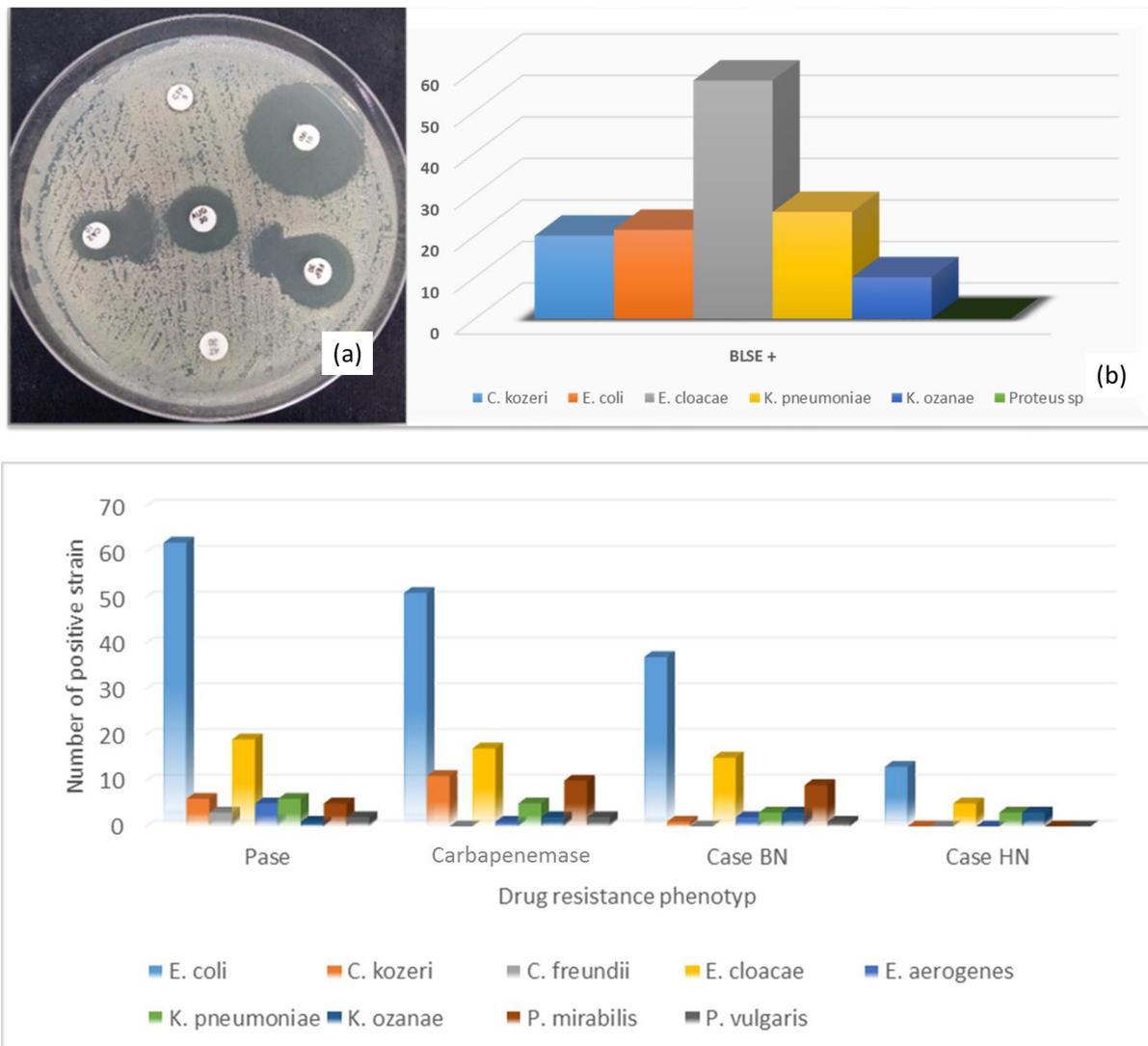


Figure 6: (a): Phenotypic profile of a test on a Petri dish (b): frequency of isolation of ESBL bacteria; (c): frequency of isolation of antibiotic resistance phenotypes by *enterobacteria* isolated.

