Infection and Drug Resistance

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ORIGINAL RESEARCH

Epidemiology of Community Origin *Escherichia coli* and *Klebsiella pneumoniae* Uropathogenic Strains Resistant to Antibiotics in Franceville, Gabon

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Introduction: Urinary tract infection is one of the major causes of consultation, microbiologic exploration, intensive use of antibiotics worldwide, and the second leading cause of clinical consultation in community practice. Many bacteria play a role in the urinary tract infections etiology, including *Enterobacteriaceae* such as *Escherichia coli* (*E. coli*) and *Klebsiella* spp.

Objective: The study's main objective was to examine the epidemiology of *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*) uropathogenic strains resistant to antibiotics in Franceville.

Methodology: The study was carried out between January 2018 and June 2019 in Franceville South-East Gabon. We examined a total of 1086 cytobacteriological urine samples. The identification of *E. coli* and *K. pneumoniae* strains was carried out using the Vitek-2 compact automated system and the antibiogram with the disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing recommendations.

Results: The prevalence of urinary tract infections was 29.2% (317/1086), of which 25.1% and 4.1% were mono-infections and co-infections, respectively. The prevalence of UTIs with *E. coli* was 28.7% (91/317) with a predominance of isolation in women. *K. pneumoniae* was responsible for 16.2% (61/317) of UTIs. *E. coli* and *K. pneumoniae* Uropathogenic strains showed resistance to beta-lactams, quinolones and cotrimoxazole, whereas Nitrofurantoin, Amikacin, Imipenem and Ertapenem were the most active antibiotics against *E. coli* and *K. pneumoniae* uropathogenic strains.

Conclusion: This study showed a high prevalence of urinary tract infections with a major implication of *E.coli* and *K. pneumoniae* strains. *E. coli* and *K. pneumoniae* presented high frequency of resistance to antibiotics, highlighting the need to adapt their use accordingly at the local level.

Keywords: urinary tract infection, antibiotic resistance, *E. coli*, *K. pneumoniae*

Introduction

Urinary tract infections (UTIs) are the second most common ailment in community medical practice. Each year, around 150 million people are diagnosed worldwide with urinary tract infections costing more than 6 billion US dollars to the global economy.[1] UTIs are a major global public health problem, because of the health costs they cause and the selection of multidrug-resistant strains both in hospitals and in community settings.[2] These are common infections with an alarming

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increase in resistance to last-resort antibiotics.[3] Many bacteria are involved in the etiology of urinary tract infections, including enterobacteria such as *E. coli* and *Klebsiella* spp.[4] These bacteria are the most common pathogens in UTIs. However, less common agents may also cause UTIs, such as non-fermenters and fungi.[5,6]

Several studies conducted in Europe and the United States of America have shown a constant increase in the rate of resistance of uropathogenic bacteria to commonly prescribed antibiotics leading to reduced therapeutic efficacy.[7]

The main risk factor for resistance to an antibiotic is repeated previous exposure to the same antibiotic. Indeed, the misuse of an antibiotic or a class of antibiotics is the cause of development of bacterial resistance, possibly extending to other families of antibiotics because of the cross-transmission of mobile genetic elements carrying antibiotic resistance genes.[8,9] These bacterial resistances develop more easily in the digestive microbiota due to a large number of bacteria (greater than 10^9 bacteria per gram of stool), promoting contact as well as the emergence of resistant mutants. UTIs are most often of ascending origin by contamination from the perineal flora, reflecting the digestive flora. Therefore this selection pressure has a definite clinical impact.[8]

Early treatment of UTIs decreases their severity rate, which, in most cases, involves antibiotic treatment prescribed empirically.[10] In order to administer appropriate therapy, it is essential to identify the main bacteria commonly involved in urinary tract infection and their antibiotic resistance profile.[11] In Gabon, the lack of treatment regimen standardization and clinical bacteriology laboratories make essential studies on uropathogenic germs and their susceptibility and/or resistance to antibiotics providing a local profile of bacterial resistance.

This study aimed to determine the epidemiology of *Escherichia coli* and *Klebsiella pneumoniae* uropathogenic strains isolated from patients in consultation at the Interdisciplinary Center for Medical Research of Franceville in South-East Gabon and to determine their resistance profile to common antibiotics.

