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Antibiotic Resistance Profile of Gram-Negative Bacilli Isolated from Urinary Tract Infections at Laquintinie Hospital in Douala, Cameroon

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Abstract: Urinary tract infections, usually caused by bacteria that originate from the intestinal flora or the perineal flora, constitute a major public health problem for Africa in general, especially in Cameroon. Known failures with empiric therapy are becoming increasingly problematic. The same is proven true for the consistent bacterial resistance to antibiotics. The purpose of this study was to determine the frequency of Gram-negative bacterial strains isolated from urine samples at Laquintinie Hospital of Douala and to establish their antibiotic resistance profile. Over a period of three months, four hundred and thirty-six (436) urine samples were taken and analyzed at the bacteriology laboratory of the Laquintinie hospital in Douala. The Bacteria were isolated according to standard methods and identification using the API20E gallery. The antibiotic sensitivity profile of the isolated bacteria was done by the disk diffusion method according to the recommendations of the Antibiogram Committee of the French Society of Microbiology 2018. One hundred and fifty-one (151) bacterial strains were isolated, mostly from women (58.67%) with a male or female (M/F) ratio of 0.7. We noted a predominance of *Escherichia coli* strains (58.28%), followed by *Klebsiella Pneumoniae* (22.51%), *Proteus mirabilis* (4.64%), *Pseudomonas aeruginosa* (3.97%), *Enterobacter cloacae* (2.65%), *Acinetobacter* (1.98%), *Enterobacter sakazakii* (1.32%) and the rest 4.65%. All the bacterial strains isolated showed strong resistance to beta-lactam (greater than 50%) but were predominantly susceptible to Imipenem (96.39%) and Amikacin (90.47%). *Escherichia coli* was particularly resistant to beta-lactams such as amoxicillin (87.5%), amoxicillin + clavulanic acid (85.24%), Cefuroxime (65.57%), Cefixime (54.83%) and Ceftriaxone (52.70%). The bacterial strains of *Klebsiella pneumoniae* obtained a total resistance to amoxicillin (100%), clavulanic acid + amoxicillin (69.23%), Cefixime (68.42%), Ceftriaxone (52.70%), and Cefuroxime (52.00%). *Escherichia coli* (58.28%) and *Klebsiella pneumoniae* (22.51%) are the two main bacterial species that are the most isolated. Resistance of *Escherichia coli* and *Klebsiella pneumoniae* to antibiotics is a growing phenomenon. Monitoring for bacterial resistance to antibiotics is imperative in our context. Develop more effective prevention strategies, screening, prophylactic isolation.

Keywords: Urinary Tract Infection, Gram-Negative Bacilli, Antibiotic, Sensitivity Profile

1. Introduction

Urinary tract infections are one of the leading causes of consultation [1]. Microbiological examinations and intensive use of antibiotics are therefore extremely frequent in both community and hospital settings [2]. After respiratory infections, they rank second among the reasons for consultation and prescription of antibiotics and represent around 40% of nosocomial infections [2]. As such, they constitute a significant public health concern for our country. The germs most often involved are the Gram-negative bacilli, natural hosts of the intestine and the environment; among the latter: *Escherichia coli* is the most offending germ. *E. coli* is responsible in 85% of cases; *Klebsiella pneumoniae* comes second with 10% of cases, *Proteus mirabilis* comes third with 4% of cases, other Gram-negative bacilli (*Pseudomonas aeruginosa*) may be less often involved [1, 3]. Dependent on epidemiological data and results of cytobacteriological examination of urine, treatment occurs with the administration of antibiotics empirically. Known failures with empiric treatment are becoming more and more worrying; the same is true for the frequency of resistance of *Enterobacteriaceae* to antibiotics. Hostile environment to cope with the selection pressure exerted by antibiotics: creates resistance. This resistance is a significant factor complicating the treatment of urinary tract infections and the dissemination of multi-resistant strains due to excessive use of antibiotics in human medicine [4, 5]. Faced with this resistance, which is a significant factor complicating the treatment of urinary tract infections, this study's objective was to determine the antibiotic resistance profile of *Enterobacteriaceae* isolated from urinary tract infections at Laquintinie hospital in Douala.

