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Phenotypic Resistance of Bacteria Isolated from Urinary Tract Infections at the Protestant Hospital of Ngaoundere (Cameroon)

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Abstract

Aims and objectives: The frequent and unprescribed use of antibiotics has led to the development of resistance by microorganisms responsible for urinary tract infection (UTI). In order to facilitate the follow-up of this microbial resistance, the aim of this study was to determine the bacteria resistant phenotypes. **Method:** To achieve the following objectives, this study was conducted from June to August 2023. The isolation and identification were performed by standard methods why susceptibility testing was done by Kirby-Bauer disk diffusion technique according to CLSI guidelines. Double-disk synergy test was applied to determine the presence of extended-spectrum β-lactamase (ESBL) produced by bacteria. The Imipenem EDTA Combined Disc Test (CDT) for Metallo beta lactamase (MBL) screening, the D-zone test to detect macrolides, lincosamides and streptogramins type B (MLS_B) and Meticillin resistant *Staphylococcus aureus* (MRS A) which was assessed using a Cefoxitin (30 µg) disc. **Results:** A total of 40 bacteria were identified with a prevalence of 37.03%. Overall, *E. coli* was the predominant isolate 14 (35%), followed by *Staphylococcus aureus* 10 (25%) and *Klesbsiella pneumonia* 4 (10%). *Pseudomonas aeruginosa, Salmonella arinosa* and *Enterobacter* were the most sensible (100%) bacteria against ciprofloxin, ceftriaxone and cefixime. Almost all bacteria were resistant to Amoxicillin/clavulanic acid (>50%). The isolates were in the majority resistant to imipenem. ESBL-producing Enterobacteriaceae represented 25.92%, with a higher rate among *E. coli*. No MBL production was found among the isolates while 38.46% represented $cMLS_B$, 15.38% represented $iMLS_B$, 23.07% represented MS_B and 23.07% represented MRSA. **Conclusion:**

Clinical strains of UTI from Protestant Hospital of Ngaoundere exhibiting ESBL, $cMLS_B$, $iMLS_B$, MS_B and MRSA. The regular updating of antibiotic resistance statistics of isolated strains allows for a better adaptation of probabilistic antibiotic therapy.

Keywords

Enterobacteriaceae, Resistance Profile, Phenotypic Detection

1. Introduction

Urinary tract infections (UTIs) are frequent in human and are a very common cause of consultation and medical prescription in everyday practice. The infection can affect several parts of the urinary system, but the most common type is a bladder infection (cystitis). Kidney infections (pyelonephritis) are another type of UTI, less common but more serious than bladder infections [1] [2].

Worldwide, UTIs' prevalence is estimated at around 150 million people per year [3]. In 2019, more than 404.6 million had UTIs globally and nearly 236,786 people died of UTIs. At the Global Burden of Disease regional level, the highest age-standardised incidence rate in 2019 occurred in Tropical Latin America (13852.9 per 100,000 population) [4]. The prevalence of UTIs in Africa varies from country to country and geographical location. For instance, the prevalence rate is 15.9% in Ghana, 4.5% in Senegal, and 12.3% in Nigeria. On average, the overall prevalence of UTIs in the nine countries of sub-Saharan Africa was 32.12% [5]. In Cameroon, uropathogens were recovered from 137 (58.3%) specimens, with prevalence rates in Buea and Bamenda being 65.9% and 54% respectively [6].

The risk factors for urinary tract infection vary with country and geographical location. Personal hygiene, prostate problems, compromised immunity, sex, diabetes, and use of spermicidal contraception are some of the risk factors for UTIs [7]. UTIs are more common in women than in men [8]. UTIs involve a wide range of clinical manifestations, including acute, chronic, uncomplicated, complicated, asymptomatic, symptomatic and recurrent [9]. The microbial agents most frequently involved have a digestive origin, such as enterobacteria (*E. coli, Proteus mirabilis, Klebsiella pneumonia, Enterobacter cloacae,* etc) and Enterococci [10].