Materials and Methods

Patients

The study was carried out between January 2018 and June 2019. It involved all patients from the community requesting a cyto-bacteriological examination of urine (CBEU) by the laboratory of medical analyzes, which is the only bacteriology laboratory in the city of Franceville, capital of the Haut-Ogooué province, the second-most populous province of Gabon bordering the Republic of Congo.

Patients of all ages and both sexes who presented for a CBEU were considered eligible for the study.

Sample Collection

Urine samples were preferably collected in the morning or after an absence of urination for about 4 hours, either in the laboratory or at home, under strict aseptic conditions. Urine was collected in a sterile, single-use urine container.

For children who could use the toilet on their own, urine samples were collected using a sterile adhesive collection bag under the supervision of a guardian. The urine vial was then sealed, identified, indicating the time of collection and then transported by the patient at room temperature to the laboratory. The socio-demographic and clinical data for each patient were collected through a structured questionnaire.

Culture and Identification of Bacterial Isolates

It consisted of inoculating ten (10) µL of total urine aseptically using a sterile single-use loop in the level 2 microbiological safety station. The inoculation was carried out systematically on agar media, CLED (Cystine-Lactose -Electrolytes-Deficient; Biomérieux, France), Mac Conkey (McC, Biomérieux, France), Eosin Methylene Blue (EMB, Biomérieux, France) after briefly homogenizing the urine. Urine samples were inoculated within two (2) hours of collection to avoid false positives. The inoculated media were systematically incubated aerobically in a bacteriological oven at 35°C for 18 to 24 hours. According to Kass criteria a number $\geq 10^5$ colony forming units (CFU)/mL of urine was considered positive; a colony number $< 10^5$ CFU/mL or with more than two (2) types of bacterial colonies were considered contaminated.[12]

The identification of *E. coli* and *K. pneumoniae* strains was done after Gram stain, oxidase test and conventional biochemical tests (VITEK-2 automated system, Biomérieux, France). Only *E. coli* and *K. pneumoniae* strains isolated in monoinfected culture were retained in the study.

Antibiotic Sensitivity Test

All *E. coli* and *K. pneumoniae* isolates were tested for sensitivity to the following antibiotics: ampicillin,

ticarcillin, amoxicillin-clavulanic acid, cefalotin, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, ertapenem, gentamicin, tobramycin, amikacin, ofloxacin, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, nitrofurantoin according to the diffusion disc method (Kirby-Bauer) on Mueller-Hinton (MH) agar according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, V. 2.0 2018).[13] Briefly, MH agars were seeded with a standardized suspension (0.5 McFarland) of each *E. coli* and *K. pneumoniae* isolate for a 24 hour MH agar culture. The antibiotic discs were firmly placed on the surface of the seed plates. The culture media were then incubated for 24 hours at 35°C. The diameters of antibiotics inhibition zones were interpreted according to the recommendations of the EUCAST.[13] Multi-drug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.[14]

Statistical Analysis

All the data collected was entered in a Microsoft Excel 2013 file and then analyzed by the R software (version R × 64.3.4.3). The differences were considered significant for $p < 0.05$.

Ethical Considerations

Informed and written consent was obtained from each adult patient prior to inclusion in the study. With regard to minors, consent has been obtained from their parents or legal guardians.

The research licence for this study was obtained from the Scientific Commission on Research Authorisations of the National Centre of Scientific and Technological Research (CENAREST) (permit 7 no. AR0033/17/MESRSFC/CENAREST/CG/CST/CSAR, dated 4 July 2017). This study was conducted in accordance with the Declaration of Helsinki.

Results

Characteristics of Patients

Of 1086 CBEUs performed, 61.7% (670/1086) were from female patients. The mean age of patients was 24.7 ± 18.9 years. More than half of these were aged 18 to 49 (53.7%). The vast majority of patients came from the city of Franceville (72.2%) and did not present any particular morbid ground (94.3%). Among these patients, 58.8% had urinary signs, and 86.8% of samples were collected mid-stream (Table 1).

Prevalence of Urinary Tract Infections Due to *E. coli* and *K. pneumoniae*

The overall prevalence of UTIs was 29.2% (317/1086), of which 25.1% (273/1086) were mono-infections while 4.1% (44/1086) were co-infections. Contaminations represented 23.2% (252/1086) of cultures, whereas 0.3% (3/1086) of CBEUs showed significant bacteriuria without leukocyturia.