2. Materials and Methods

2.1. Collection, Transport and Conservation

2.1.1. Collection

To those who were consenting and respected the collection conditions (no antibiotic, first urine of the morning, or urine having stayed at least 3 hours in the bladder except in babies), the urine was collected in the middle of the stream in sterile jars for single use. After careful hygiene of the perineal region in infants, urine collection was done by attaching a pediatric urine collection bag (Urinocol) to the urethral meatus. This bag had to be changed every 30 minutes after cleaning.

2.1.2. Transport and Conservation

The urine containers were clearly labeled and immediately transported to the laboratory, accompanied by a sheet containing all the necessary information (date, patient code, and time of collection), and then analyzed immediately. If not analyzed directly, samples could be stored no more than 2 hours at room temperature or no more than 24 hours at 4°C.

2.2. Bacteriological Analysis

2.2.1. Plating, Bacterial Isolation, and Identification

The 10µl calibrated loop technique was used. Thus, using a calibrated and sterile inoculation loop, 10µl of urine were deposited on Cystine Lactose-Electrolyte-Deficient Agar (CLED) and Eosin Methylene blue agar (EMB) medium, then spread, forming fine streaks. The dishes were then incubated in an oven at 37°C for 24 hours. After isolation of the bacteria and then Gram staining, the pure bacterial isolates were identified using commercial biochemical gallery (API 20E gallery to identify *Enterobacteriaceae*).

2.2.2. Study of Sensitivity to Antibiotics

The technique used was the disk diffusion method (antibiotic disks) on the agar medium. The antibiograms of strains were carried out on Mueller Hinton media following the technique recommended by the Antibiogram Committee of the French Society of Microbiology (AC-FSM 2018). The agar plates were inoculated using a sterile loop from a bacterial suspension standardized to the scale of 0.5 Mcfarland (an optical density equal to 0.2 to 650nm) per the recommendations of the AC-FSM 2018. Discs of antibiotics were placed on the surface of the agar using a sterile metal clamp. The antibiogram was carried out by measuring the diameters of the zones of inhibitions using a caliper. The results were compared to the critical values then the bacteria were classified: Sensitive, Intermediate, or Resistant according to AC-FSM 2018 standards.

2.3. Data Processing and Analysis

The data were collected, processed, and analyzed by Excel software (version 2010). The results were presented in tabular, graphical, or narrative form.

3. Results

3.1. Distribution of the Study Population by Gender and Age

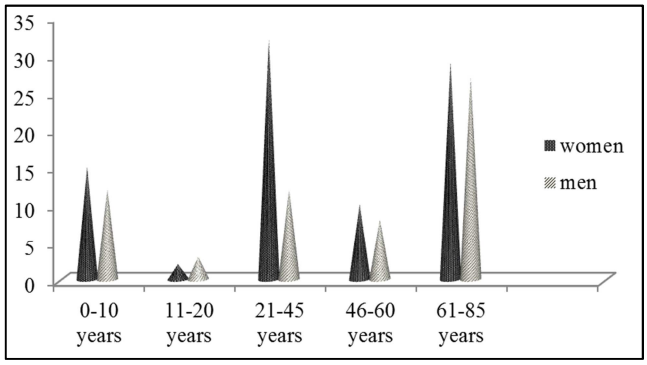

Figure 1. *Distribution of the study population by gender and age.*

Of the 150 study participants, women were more represented (n = 88) than men (n = 62) with respective rates of 58.67% and 41.33%. The ages ranged from 15 days old to

85 years old with an average of 17 years old; the age group, 61-85 years old, was the most represented with 56 participants (Figure 1).