Moreover, the overusing of antibiotic therapy has contributed to the emergence of bacterial infections resistance. According to these facts, the high administration of antibiotics in and outside hospital without precautions has a major socioeconomic impact and is responsible for the increasing cases of antibiotic resistance in nowadays population [11].

Antibiotic susceptibility test is mandatory to be known, in order to ensure appropriate therapy according to the organisms that cause UTIs. Therefore, it is recommended for physicians to acknowledge more information about local susceptibility pattern of uropathogens. In Cameroon, Ngaoundere, to our knowledge,

due to the lack of multi-resistant bacteria surveillance, the extent of circulating bacteria resistant strains (phenotypes) is unknown, thus the reason for this study. This study is part of a research work, conducted by Department of Biomedical Sciences and it is of epidemiological, theurapeutic and etiological diagnostic interest.

2. Materials and Methods

2.1. Patients and Sampling

Patients admitted to the Protestant Hospital of Ngaoundere with UTIs and antibiogram were included. Before inclusion in the study, an informed consent was signed by all participants. This cross-sectional study was conducted from April 2023 to August 2023. Sociodemographic data for these patients were recorded based on the documented of clinical diagnostics in their medical files. Sampling was performed during the study periods from patient who did not receive antimicrobial drugs at least seven days. Urine samples were collected at morning before first urination or 4 hours after a last miction. The urine sample were immediately transferred to the microbiology laboratory in the Protestant Hospital of Ngaoundere.

2.2. Bacterial Culture and Characterization

The samples were processed according to the standard procedures and germs were identified according to the European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) [12]. In brief, specimens were inoculated to medium (Cled) plate and incubated at 37˚C for 18 H to 24 H. Microscopical examination of Gram-stained smears confirmed. Bacterial colonies were subjected to biochemical reaction (oxidase, catalase, API 20E). Susceptibility of isolated bacteria to various classes of antibiotics was determined on Mueller Hinton agar (MHA) by Kirby-Bauer disc diffusion method, using commercial disks according to Clinical and Laboratory Standards Institute (CLSI) [13]. The isolates germs were determined against Amoxicillin/clavulanic acid (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefuroxime, cefixime, imipenem (10 µg), gentamicin (10 µg), Tobamycin (10 µg), Erythromycin, azithromycin, ciprofloxacin (5 µg), Norfloxacin (30 µg), Nitrofurantoin (50 µg).

2.3. Determination of Resistant Mechanisms of Germs (Phenotypes) Responsible Against Antibiotics

The antimicrobial susceptibility testing of all isolates was done by the standard Kirby-Bauer disk diffusion method, using commercial disks according to Clinical and Laboratory Standards Institute (CLSI). When pure culture was obtained, a loopful of bacteria was then taken from a colony and transferred to a tube containing 5 mL of sterile distilled water and mixed gently until it formed a homogenous suspension. Then, the turbidity of the suspension was adjusted to the density of a McFarland 0.5 (Mary-l'Etoil, year) in order to standardize the inoculum size

[14] [15].

Antibiotic susceptibility testing was performed by Kirby-Bauer's disk diffusion method on Muller-Hinton agar whereby 50 µl of bacterium suspension prepared above was poured on to the surface of the agar. A swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar and the surface was allowed to dry for about 5 minutes before placing antibiotic disc. Individual antibiotic dispensers were placed onto the surface of the agar using a pair of sterile forceps and the latter was then incubated upside down for 24 hours at 37˚C.

Antibiotic disks used are selected in relation to the frequent treatment used by the clinicians and pharmaceutical presentation depends on the age of the patients. The antibiogram was interpreted based on CLSI guidelines as follows. The results were reported as sensitive and resistant.