The prevalence of UTIs due to *E. coli* was 28.7% (91/317) with 24.6% (78/317) of mono-infections and 4.1% (13/317) of co-infections. Regarding co-infections, *E. coli* was associated with *K. pneumoniae* (0.3%), *K. oxytoca* (0.3%), *E. faecalis* (0.9%), *Staphylococcus* spp. (1.3%), *S. agalactiae* (0.3%), *E. aerogenes* (0.3%), *E. cloacae* complex (0.63%) (Table 2).

K. pneumoniae was found in 18.9% (60/317) of urinary tract infections with 14.2% (45/317) of the strains isolated in monoculture while 4.7% (15/317) were identified in association with *K. oxytoca* (0.3%), *Enterococcus* spp.

Table 1 Socioclinical Characteristics of the Study Patients

Characteristics	Number	Percentage
Sex		
Male	416	38.3
Female	670	61.7
Age groups		
≤ 5 years	284	26.2
6–17 years	110	10.1
18–49 years	583	53.7
≥ 50 years	109	10.0
Locality		
Franceville	784	72.2
Outside Franceville	302	27.8
Underlying terrain		
None	1024	94.3
Comorbidity	62	5.7
Symptoms		
Urinary signs	638	58.7
Non-urinary signs	344	31.7
None	104	9.6
Sampling methods		
Collector pocket	142	13.1
Mid-jet	943	86.8
Urinary catheters	1	0.1

Table 2 Isolation of Uropathogenic Bacteria

Urinary Infections	Number (n=317)	Percentage (%)
Mono-infection		
E. coli	78	24.6
K. pneumoniae	45	14.2
Other Enterobacteriaceae	20	6.3
Non-fermenting GNB	11	3.5
S. saprophyticus	19	6.0
S. aureus	14	4.4
Coagulase negative *Staphylococcus*	45	14.2
E. faecalis	21	6.6
E. faecium	4	1.3
Streptococcus spp.	6	1.9
Other Gram positive cocci	5	1.6
C. albicans	5	1.6
Co-infections		
E. coli/K. pneumoniae	1	0.3
E. coli/K. oxytoca	1	0.3
E. coli/E. faecalis	3	0.9
E. coli/Staphylococcus spp.	4	1.3
E. coli/S. agalactiae	1	0.3
E. coli/E. aerogenes	1	0.3
E. coli/E. cloacae complex	2	0.63
K. pneumoniae/K. oxytoca	1	0.3
K. pneumoniae/Enterococcus spp.	7	2.2
K. pneumoniae/Staphylococcus spp.	6	1.9
K. pneumoniae/P. acidilactici	1	0.3
C. freundii/E. faecalis	1	0.3
E. cloacae/E. faecalis	1	0.3
E. cloacae/A. denitrificans	1	0.3
P. mirabilis/S. marcescens	1	0.3
P. mirabilis/C. freundii	1	0.3
S. fonticola/S. haemolyticus	1	0.3
R. ornithinolytica/Enterococcus spp.	1	0.3
E. faecalis/E. faecium	1	0.3
E. faecalis/S. saprophyticus	1	0.3
E. faecalis/A. salmonicida	1	0.3
S. epidermidis/C. albicans	1	0.3
S. epidermidis/S. haemolyticus	1	0.3
S. saprophyticus/A. baumanii	1	0.3
S. saprophyticus/Streptococcus spp.	1	0.3
S. haemolyticus/S. porcinus	1	0.3
S. haemolyticus/S. capitis	1	0.3

(2.2%), *Staphylococcus* spp. (1.9%), or *P. acidilactici* (0.3%) (Table 2).

Distribution of Urinary Mono-Infections Due to *E. coli* and *K. pneumoniae*

A total of 78 *E. coli* urinary mono-infections were identified in the CBEUs of patients. These *E.coli* mono-infections were significantly more frequent in women compared to men (82% vs 18%; p <0.001) (Figure 1). The frequency of *E. coli* UTIs was significantly lower in patients aged 6–17 years compared to those aged ≤5 years (7.7% vs 25.6%; p = 0.03) and 18–49 years (7.7% vs 52.6%; p <0.001). In addition, *E. coli* was found predominantly in patients with urinary signs compared to those without urinary signs (68% vs 32%; p <0.001) (Figure 1).

Regarding the seasons, *E. coli* UTIs were significantly more frequent in the short rainy season compared to the short dry season (35.9% vs 17.95%; p = 0.012) or the long rainy season (35.9% vs 19.23%; p = 0.02) (Figure 1).