Legend: PASE: Penicillinase; CASE BN: Low Level Cephalosporinase; CASE HN: High Level Cephalosporinase, ESBL: Extended Spectrum Betalactamases.

Multi-drug resistance bacteria

Bacteria are said to be multidrug resistant (MDR) due to the accumulation of acquired resistance to several families of antibiotics. Table 1 below shows that all bacteria isolated during this period are multidrug resistant with rates above 50%.

Table 1: Number of multi-drug resistant bacteria isolated at Laboratoire National de Santé Publique de Brazzaville during 2021.

Strains	Number of isolated bacteria	Number of BMR
Enterobacteria	289	234
<i>E. coli</i>	151	131(86,75%)
<i>E. cloacae</i>	56	42 (75%)
<i>K. pneumonia</i>	30	24 (80,4%)
<i>E. faecalis</i>	20	17 (81,2%)
<i>P. mirabilis</i>	20	13 (87,5%)
<i>C. kozeri</i>	12	8 (66,6%)
Non Fermenter Gram Negative Bacille	03	02
<i>P. aeruginosa</i>	3	2 (66,66%)
Gram Positive Cocci	182	113
<i>S. aureus</i>	179	111 (62,01%)
<i>S. agalactiae</i>	3	2 (66,66%)

4. Discussion

The emergence of antibiotic resistant and multi-resistant bacteria is a serious public health problem at national, regional, and global levels. Indeed, it constitutes a real problem in the management of patients by physicians in the prescription of appropriate treatments. Nowadays, we can assist to a continuous explosion of multi-resistant bacteria, with various resistance phenotypes, whose management is becoming gradually more difficult [5]. The present work aimed to screen the genera and species of bacteria involved in UTIs in order to establish an epidemiological profile, then to evaluate the antibiotic resistance of bacteria isolated from urine and semen at Laboratoire National de Santé Publique in Brazzaville during the year 2021.

Out of 918 urine samples and 131 sperm samples analyzed, 566 bacterial isolates were classified in 10 genera: *Staphylococcus*, *Enterobacter*, *Enterococcus*, *Streptococcus*, *Neisseria*, *Pseudomonas*, *Citrobacter*, *Klebsiella*, *Proteus*, and *Escherichia*. These results could be explained by the proximity of the

urinary tract and the anal canal in women, and the poor intimate hygiene. It has been shown that *Enterobacteriaceae* and commensal bacteria such as those of the *Staphylococcus* group may be involved in infections of the human urogenital tract [14]. Similar results were obtained by Rizwann and colleagues in New Delhi, India [15-17] in which the same bacteriological profile of the germs isolated from their fluids was observed.

This study displayed that the reasons for patients to seek for medical assistance due to urinary tract infections were similar for both women and men. These results reflect an evolution in the habits of the Congolese people, who increasingly consult a physician for an adequate follow-up.

It has been perceived during this study that urine samples from female patients were significantly higher than those from male patients. This observation allows us to speculate that male patients who consulted a physician had a history of infection and did not consult until long after the infection had set in and were probably engaged in

self-medication. In fact, infections in female patients can be explained by anatomical reasons, unlike in men. These findings highlighted the fact that further investigations must be conducted in order to reveal the epidemiological reality of urinary tract infections in Brazzaville and the Republic of Congo as a whole.

Although the average age of the patients was around 40 years. The positive male samples were mostly isolated from middle-aged men whose age was around 60 years, unlike women who seek for medical assistance at a young age. Furthermore, contrasting to many studies conducted on the epidemiology of UTIs, this study showed that the most isolated strain was *S. aureus*. Its isolation could be explained by the fact that this bacterium is a commensal bacterium present in the flora of the skin, so the increase of sexual activity and the non-observance of body hygiene rules could favor the explosion of contamination by this bacterium. This research also showed that for bacteria of the *Enterobacteriaceae* family, the most isolated strain was *E. coli*. This result confirms that of Isca and colleagues in a study conducted in children in Romania where they isolated *E. coli* in 38.84% of urine samples [18].