2.4 Determination of the Resistant Phenotypic Profiles

2.4.1. Screening of *β*-Lactamases Enzymes

1) Screening of Extended-Spectrum *β*-Lactamase (ESBL) enzyme

Double-disk synergy test was applied to determine the presence of extended-spectrum *β*-lactamase (ESBL) production. Chances of ESBL enzyme screening were carried out if we observed any resistance in ceftazidime (caz ≤ 22 mm), or cefotaxime (ctx ≤ 27 mm) and cefodoxime ≤ 17 mm, ceftriazone (all third generation cephalosporins). A susceptibility disk containing amoxicillin-clavulanic acid was placed at the center of the plate, and disks containing ceftazidime (30 µg) was placed 30 mm from the amoxicillin-clavulanic acid (30 µg) disk. ESBL producing isolate was indicated by an extension of the inhibition zone of amoxicillin-clavulanic acid disk that is ≥5 mm than that of ceftazidime after 48 hours incubation at 37˚C aerobically and the result was noted [16].

2) Imipenem EDTA Combined disc test (CDT) for Metallo-betalactamase (MBL)

If any isolates from the antibiogram yielded a resistance in Imipenem or Ceftazidine or Meropenem, we proceeded to test for MBL production. An exactly 0.5 McFarland suspension of the pure bacteria culture was inoculated on the surface of Mueller-Hinton agar plate and then two imipenem (10 µg) and imipenem (10 µg) plus EDTA (20 µl of 0.1 M) disks was placed at distance of 25 mm apart and incubate the plate overnight at 37˚C. If the zone of inhibition (ZOI) of imipenem (10 µg) plus EDTA (20 µl) disks was greater than or equal to 5 mm than that of imipenem (10 µg), then the organism is an MBL producer [17].

2.4.2. Meticillin Resistant *Staphylococcus aureus*

An exact 0.5 McFarland suspension of the pure bacteria culture was inoculated on the surface of Mueller-Hinton agar plate and cefoxitin (10 µg) disks was placed and the plate incubate overnight at 37˚C. If zone of inhibition (ZOI) of Cefoxitin was less than or equal to 21 mm, the isolate was a Meticillin resistant *Staphylococcus aureus* (MRSA) strain (https://universe84a.com/collection/beta-lactamases/).

2.4.3. D-Zone Test (Erythromycin-Resistant *Staphylococcus aureus* (cMLS$_B$, iMLS$_B$ and MS Strains)

For detecting erythromycin and clindamycin resistance, 15 µg erythromycin disks and 2 µg clindamycin disks were used. Interpretation of the diameters of zones of inhibition was as follows: for erythromycin = 23 mm; S, 14 - 22 mm; I, = 13 mm; R, and for clindamycin = 21 mm; S, 15 - 20 mm; I, = 14 mm; R. Intermediate resistant strains were considered resistant. Erythromycin-resistant *S. aureus* strains were selected for further studies [5]. D-testing was performed for erythromycin-resistant *S. aureus* strains according to the guidelines of the CLSI. Suspension equivalent to 0.5 McFarland of each freshly cultured isolate in sterile distilled water was prepared and used for inoculation of Mueller-Hinton agar plates. Erythromycin and clindamycin disks were placed on inoculated plates 15 mm apart (edge-to-edge). Plates were read after 18 h - 24 h incubation at 35˚C and the shape of the clindamycin zone was verified. Strains resistant to both antibiotics were considered to have cMLS$_B$ resistance. Strains with flattening of the susceptible zone of inhibition to clindamycin adjacent to the erythromycin disk (D-shape) were considered to contain iMLS$_B$-resistance, while strains with circular zones were considered to contain MS resistance [18].

3. Results

3.1. Demographic Characteristics of Patients

Overall, 108 patient's urine samples were included in the current study. It shows that out of the 108, 30.56% (33) consisted of males while 69.44% (75) were females, a sex ratio of 2:27 in favour of the females (Table 1). The overall prevalence of UTI infection is 37.03%. Of them 23 (57.5%) females and 17 (42.5%) males were infected.

Table 1. Age and gender distribution in acquired UTI patients.