A total of 45 *K. pneumoniae* UTIs were identified in patients' CBEUs. *K. pneumoniae* was found significantly more often in women than in men (66.7% vs 33.3%; p = 0.002). It was associated with patient age being significantly lower in the 6–17 years age group compared to the ≤5 years (4.4% vs 55.6%; p <0.001) and 18–49 years age groups (4.4% vs 28.9%; p = 0.002) (Figure 1). In addition, the distribution of UTIs due to *K. pneumoniae* was associated with seasonality and was more frequent during the short rainy season compared to the long dry season (37.8% vs 13.3%; p = 0.008) (Figure 1).

Antibiotic Resistance Assessment of *E. coli* and *K. pneumoniae* Isolates

Of the 123 strains of *E. coli* and *K. pneumoniae* isolated, the highest rates of antibiotic resistance were observed with Ampicillin (78%), Ticarcillin (75%), Trimethoprim-Sulfamethoxazole (63%), Cefalotin (47%), Amoxicillin-clavulanic acid (44%), Cefotaxime (41%), and Nalidixic acid (40%). The majority of bacterial strains isolated (56%) were multidrug resistant (MDR) (Table 3).

Overall, beta-lactam resistance was significantly more frequent in MDR strains compared to non-MDR strains for Ampicillin (p <0.001), Ticarcillin (p <0.001), Amoxicillin-clavulanic acid (p< 0.001), Cefalotin (p <0.001), Cefoxitin (p <0.001), Cefotaxime (p <0.001), Ceftazidime (p <0.001) and Cefepime (p = 0.026) (Table 3). This was also observed with quinolones including Nalidixic Acid (p <0.001), Ciprofloxacin (p <0.001) and Ofloxacin (p <0.001); and with aminoglycosides such as Gentamicin (p <0.001), Tobramycin (p <0.001) and Amikacin (p = 0.026) (Table 3). Furthermore, resistance to Trimethoprim-Sulfamethoxazole was more prevalent in MDR strains compared to non-MDR strains (p <0.001) (Table 3).