3.2. Frequency of Bacterial Strains Isolated from Urine Samples

The most frequent 14 bacterial strains isolated were *E. coli* (58.28%) followed by *K. pneumoniae* (22.53%), as indicated in Table 1 below.

Table 1. *Bacterial strains isolated.*

Isolated germs	Frequency	Percentage (%)
E. coli	88	58.28
Proteus mirabilis	7	4.63
Acinetobacter spp	3	1.99
Pseudomonas aeruginosa	6	3.97
Klebsiella pneumoniae	34	22.53
Enterobacter cloacae	4	2.65
Aeromnas cavae	1	0.66
Enterobacter sakazakii	2	1.32
Citrobacter freundii	1	0.66
Citrobacter diversus	1	0.66
E. fergusonii	1	0.66
Pseudomonas fluorescens	1	0.66
Kluyvera spp	1	0.66
Aeromonas sobria	1	0.66
TOTAL	151	100

3.3. Resistance Profile of Bacterial Strains Isolated to Antibiotics

All the bacterial strains isolated exhibited a resistance more significant than 50.00% to beta-lactam: aminopenicillin of group A (Amoxicillin, 92.20%; amoxicillin + Clavulanic acid 93.51%,) and to a second-generation cephalosporin (Cefuroxime 92.86%) and third-generation (Cefixime, 68.94%, Ceftriaxone 61.60%). Resistance also includes intermediate strains. All bacterial isolates were predominantly susceptible to Imipenem 93.04% and Amikacin 80.41% (Table 2).

Table 2. *Resistance profile of the bacterial strains isolated to the antibiotics tested.*

Antibiotics	Acronyms	S (%)	I (%)	R (%)
Cefuroxime	CXM	6.50	27.86	65.00
Cefixime	CFM	31.06	5.84	63.10
Ceftazidime	CAZ	46.42	7.15	46.42
Ceftriaxone	CRO	38.39	4.46	57.14
Amikacin	AK	80.41	13.40	6.19
Tobramycin	TOB	62.68	16.41	20.90
Gentamicin	CN	43.03	15.19	41.77
Netilmicin	NET	79.48	18.82	7.69
Clavulanic Acid + Amoxicillin	AMC	6.48	12.96	80.55
Amoxicillin	AX	7.27	7.27	85.45
Aztreonam	AT	60.37	15.10	24.52
Imipenem	IMP	93.04	1.74	3.47
Levofloxacin	LEV	61.97	11.27	26.76
Ofloxacin	OFX	30.76	2.11	48.07
Ciprofloxacin	CIP	52.38	2.38	45.24
Fosfomycin	FF	57.47	24.13	18.40
Nitrofurantoin	NIT	52.11	21.12	26.76

3.4. Resistance Profile of E. coli to the Antibiotics Tested

E. coli and *K. pneumoniae* were the main bacteria isolated. For this reason, we wanted to highlight their resistance profile to the antibiotics tested. *E. coli* showed strong resistance to Clavulanic Acid + Amoxicillin (98.35%), to Amoxicillin (95.83%), and good susceptibility to Imipenem (93.94%), to Amikacin (75.00%), Nitrofurantoin (75.61%), as presented in Table 3.

Table 3. *Antibiotic resistance profile of E. coli.*

Antibiotics	Acronym	S (%)	I (%)	R (%)
Cefuroxime	CXM	6.56	27.87	65.57
Cefixime	CFM	35.48	9.68	54.83
Ceftazidime	CAZ	38.71	9.67	51.61
Ceftriaxone	CRO	41.49	5.40	52.70
Amikacin	AK	75.00	16.66	8.33
Tobramycin	TOB	60.87	15.22	23.91
Gentamicin	CN	43.75	14.58	41.66
Netilmicin	NET	77.27	9.09	13.63
Clavulanic Acid + Amoxicillin	AMC	1.64	13.11	85.24
Amoxicillin	AX	4.16	8.33	87.50
Aztreonam	AT	57.14	21.42	21.42
Imipenem	IMP	93.94	3.03	3.03
Levofloxacin	LEV	53.49	13.95	32.56
Ofloxacin	OFX	24.18	20.69	55.17
Ciprofloxacin	CIP	47.42	4.35	47.42
Fosfomycin	FF	70.59	17.64	11.76
Nitrofurantoin	NIT	75.61	14.63	9.75