However, a recent work by Tristan and colleagues isolated the majority of *K. pneumoniae* (41%) from the urogenital tract [19]. This difference could be explained by the endemic self-medication in Brazzaville which could eliminate these bacteria from the urogenital tract and favor the occurrence of other genera that would be less targeted. This may also explain why fewer samples taken from women were positive.

Resistance to antimicrobial agents is an urgent global health challenge. It's threatening the successful prevention and treatment of bacteria, viruses, parasites and

fungal infections. The high frequency of infections caused by bacteria resistant to antibiotics makes empiric treatment more difficult. The choice of treatment depends on the microbiological spectrum and the antibiogram of each patient. Most UTIs are generally treated without bacteriological testing. Among the antibiotics used, penicillins (beta-lactams), cephalosporins, fluoroquinolones and carbapenems generally remain the initial choices. However, these broad-spectrum antibiotics should always be replaced by a narrow-spectrum and targeted antibiotic.

This study provides a synopsis of the antibiotic resistance of bacteria isolated from the urogenital tract at Laboratoire National de Santé Publique in Brazzaville. Undeniably, the sensitivity of bacteria was tested on five (5) families commonly prescribed in different hospitals of Brazzaville, specifically: Beta-lactams, Quinolones-Fluoroquinolones, Aminoglycosids, Cyclin, and Macrolids.

The general trend showed a relative resistance to all the antibiotics tested. Indeed, beside the resistance to Penicillins characteristic of *Enterobacteriaceae*, we noted an emerging resistance to Imipenem by strains of *P. mirabilis*, *E. coli*, and *C. kozeri*. These results confirm facts from the literature saying that Imipenem resistance is now affecting species that may be typically community-based [20]. In contrast, these results are at odds with those of Daoud in Lebanon, who showed that *E. coli* strains are susceptible to Imipenem [21]. The resistance to Imipenem observed in this study is an indicator of the ability of these strains to produce metallo-beta-lactamases that confer this property to bacteria. These bacteria may acquire metallo-beta-lactamases as a result of horizontal transfer between bacteria and as a consequence of

drug pressure [22]. In most cases, resistance rates were below 20% for all beta-lactam antibiotics tested. Our results are similar to those of Cecili and colleagues in 2015 [23] in Cameroon, who made the same observation in a study evaluating antibiotic resistance in *Enterobacteriaceae* isolated in Douala. Resistance to 3rd generation Cephalosporins was relatively low, as we observed for all Beta-lactam antibiotics. Similar results were reported by Hashemi and colleagues in a resistance study conducted in Iran in 2013 [24]. However, our results differ from those reported in Annaba, Algeria by Ramoul in 2013 [25]. We have nevertheless observed the appearance of bacteria multiresistant to Beta-lactams. The emergence of these strains would be the consequence of drug pressure due to self-medication, which remains a major public health problem and one more threat in the emergence of these strains, which will undoubtedly end up dipping clinicians into a therapeutic deadlock. This result could explain the fact that the number of positive samples among male and female patients was similar. Additionally, it also shows an explosion of self-medication, hence the multidrug resistance and medical assistance as a last resort. Likewise, a strain of *N. gonorrhoeae* resistant to Imipenem was also isolated, demonstrating a further indication of the consequences of drug pressure. In fact, it is a sexually transmitted infection and, under these conditions, the strain probably has an epidemiological history which links it to this antibiotic.

For sperm cultures, 63.36% of the samples were positive and were mostly the consequence of infections of the male urinary tract. The point is that it gradually

concerns young boys. It is also a silent epidemic that needs to be taken into account by the competent health authorities.

