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Prevalence and resistance profile of *Escherichia coli* and *Klebsiella pneumoniae* isolated from urinary tract infections in N'Djamena, Tchad

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ABSTRACT

Bacterial resistance to antibiotics is a worldwide problem. In Chad, statistical data are scarce. The reason why this study was undertaken from June 2014 to December 2016, to identify the main *Enterobacteriaceae* responsible for urinary tract infections and their susceptibility to antibiotics. Germs were isolated and identified by standard microbiology methods and tested with antibiotics according to Kirby-Bauer technique. Data collected was analyzed using Excel and Statistical Package for Social Sciences (SPSS) version18.0. Out of the 503 urine samples analyzed, 93 *Enterobacteriaceae* were isolated (18.5%) of which 60 (64.5%) were *Escherichia coli*, 23 (24.73%) *Klebsiella pneumoniae* and 10 (10.75%) were other *Enterobacteriaceae*. Bacterial resistances to the following were observed: amoxicilline (96.66% - 100%), cefoxitine (13.33% - 30.4%), cefotaxime (33.3% - 56.52%), gentamycine (28.33% - 39.13%), and nalidixic acid (31.66% - 43.47%), trimethoprim - sulfamethoxazole (65% - 95.65%) and fosfomycine (8.33% - 13.04%). The present study identified two bacteria associated with urinary tract infections and their resistances to antibiotics commonly used in Chad. It is important to rationalize the use of antibiotics that have good antibacterial activity. Diversified studies in human and veterinary medicine are needed to better control the emergence of new resistance in N'Djamena.
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Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, urinary tract infection, resistance to antibiotics, N'Djamena.

INTRODUCTION

The resistance of *Enterobacteriaceae* to antibiotics is a worldwide problem (Callaway et al., 2010; Bonny et al., 2014; Nyawira et al. 2014; Ventola, 2015). In Chad, various sources report bacterial resistance to antimicrobials. However, these data need to be reinforced and consolidated in order to have a mapping reflecting the reality. In particular, the phenomenon of resistance is quite disturbing with the emergence of extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBLs). These strains are responsible for nosocomial and community-acquired infections (Arpin et al., 2003; Woodford et al., 2004). They are often

resistant to ß-Lactam commonly used in medicines. It appears that only cephamycin and carbapenem appear to be active against these infections (Bradford et al., 2001). Cases of resistance to ß-Lactamines are sometimes associated with resistance to other classes of antibiotics. These are aminoglycosides, quinolones and sulfonamides. These multi bacterial resistances are a challenge for clinicians because they considerably limit the therapeutic choice in the care of patients. *Enterobacteriaceae* are the most incriminated. These bacteria are often involved in urinary tract infections, septicemia with urinary starting point, deep abscesses and various infections. Since the prevalence of ESBLs may vary according to locality, organism and year, it is important for each country to continue to detect strains and the resistance profile of these bacteria to commonly prescribed antibiotics (Okorondu et al., 2013). Research in clinical samples is important and can better define the strategies for effective prevention of these formidable strains in hospitals. The objective of this study was to determine the prevalence of the two main *Enterobacteriaceae* isolated from urinary tract infections and their susceptibility to antibiotics commonly used in Chad.

MATERIALS AND METHODS
Study design and period

This prospective study was conducted in N'Djamena from June 2014 to December 2016 at the microbiology laboratory of the Mother and Child Hospital (HME). L'HME is a public reference hospital located in N'Djamena in the 3rd district. This hospital has a gynecology-obstetrics, pediatrics, neonatology, surgery; medical imaging and biology laboratory. It has 300 beds and employs a total of 697 people.

Determination of the sample size

The sample size were determined by the formula commonly used: $n = \frac{\varepsilon^2 p(1-p)}{i^2}$.
With: n = sample size; ε = value corresponding to a given confidence level (1.96 for a confidence level of 95%-value

commonly used); p = percentage (0.02) according to Yandai et al. (2014); i = standard error (0.05).

Culture and identification of bacteria

Urine samples were cultured on Cystine Lactose Electrolyte Deficient (CLED) plates and incubated at 37 °C for 18 to 24 hours. All bacteria developing on CLED agar were identified by their characteristic appearance, Gram strains, mobility, biochemical reactions (lysine decarboxylase, carbohydrate fermentation, indole production, methyl red, voges proskauer, citrate) using API® 20 E identification system (Biomerieux, Marcy l'étoile, France).