3.5. Resistance Profile of K. pneumoniae to the Antibiotics Tested

The bacterial strains isolated from *K. pneumoniae* were resistant to Amoxicillin (100%) and sensitive to Imipenem (100%), to Amikacin (84.21%) to Netilmicin (100%) As shown in the table 4 following.

Table 4. *Antibiotic resistance profile of K. pneumoniae.*

Antibiotic	Acronym	S (%)	I (%)	R (%)
Cefuroxime	CXM	24.00	24.00	52.00
Cefixime	CFM	31.58	0.00	68.42
Ceftazidime	CAZ	52.94	5.88	41.17
Ceftriaxone	CRO	26.31	5.26	62.42
Amikacin	AK	84.21	15.69	0.00
Tobramycin	TOB	57.14	42.86	0.00
Gentamicin	CN	52.63	15.78	31.57
Netilmicin	NET	100	0.00	0.00
Clavulanic Acid + Amoxicillin	AMC	11.53	19.23	69.23
Amoxicillin	AX	0.00	0.00	100
Aztreonam	AT	75.00	8.33	16.66
Imipenem	IMP	100	0.00	0.00
Levofloxacin	LEV	78.57	7.14	14.28
Ofloxacin	OFX	40.00	40.00	20.00
Ciprofloxacin	CIP	50.00	0.00	50.00
Fosfomycin	FF	45.00	45.00	10.00
Nitrofurantoin	NIT	20.00	35.00	45.00

4. Discussion

A total of 151 strains were isolated from the urine of 151 patients belonging to 14 species of Gram-negative bacilli. We noted a predominance of the female sex in this

population with 58.67% against 41.33% of the male sex (i.e., a sex ratio of M / F of 0.70). This result is similar to that found by Mariko et al. [6], which was a sex ratio of M / F of 0.60. Risk factors such as the proximity of the urethral, vaginal, and anal openings, and the shortness of the urethra may explain this predominance of urinary tract infection in women [7]. We also found that the age range 61 to 85 was the most infected (37.34%). The levels of female hormones decrease with age. This hormonal decrease can lead to thinning and dryness of the walls of the urinary tract. What is more, this protective mucous membrane loses its acidity, reducing its ability to fight infection. Also, this could be explained by age with a weak or defective immune system and insufficient hygiene practices. This result goes against that of Dossim [8], who reports that the majority of strains were isolated from children aged 0 to 10 years (51.80%), and Zanella et al. [9], who studied epidemiology and diagnosis of urinary tract infections.

The identification of bacterial strains showed us that the most frequently isolated species were *E. coli* (58.28%) and *K. Pneumoniae* (22.53%). This result is appreciably similar to that of Ebongue [10] at the General Hospital of Douala with a percentage of 48.5% in *E. coli* and 32.8% *K. pneumoniae*, or those of Lyonga *et al*. [11], the World Health Organization (WHO) study found 36.60% of *E. coli* and 33.00% for *K. pneumoniae*, or that of Sekhsokh [12] at the Microbiology laboratory, Mohammed-V military training hospital who reported that the most incriminated germs were *E. coli* (44.70%) and *K. pneumoniae* (20.42%). Similarly, the work carried out in Tunisia by Ben Haj Khalifa [13] and in Lebanon by Imad Al Kassaa *et al.* [14] show that *E. coli* and *K. pneumoniae* were the germs most frequently isolated in urinary tract infections. This result is superimposable on those observed in Cameroon, Africa, and Europe [15, 16]. These results are because, in most cases, the bacteria that invade the lower urinary tract originate from our bodies *E. coli* usually lives in the intestine and can infect the urinary tract when feces contact the urethra.