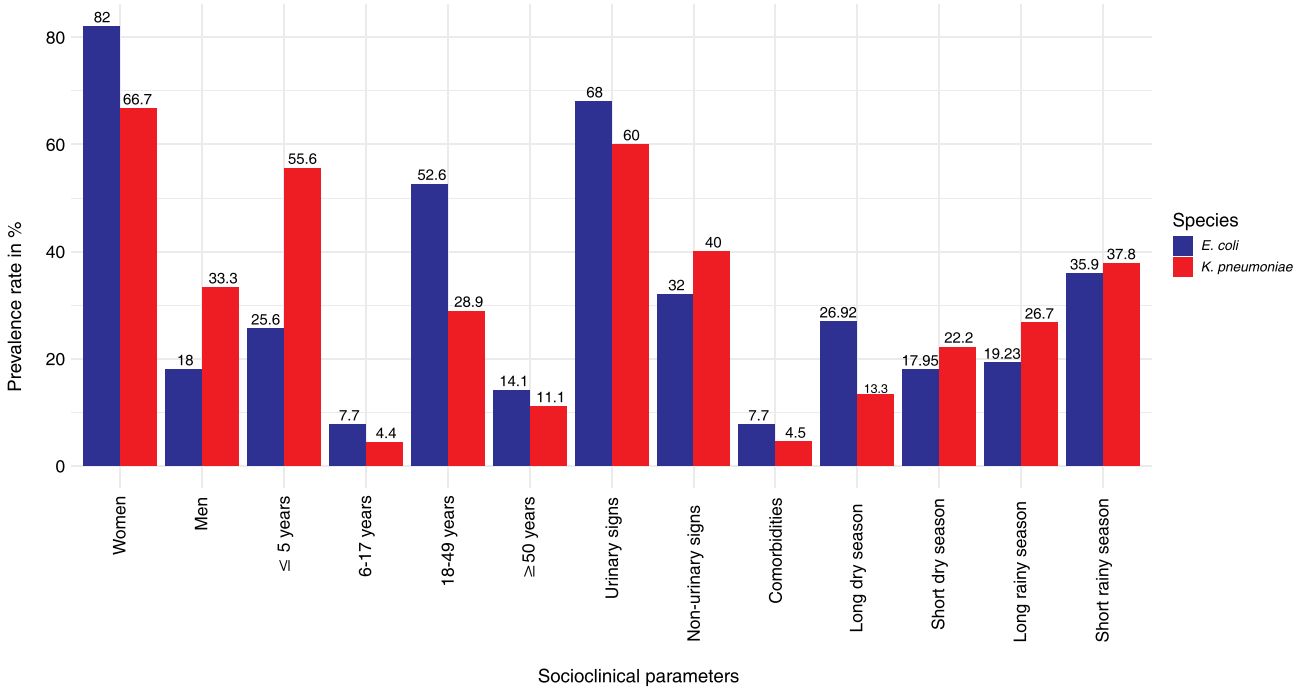

Figure 1 Distribution of *E. coli* and *K. pneumoniae* urinary mono-infections according to socioclinical parameters.

Among 78 *E. coli* strains, the highest resistance to beta-lactams was observed with Ampicillin (65%), Ticarcillin (61%), Amoxicillin-clavulanic acid (45%), Cefalotin (41%) and Cefotaxime (33%) while the lowest frequency of resistance was found with Imipenem (2%), Ertapenem (2%) and Cefepime (7%) (Table 3). The majority of *E. coli* strains (55%) was MDR. The MDR phenotype Ampicillin/Nalidixic acid/Trimethoprim-Sulfamethoxazole was the most prevalent (70%) in *E. coli* strains. Resistance to Ampicillin ($p < 0.001$), Ticarcillin ($p < 0.001$), Amoxicillin-clavulanic acid ($p < 0.001$), Cefalotin ($p < 0.001$), Cefoxitin ($p = 0.025$), Cefotaxime ($p < 0.001$), Ceftazidime ($p < 0.001$), Nalidixic Acid ($p < 0.001$), Ciprofloxacin ($p = 0.002$) and Ofloxacin ($p < 0.001$) were more common in MDR-*E. coli* compared to non-MDR -*E. coli*-isolates (Table 3). Antibiotics multidrug-resistance has been observed more frequently in women compared to men ($p < 0.0001$).

Of 45 *K. pneumoniae* strains tested, the highest rates of antibiotics resistance were observed with Cefalotin (58%), Cefotaxime (56%), Amoxicillin-clavulanic acid (46%) and Ceftazidime (47%) whereas the lowest frequency of resistance was obtained with Cefepime (4%), Imipenem (4%) and Ertapenem (4%) (Table 3). *K. pneumoniae* strains were 57% MDR, predominantly isolated from patients ≤ 5 years ($p = 0.002$). The MDR phenotype Amoxicillin-clavulanic acid/Cefotaxime/Trimethoprim-Sulfamethoxazole was most common (52%) in *K. pneumoniae* strains. Resistance to Amoxicillin-clavulanic acid, Cefalotin, Cefoxitin, Cefotaxime, Ceftazidime, nalidixic acid and Ofloxacin was significantly more frequently observed in MDR-*K. pneumoniae* compared to non-MDR-*K. pneumoniae* isolates (Table 3).

We also compared the resistance of *E. coli* and *K. pneumoniae* strains to antibiotics. *K. pneumoniae* strains of were more resistant to cefotaxime compared to those of *E. coli* (56% vs 33%; $p = 0.024$) (Table 3). Regarding quinolones, *E. coli* strains were more resistant to nalidixic acid (51% vs 22%; $p = 0.002$) and to Ciprofloxacin (34% vs 13%, $p = 0.01$) compared to *K. pneumoniae* strains while resistance to Ofloxacin was similar between the two types of bacteria (39% vs 28%; $p = 0.226$) (Table 3).

For aminoglycosides, the resistance of strains of *E. coli* and *K. pneumoniae* was evaluated with antibiotics such as Gentamicin (25% vs 28%), Tobramycin (28% vs 24%) and Amikacin (7% vs 4%). It was similar for the three antibiotics between *E. coli* and *K. pneumoniae* (Table 3). Resistance of *K. pneumoniae* to Cefotaxime (80% vs 43%; $p = 0.044$) and Trimethoprim-Sulfamethoxazole (73% vs 53%; $p < 0.0001$) was significantly higher in men compared to women. Among MDR isolates, we