Fluoroquinolones are first-line antibiotics in the treatment of urogenital infections due to their extended spectrum of action against gram-positive and gram-negative bacteria [26]. This makes these molecules the privileged candidates for the treatment of these infections. Our results are divergent to those of Hashemi *et al.*, [24] and Hooper *et al.*, [26], insofar as all bacteria showed a very high degree of sensitivity to fluoroquinolones, of the order of more than 70% overall. An increased susceptibility was observed to Ciprofloxacin compared to Norfloxacin for all bacteria tested. Nevertheless, the appearance of resistance alerts us to the fact that resistant strains are emerging, particularly for bacteria of the genus *Staphylococcus*, which, like certain *Enterobacteriaceae*, do not possess a plasmid and acquire the resistance genes as a result of drug pressure. In addition, because of their ability to cause sepsis and localized infections in several parts of the human body, this configuration makes them susceptible to further development of resistance mechanisms in response to drug pressure.

This study made it possible to show a high sensitivity of the isolated germs to Aminoglycosides and Macrolides. Molecules of the Aminoglycoside family tested on the isolates studied had strong antibacterial activity. These results are contrary to those reported by Cécile *et al.*, in 2015 in Cameroon in Douala [23] who worked on *Enterobacteriaceae*. As *E. coli* which is naturally resistant to erythromycin, we noted a very high sensitivity to this antibiotic. Nevertheless, due to the resurgence of multi-drug resistant strains, it suggests that measures need to be taken, in

accordance with the recommendations of the EUCAST 2021 concerning prescriptions for antibiotics in the Republic of Congo.

Epidemiological data show that the abusive use of antibiotics such as Penicillins, Cephalosporins, and fluoroquinolone antibiotics increases the risk of the emergence of extended-spectrum Bêta-lactamase-producing strains of *Enterobacteriaceae* in human urogenital infections [10, 27, 28]. These strains are often isolated in hospitals where antibiotics of these groups are over-prescribed or simply where patients are in severe conditions that require intensive antibiotic use.

This study constitutes an important work because it aims to highlight the emergence of these bacteria in community settings. This work also consists of highlighting the scourge of dispensing drugs without a prescription, certain unnatural practices of

5. Conclusion

This study explored the antibiotic resistance of bacteria isolated from urine and semen at the National Public Health Laboratory in Brazzaville. This study provides an overview of the frequency of isolation of strains, sensitivity, and resistance phenotypes of bacteria isolated from January to December 2021. Out of this study, it appears that the genus *Staphylococcus* was the most isolated from urine and semen samples. The *E. coli* strain was the most isolated species in the *Enterobacteriaceae* family. The study of resistance during this research period showed a high distribution of multidrug resistant bacteria. Similarly, the bacteria isolated showed worrying antibiotic resistance phenotypes (Carbapenemase, Cephalosporinase,

the medical profession insofar as certain antibiotics are prescribed without prior antibiogram.

The detection of ESBL-positive strains was done phenotypically on petri dishes, in fact as the characteristic champagne cork inhibition zones on the dish reflect the synergy. The genes responsible for these phenotypes are transferable, which is a significant factor to take into account. This resistance can also extend to other classes of antibiotics such as cyclins and phenicols [29]. The rate of beta-lactamase producing strains isolated during this study period is lower than that found by Chamoun and colleagues in Lebanon, which was 32% against 28% in the present study [30]. Similarly, this study also showed that the majority of beta-lactamase producing strains were *E. cloacae* followed by *E. coli*, which is also not in agreement with those found by Chamoun and colleagues [30].

Penicillinase, MRSA, ESBL) and this dictates that the question of antibiotics use in therapeutics must be framed in the Republic of Congo. It was also shown that more than half of the reasons for medical assistance were of infectious origin. The same resistance was observed for bacteria isolated from urine, highlighting a thorny public health problem linked to fertility.

In order to prevent the development of resistance to antimicrobial agents in Republic of Congo, the overuse or misuse of antibiotics should be limited and sales control measures must be established. Further research in this field is needed to describe antibiotics' resistance in

community-acquired infections and hospital-acquired UTI.

6. Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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