Gender	Age groups				
	≤20; n (%)	[20 - 40]; n (%)	[40 - 60]; n (%)	≥60; n (%)	Total; n (%)
Male	13 (12.04)	8 (7.41)	8 (7.41)	4 (3.70)	33 (30.56)
Female	6 (5.55)	61 (56.48)	8 (7.41)	0 (0)	75 (69.44)
Total	19 (17.59)	69 (63.89)	16 (14.82)	4 (3.70)	108 (100)
	Positive cases				
Male	11	3	0	3	15.74%
Female	1	20	0	2	21.29%
Positive case	12	23	0	5	40 (37.03)

3.2. Bacterial Isolates

In our study, *E. coli* was the most encountered *Enterobacteriae* with 35% (14/40) followed by *K. pneumoniae*, 10% (4/40), while *S. aureus* on the other hand was the most encountered Gram-positive cocci, 25% (10) as shown in Table 2 below.

Table 2. Prevalence of each isolate obtained.

Isolate germs	N° of isolates	N° (%)
Escherichia coli	14	35
Staphylococcus aureus	10	25
Klebsiella pneumoniae	4	10
Salmonella arizona	1	2.5
Pseudomonas	1	2.5
Enterobacter cloacae	1	2.5
Neisseria gonorrhoeae	3	7.5
Other gram negative rods	6	15
Total	**40**	**100**

3.3. Antimicrobial Susceptibility

Most bacterial isolates were found resistant to imipenem (72.5%), amoxicillin/clavulanic acid 57.5%, ceftazidime (45%) but maximum sensitive to tobramycin (82.5%), ciprofloxacin (7.5%), cefixime (12.5%), cefuroxime (12.5%). Table 3 shows the antibiotic susceptibility pattern of the all-bacterial isolates in this study. *E. coli* yielded a higher resistance rate for Amoxicillin/clavulanic acid (57.1%), ceftazidime (50.7%), and imipenem (71.4%) among *K. pneumoniae* more than 50% resistance was seen to gentamycin (75%), imipenem (100%), Amoxicillin/clavulanic acid (75%), ceftazidime (50%). *S. aureus* yielded a higher resistant rate to imipenem (100%) followed by erythromycin (70%) and azithromycin (50%).

Table 3. Antimicrobial resistant rates of Enterobacteriaceae (n = 30) and Staphylococcus (n = 10) collected from clinical urine specimens of patients.

Organisms / Antibiotics	*E. coli* (n = 14)	*K. pneumoniae* (n = 4)	*Staphylococcus aureus* (n = 10)	*Pseudomonas aeruginosa* (n = 1)	*Salmonella arizona* (n = 1)	*Enterobacter cloacae* (n = 1)	*Neissera gonorrhoeae* (n = 3)	Other gram negative rods (n = 6)	Overall resistance
Augmentin	57.1 (8)	75 (3)	40 (4)	100 (1)	100 (1)	100 (1)	66.7 (2)	50 (3)	57.5 (23)
Ceftazidime	50 (7)	75 (3)	30 (3)	100 (1)	0	0	66.7 (2)	33.3 (2)	45 (18)
Cefepime	7.1 (1)	0	20 (2)	100 (1)	0	0	0	66.7 (4)	20 (8)
Ceftriazone	4.9 (6)	25 (1)	10 (1)	0	0	100 (1)	0	__	22.5 (9)

Continued

Cefixime	21.4 (3)	50 (2)	20 (2)	0	0	0	33.3 (1)	__	12.5 (5)
Cefuroxime	7.1 (1)	25 (1)	10 (1)	100 (1)	100 (1)	0	0	16.7 (1)	12.5 (5)
Cefotaxime	21.4 (3)	50 (2)	10 (1)	100 (1)	__	0	0	__	17.5 (7)
Erythromycin	14.3 (2)	__	70 (7)	__	__	100 (1)	33.3 (1)	__	27.5 (11)
Azithromycin	21.4 (3)	0	50 (5)	__	0	0	0	__	20 (8)
Norfloxacin	0	0	40 (4)	0	__	__	0	__	10 (4)
Ciprofloxacin	21.4 (3)	0	0	0	0	0	0	__	7.5 (3)
Imipenem	71.4 (10)	100 (4)	100 (10)	100 (1)	100 (1)	100 (1)	66.7 (2)	__	72.5 (29)
Tobamycin	14.3 (2)	__	__	__	__	__	33.3 (1)	__	7.5 (3)
Gentamicin	14.3 (2)	75 (3)	0	100 (1)	__	0	__	__	15 (6)