Table 3 Resistance of *E. coli* and *K. pneumoniae* Strains to Antibiotics

Antibiotics	*E coli* and *K. pneumoniae* (n=123)			*p*-value	*E. coli*			*p*-value	*K. pneumoniae*			*p*-value	*p*-value*
	All Isolates	MDR Status of Isolates			All Isolates	MDR Status of Isolates			All Isolates	MDR Status of Isolates			
	(n=123)	MDR (n=69)	Non-MDR (n= 54)		(n = 78)	MDR (n = 43)	Non-MDR (n = 35)		(n = 45)	MDR (n = 26)	Non-MDR (n = 19)		
β-lactams													
Ampicillin	96 (78%)	67 (97%)	29 (53%)	**<0.001**	51 (65%)	41 (95%)	10 (28%)	**<0.001**	45 (100%)	26 (100%)	19 (100%)	-	-
Ticarcillin	93 (75%)	64 (92%)	29 (53%)	**<0.001**	48 (61%)	38 (88%)	10 (28%)	**<0.001**	45 (100%)	26 (100%)	19 (100%)	-	-
Amoxicillin-clavulanic acid	55 (44%)	48 (69%)	7 (12%)	**<0.001**	34 (45%)	29 (67%)	5 (14%)	**<0.001**	21 (47%)	19 (73%)	2 (10%)	**<0.001**	NS
Cefalotin	58 (47%)	54 (78%)	4 (7%)	**<0.001**	32 (41%)	29 (67%)	3 (8%)	**<0.001**	26 (58%)	25 (96%)	1 (5%)	**<0.001**	NS
Cefoxitin	22 (17%)	20 (28%)	2 (3%)	**<0.001**	11 (14%)	10 (23%)	1 (2%)	**0.025**	11 (24%)	10 (38%)	1 (5%)	**0.027**	NS
Cefotaxime	51 (41%)	50 (72%)	1 (1%)	**<0.001**	26 (33%)	26 (60%)	0 (0%)	**<0.001**	25 (56%)	24 (92%)	1 (5%)	**<0.001**	**0.024**
Ceftazidime	45 (36%)	43 (62%)	2 (3%)	**<0.001**	24 (30%)	23 (53%)	1 (2%)	**<0.001**	21 (47%)	20 (76%)	1 (5%)	**<0.001**	NS
Cefepime	8 (6%)	8 (11%)	0 (0%)	**0.026**	6 (7%)	6 (13%)	0 (0%)	NS	2 (4%)	2 (7%)	0 (0%)	NS	NS
Imipenem	4 (3%)	4 (5%)	0 (0%)	NS	2 (2%)	2 (4%)	0 (0%)	NS	2 (4%)	2 (7%)	0 (0%)	NS	NS
Ertapenem	3 (2%)	3 (4%)	0 (0%)	NS	2 (2%)	2 (4%)	0 (0%)	NS	1 (2%)	1 (3%)	0 (0%)	NS	NS
Quinolones and fluoroquinolones													
Nalidixic acid	50 (40%)	43 (62%)	7 (12%)	**<0.001**	40 (51%)	33 (76%)	7 (20%)	**<0.001**	10 (22%)	10 (38%)	0 (0%)	**0.007**	**0.002**
Ciprofloxacin	33 (26%)	28 (40%)	5 (9%)	**<0.001**	27 (34%)	22 (51%)	5 (14%)	**0.002**	6 (13%)	6 (23%)	0 (0%)	NS	**0.01**
Ofloxacin	44 (35%)	38 (55%)	6 (11%)	**<0.001**	31 (39%)	26 (60%)	5 (14%)	**<0.001**	13 (28%)	12 (46%)	1 (0%)	**0.008**	NS
Aminoglycosides													
Gentamicin	33 (26%)	32 (46%)	1 (1%)	**<0.001**	20 (25%)	19 (44%)	1 (2%)	**<0.001**	13 (28%)	13 (28%)	0 (0%)	**<0.001**	NS
Tobramycin	33 (26%)	32 (46%)	1 (1%)	**<0.001**	22 (28%)	21 (48%)	1 (2%)	**<0.001**	11 (24%)	11 (24%)	0 (0%)	**0.004**	NS
Amikacin	8 (6%)	8 (11%)	0 (0%)	**0.026**	6 (7%)	6 (13%)	0 (0%)		2 (4%)	2 (4%)	0 (0%)	NS	NS
Sulfonamides													
Trimethoprim-sulfamethoxazole	78 (63%)	61 (88%)	17(31%)	**<0.001**	51 (65%)	38 (88%)	13 (35%)	**<0.001**	27 (60%)	23 (88%)	4 (20%)	**<0.001**	NS
Nitrofurans													
Nitrofurantoin	2 (1%)	2 (2%)	0 (0%)	NS	1 (1%)	1 (2%)	0 (0%)	NS	1 (2%)	1 (3%)	0 (0%)	NS	NS