Concerning resistance, considering as resistant strain a non-sensitive, a strain categorized as resistant or intermediate. Thus, we obtained a solid resistance to beta-lactams: aminopenicillins of group A (Amoxicillin, 92.2%; Amoxicillin + Clavulanic acid 93.51%); to CG2 (Cefuroxime 92.86%) and CG3 (Cefixime, 68.94%, Ceftriaxone 61.60%). These acquired resistances would be the consequence of the selection pressure due to the wide use of these antibiotics and their genetic determinism, which means that they have a tremendous power of dissemination [17]. 'Amoxicillin + Clavulanic acid (93.51%). Perhaps it is due to the prescription of Oxapenins, particularly in ambulatory medicine, even before having the results of the Cytobacteriological Examination of Urine [18] is not sufficient to restore the sensitivity of amoxicillin in strains producing penicillinases [19]. This resistance could be explained by a decrease in the activity of the beta-Lactamase inhibitor; this decrease may result from an overproduction of penicillinases or the inactivation of the inhibitor itself [20].

Resistance to Ceftriaxone (61.60%) is very different from that noted in a Tunisian study of 13.80% [3]. The emergence of bacterial strains resistant to this antibiotic is increasingly observed; this could be explained by the continued increase in the frequency of ESBL-producing strains in our hospitals [3]. Resistance to Ceftazidime (53.57%) is more excellent than that found by Tsolaki et al. [21], which was 18.30%, and those found by Moubareck *et al*. [22], which was 27.50%, as well as those found by Arai in 2001 at the American University Hospital. These results are similar to that of Zomahoun (75.00%) and those reported by the literature in Africa [7]. This increase in resistance may result fromacquiring resistance factors generally secondary to this antibiotic's massive and uncontrolled use [23]. Carbapenems (Imipenem) and monobactams (Aztreonam) showed a sensitivity of 93.04% and 60.37%. These results show similarities to those of Kassaa *et al*., [14] Lebanon report, which found 96.40% sensitivity with imipenemcompared to 6.40% and 77.90% to aztreonam. Kenmegne mean while reported a rate of 100% at the Imipenem. The resistance rates noted (6.90% and 39.63%) are due to the production of Extended Spectrum Beta-Lactamase (ESBLs) [20].

Aminoglycosides keep good sensitivity activity. Among them, amikacin and Tobramycin were the most effective with 80.41% and 62.68% sensitivities. This was reported by Duszynska et al. [24] with a rate of 86.00% for amikacin. The incidence of the emergence of aminoglycoside resistance has increased due to ESBL-producing strains [25], reported in other Tunisian studies [26].

We also noted a strong resistance of *E. coli* to b-lactams (amoxicillin 98.83, amoxicillin-clavulanic acid 98.35%). As for the bacterial strains of *K. pneumoniae*, we obtained a total resistance to amoxicillin 100% and clavulanic acid + amoxicillin 88.86%. Resistance is probably a consequence of inappropriate prescription, misuse of antibiotics, self-medication, and the resurgence of illicit points of sale and poor storage of antibiotics [23]; or by the fact that the genes responsible for resistance can be carried by the production of several enzymes [15]. In addition, the high level of resistance to antibiotics suggests a high risk of transition of multi-resistant bacteria [27–29]. In addition, the high level of resistance to antibiotics suggests a high risk of transition of multi-resistant bacteria [27–29].

5. Conclusion

From all the results obtained from this work, it emerges that *Escherichia coli* (58, 28%) and *Klebsiella pneumoniae* (22.51%) are the two leading bacterial species that are the most isolated, and they all have a resistance more significant than 50.00% with beta-lactams and third generation cephalosporins. With the increasing resistance of *Escherichia coli* and *Klebsiella pneumoniae* species to antibiotics, it is imperative to establish surveillance for bacterial resistance to antibiotics in our context. Develop more effective prevention strategies, screening, prophylactic isolation.

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