3.4. ESBL and MBL *β* Lactamases Production

Out of 27 Enterobacteriaceae screened for ESBL production, 25.92% (7) were positive indicated by an extended of the zone of inhibition ≥ 5 mm of amoxicillin/clavulanic acid than ceftazidine. The highest frequency of ESBL enzymes was found among *K. pneumoniae* isolates (50%) (Table 4).

Table 4. Frequency of ESBL, MBL among Enterobacteriaceae members detected by DDST and imipenem EDTA combined disc test (CDT) phenotypic laboratory methods. *E. coli* presented the most ESBL phenotypes (57%), followed by *K. pneumoniae* (29%) and (14%) then other gram-negative rods.

Isolats	N° of isolates with ESBL	ESBL (%)	N° of isolates with MBL	MBL (%)	overall phenotype %
E. coli	4 (4/14)	28.57	0	0	57.14
K. pneumoniae	2 (2/4)	50	0	0	28.57
Other Gram-negative rods	1 (1/9)	16.67	0	0	14.28
TOTAL	7 (7/27)	25.92	0	0	99.99

ESBL: Extended spectrum beta lactamase MBL: Metallo beta lactamase.

3.5. MRSA and MLSB Production

Among the 10 *Staphylococcus aureus* isolate tested, 23.07% (3/10 strains) expressed the MRSA strain, with a ZOI ≤ 21 mm of Cefoxitin. For the D-test, 38.46% (5/10 strains) were found to exhibit the constitutive MLS_B resistance, and 15.38% (2/10 strains) the inducible MLS_B resistance, while 23.076% (3/10) expressed the MS_B resistance (Table 5).

Table 5. Frequency of $iMLS_B$, $cMLS_B$, MS_B and MRSA in *Staphylococcus aureus* detected by different phenotypic laboratory methods.

Tests	Phenotypes	No. of strains with resistance phenotype	No. (%)
D-TEST	$cMLS_B$	5	50
	$iMLS_B$	2	20
	MS_B	3	30
MRSA test	MRSA	7	70

D-test: disk-test; $iMLS_B$: Induicible Macrolide Lincosamide Streptogramin B; $cMLS_B$: Constitutive Macrolide Lincosamide Streptogramin B; MS_B: Macrolide Streptogramin B; MRSA: Meticillin resistant *Staphylococcus aureus.*

4. Discussion

In this research, 108 samples of urines were collected. Among these, 40 (37.03%) bacteria were isolated. This result is slightly similar to the one obtained in Nigeria (22%) [18]. The female gender (57.5%) was the most affected by UTIs than the male gender (42.5%). This can be due to the women's genital urinary anatomy, particularly the short urethra with proximity to the perianal area, which increases the chances of UTIs more than it is in men [19]. The vast majority of germs belonged to *E. coli at* 35%, *K. pneumoniae* 10%, *S. aureus* 25%.

The epidemiological profile of bacteria varies from one region to another. Therefore, knowledge of the local epidemiology and its evolution remains essential for the choice of an effective first-line antibiotic therapy adapted to each locality. The results of our study highlighted an involvement of *E. coli* strains (35%) in enterobacteria UTIs. This is clearly justified by the high prevalence of *E. coli* in these infections.