Notes: *Comparison of resistance rates between *E. coli* and *K. pneumoniae*. In bold: significant P-value.
Abbreviation: NS, not significant.

identified one strain of *E. coli* and *K. pneumoniae* resistant to 14/18 (77%) and 13/18 (72%) of antibiotics tested.

Discussion

The overreaching goal of this study was to describe the epidemiology of *E. coli* and *K. pneumoniae* uropathogenic strains isolated in Franceville in South-eastern Gabon and to determine their resistance profiles to common antibiotics.

The epidemiological profile of uropathogenic bacteria varies from one region to another.[15] As a result, the knowledge of the local epidemiology, as well as its evolution is crucial in the selection of an effective first-line antibiotic therapy adapted to each region.[15] Of the 1086 patients' CBEUs, the prevalence of urinary tract infections was 29.2%. This prevalence is lower than 59.8% reported in a previous study in Cameroon.[16] *E. coli and K. pneumoniae* represented 44.9% of the germs involved in the etiology of urinary tract infections with 28.7% and 16.2% respectively. Such prevalence is similar to 24.5% for *E. coli* and 18.4% for *K. pneumoniae* reported in an earlier study in Nigeria.[17] However, the prevalence of *E. coli* found in UTIs is significantly lower than those between 60% and 90% reported in Chad;[18] Madagascar;[19] Rwanda[20] and Morocco.[21] We found that urinary tract infections due to *E. coli* mainly affected women, which is in agreement with similar studies carried out in Cameroon[22] and Nigeria.[23] This could be explained on the one hand by the proximity of the urethral, anal and vaginal orifices in women and on the other hand by poor hygiene practices as well as pregnancies responsible for a biased immune response favorable to microbial agents development in pregnant women.[22,24] We also observed that the urinary tract infection with *K. pneumoniae* was more frequent among young patients (≤ 5 years) and the older patients (≥ 50 years). Our results are in agreement with those reported in a previous study.[22] These two groups of patients are at higher risk.[22] Indeed, in the elderly, the risk of urinary tract infection increases due to the decline of the immune system efficiency and decreased functional autonomy.[8] In children aged ≤ 5 years, UTI incidence is about 5% in girls and 20% in uncircumcised boys. For febrile infants in the first two months of life and during the first 6 months, the risk of developing an UTI is 10 to 12 times higher in uncircumcised boys.[25] This susceptibility of developing UTIs could be attributed to a less robust immune system.[25]

E. coli and *K. pneumoniae* isolated strains have shown strong resistance to several antibiotics. The resistance of *E. coli* to Ampicillin and Ticarcillin was 65% and 61%, respectively. Similar resistance rates have been reported on an outpatient basis in West Africa,[26] Madagascar,[19] the Central African Republic[27] and Chad.[18] These high resistance rates could explain why aminopenicillins and carboxypenicillins are no longer recommended for the probabilistic treatment of UTIs.[28]

The resistance rates of *E. coli* and *K. pneumoniae* to amoxicillin-clavulanic acid were 45% and 47%, respectively. These prevalence are higher than those ranging between 25 and 35% reported in France.[8] However, this resistance rate is similar to that of 50% reported in Rwanda in patients of community origin.[20] The resistance of these two bacteria to amoxicillin-clavulanic acid reported in this study is much lower than that observed in Senegal, 60.17% for *E. coli* and 73.18% for *K. pneumoniae*, respectively.[29]

K. pneumoniae strains have shown a higher resistance rate of 76.09% to amoxicillin-clavulanic acid in Pakistan.[30] These high rates of antibiotic resistance could be explained by the broad probabilistic prescription of these antibiotics, particularly in outpatient medicine in the absence of CBEUs results.[22]