The study of antibiotic resistance of uropathogenic bacterial strains has highlighted variable resistance rates to the antibiotics tested, particularly to the main molecules used in the treatment of UTIs. The resistance rate was high to Amoxicillin/clavulanic acid (57.5%) and Ceftazidime (45%). A similar resistance rate was noted at Garoua [20] and Madagascar [21]. Even higher rates of resistance to Amoxicillin/clavulanic acid, up to 92%, have been reported by other studies [22]. These high resistance rates of enterobacteria justify that these β lactams are no longer recommended in probalistic treatment. The acquisition of resistance to Amoxicillin/clavulanic acid, a very widely used antibiotic in Cameroon, is a global phenomenon reported at very variable rates. In our study, the resistance of *E. coli* to Amoxicillin/clavulanic acid is 57.1% compared to 82.4% in India [23].

The mechanism of acquired resistance to B-lactams is enzymatic in nature by production of broad-spectrum B-lactamases. The involvement of enterobacteria producing broad-spectrum B-lactamases constitutes a public health problem. These high rates of ESBL production by enterobacteria confirm both the high

production of ESBL by *E. coli* strains and the wide spread of this resistance phenomenon in the community and hospital environments.

In this study, it is found that the prevalence of ESBL resistance among clinical isolates of Enterobacteriaceae in our hospital institutions was 25.92%. A study conducted in the city of Garoua, capital of the Northern region of Cameroon, found an overall high prevalence of uropathogenic E-ESBL was 19.3% among small children with suspected urinary tract infection [24]. Comparing the results of the two studies indicates that the prevalence of ESBL resistance among Enterobacteriaceae isolates in our institution has increased. Of all the 27 Enterobacteriaceae strains isolated, no MBL phenotypes were observed during our study as all the isolates displayed a 100% resistance to imipenem. In Cameroon, due to the lack of national multi resistant bacteria surveillance, the extent of circulating E-ESBL strains is unknown.

Also in our present study, *Stapylococcuss aureus* (25%) was the most frequent gram-positive cocci recovered from patients' clinical urine sample. Out of the 10 *Staphylococcus aureus* isolate, 23.07% expressed MRSA phenotypes, 38.46% displayed $cMLS_B$, 15.38% were $iMLS_B$ and 23.07% were MS_B phenotype. A study at a Korean hospital by Modukuru *et al.* (2021) [25] determined on *S. aureus* that 17.82% had inducible MLS_B, 27.01% had constitutive MLS_B, 55.17% had the MS phenotype. Thus, in all the staphylococci isolates, the level of constitutive MLS_B resistance was higher than the level of inducible MLS_B resistance.

5. Conclusion

The prevalence of UTIs was 37.03%. Good antibiotic activity was shown by aminoglycosides and quinolones. *E. coli, K. pneumonia* and *S. aureus* were the most encountered microorganisms. ESBL producers amongst the Enterobacteriaceae members and $cMLS_B$ in *S. aureus* were the most frequent and this might result in an increase in the resistant patterns of resistant isolates in our study area. The emergence and dissemination of multidrug-resistant uropathogenic bacteria constitute a real public health problem for the empirical management of urogenital infections. Updating data and monitoring the sensitivity of bacteria to antibiotics remains a key tool to reduce the extent of the phenomenon of multidrug-resistant bacteria.

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Authors' Contributions

Benjamin TANGUE TALOM: **Methodology, formal analysis, writing—reviewing and editing, visualization**. Berinyuy MONIRATOU: **Conceptualization, methodology, writing—original draft**. Zelda Inès EGUEN: **Methodology, writing—**

original draft. Michel Archange TAGNE FOKAM: **Formal analysis, reviewing and editing**. Carolle Sylvie DONGMO MEFFO**: Formal analysis, reviewing and editing**. Simeon Pierre CHEGAING FODOUOP**: Methodology validation, writing—reviewing and editing, supervision**. Jules-Roger KUIATE**: Methodology validation, writing-reviewing and editing, supervision, funding acquisition. All authors have read and approved the final manuscript**.

Ethics Statement

The studies were approved by the Ethics Committee of the University of Ngaoundere (Ref: N˚2023/0145/UN/R/DFS/CD-DSBM). The confidentiality of the study subjects' information was maintained in accordance with national and international regulations. Written informed consent to participate in this study was provided by patients or the participants' legal guardian/next of kin.

Conflicts of Interest

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

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