The resistance rate of *E. coli* to 3rd generation cephalosporins was 33% and 30% for cefotaxime and ceftazidime, respectively. These resistance rates were higher than those ranging from 0 to 5% observed in the Central African Republic,[27] Sudan,[31] and Madagascar.[19] The resistance of *E. coli* to ceftazidime was similar to that of 29.1% obtained in Rwanda.[20] On the other hand, the resistance of the strains of *K. pneumoniae* to 3rd generation cephalosporins was 56% for Cefotaxime and 47% for Ceftazidime. These resistance rates are in line with those reported by studies in Pakistan and Senegal, which were respectively 54.35% for 3rd generation cephalosporins[30] and 57.85% for Cefotaxime.[29] A very high 84% resistance rate of *K. pneumoniae* to Ceftazidime has been reported in India.[32]

This *E. coli* strains resistant to third-generation cephalosporins (C3G) could be explained by their acquisition of a plasmid-extended-spectrum beta-lactamase (ESBL).[8,24] Previous studies carried out in Gabon reported high prevalence of bacterial strains producing ESBLs.[33,34] In addition, the Global Antimicrobial Resistance Surveillance Report found that five out of six (5/6) and six out of six regions (6/6) reported *E. coli* and *K. pneumoniae*

resistance > 50% to C3Gs, respectively.[35] These findings were in agreement with the results of this study.

Carbapenems (Imipenem and Ertapenem) had good efficacy on *E. coli* and *K. pneumoniae* strains with resistance rates of 2% and 4%, which corroborated the results obtained by Leopold et al.[36]

Quinolones retained acceptable activity against *K. pneumoniae* strains with resistance rates of 13% to 28%. However, this resistance was higher in strains of *E. coli* ranging from 34% to 51%. An earlier study conducted in sub-Saharan Africa reported *E. coli* strain resistance to quinolones ranging between 0 and 98%.[36] The first-line use of fluoroquinolones as a probabilistic treatment against these bacteria could explain the emergence of their resistance to quinolones.[37] Aminoglycoside activity was conserved across all *E. coli* and *K. pneumoniae* strains with resistance rates ranging between 4% and 28%. Amikacin has been the most active antibiotic among the aminoglycosides. Resistance to aminoglycosides observed in this study was similar to that of 16.7% and 21.8% reported in Iran[38] and between 8% and 14% observed in Morocco.[21] The apparently preserved efficacy of aminoglycosides could be explained by their frequent parenteral administration, limiting use. In contrast, the resistance of *E. coli* and *K. pneumoniae* to Cotrimoxazole were 65% and 60%, respectively. The resistance rates to Cotrimoxazole of 80% in the Central African Republic; 69.5% in Madagascar and 55% in Morocco have been reported in previous studies.[19,21,27] This high resistance of *E. coli* and *K. pneumoniae* to Cotrimoxazole could explain its exclusion in the probabilistic treatment of uncomplicated urinary tract infections. Nitrofurantoin appeared to be the most active antibiotic against *E. coli* and *K. pneumoniae* uropathogenic strains.

We found that 57% of *E. coli* and *K. pneumoniae* isolated were MDR. The presence of these MDR strains suggests that inappropriate use of antibiotics without medical prescription is taking place. This is mainly due to the lack of clinical microbiology laboratory,[39,40] which tends to promote self-medication, increasing the risk of selection of resistant bacteria leading to the emergence of multidrug resistant bacteria.[41,42]

Conclusion

This study showed a high prevalence of urinary tract infections with great involvement of *E. coli* and *K. pneumoniae* strains. These bacteria have shown high levels of resistance to beta-lactams, quinolones and cotrimoxazole, while Nitrofurantoin, Amikacin, Imipenem and Ertapenem remain the most active antibiotics against *E. coli* and *K. pneumoniae* uropathogenic strains. The levels of bacterial resistance observed in this study requires the use of antibiotics adapted to the local epidemiology and the promotion of the antimicrobial resistance surveillance by the health authorities.

Abbreviations

CBEU, Cytobacteriological examination of urine; CFU, Colony forming unit; CLED, Cystine-Lactose-Electrolytes-Deficient; C3G, third generation cephalosporins; EMB, Eosine Methylene Blue; ESBL, Extended-spectrum-beta-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; GNB, Gram negative bacilli; McC, Mac Conkey; MDR, Multidrug resistance; MH, Mueller-Hinton; UTI, Urinary tract infection.

Data Sharing Statement

Data supporting the conclusions of this study will be made available on request to the corresponding author.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare that they have no conflicts of interest for this work.

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