Antibiotic susceptibility test

Antibiotic susceptibility test was performed by Kirby Bauer disk diffusion method in respect to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). Pure culture of *E. coli* and *K. pneumoniae* was inoculated on Muller-Hinton agar plate (Liofilchem, Italy) with a depth of 4 mm. Bio-Rad antibiotic disk used were: amoxicillin / clavulanic acid (AMC) (20/10 μg), cefoxitine (FOX) (30 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg) , imipeneme (IMP) (10 μg), aztreoname (TMJ) (30 μg), cefepime (FEP) (30 μg) fosfomycine (FOS) (10 μg), gentamicine (CN) (10 μg)), amikacine (AK) (30 μg), tobramycine (TOB) (10 μg), nalidixic acid (NA) (30 μg), ciprofloxacin (CIP) (5 μg), ofloxacin (OFX) (5 μg) and trimethoprim-sulfamethoxazole (SXT) (1.25 / 23.75 μg). In the analyses, intermediary resistant and resistant isolates were classified as non-susceptible. *Escherichia coli* American Type Culture Collection (ATCC 25922) were used as quality control strains.

Detection of ESBL production

Detection of ESBL production was screened on Muller-Hinton agar using a double-disc synergy test (DDST) according to the procedure of Jarlier et al. (1988). The plates were inoculated with the strains as for standard disk diffusion test according to

EUCAST (2014). Antibiotic disks containing cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), and aztreoname (30 μg) disks were placed 30 mm (center to center) from an amoxicillin/clavulanic acid disk prior to incubation. After overnight incubation at 35 - 37 °C, the production of ESBL by the tested organism was detected by the presence of characteristic distortions of the inhibition zones, indicative of clavulanate potentiation of the activity of the test drug. Negative double-disk tests were repeated with a disk spacing of 20 mm (center to center).

Statistical analyzes

All outcome data were analyzed using Statistical Package for Social Sciences (SPSS) version18.0 software and Microsoft Excel 2010. The differences between resistance patterns of germs strains were determined using Chi-square test of Pearson. All differences in which the probability of the null hypothesis was p < 0.05 were considered significant.

Ethical consideration

This study has received an authorization N° 1025, signed on October 2013 by the General Director of the Hospital of the Mother and Child. The researches were carried out on the samples received at the laboratory for the bacteriological diagnoses and the care of the patients. The results were given to doctors for the treatment of people suffering from urinary tract infections.

RESULTS
Prevalence of germs isolated

Table 1 presents the different germs isolated in our study. From a total of 503 urine samples analyzed, 93 (18.5%) strains were isolated, including 60 strains of *E. coli* (64.5%), 23 of *Klebsiella pneumoniae* (24.7%) and 10 other strains. These results show that *E. coli* and *K. pneumoniae* were two major bacteria involved in urinary tract infections.

Resistance profile of *E. coli* and *Klebsiella pneumoniae*

Resistance profile of *E. coli* and *Klebsiella pneumoniae* are shown in Table 2. For *E. coli* strains, the resistances were high to four antibiotics: amoxicillin (96.66%), trimetoprim-sulfamethoxazole (65%) and cefalotine (58.33%), ticarcilline (50%). They were low to ofloxacine and ciprofloxacin (25% - 26.6%), gentamicin and tobramycin (28, 33% - 25%). It was very low to fosfomycine (8.33%), cefoxitine (13.33%) and amikacine (15%). However, with *Klebsiella pneumoniae*, these resistances appeared to be higher in eight antibiotics: amoxicilline (100%), trimetoprim-sulfamethoxazole (95.65%), cefalotine (69.56%), cefazoline (65%, 21%), amoxicilline + clavulanic acid (52.17%), ticarcilline (52.17%), cefotaxime (56.52%) and piperacilline (47.82%). For the two germs, resistance was average with some antibiotics and low with fosfomycine. Imipeneme was the only active molecule on all isolates studied.

Phenotype of β-lactamase resistance of *E. coli* and *Klebsiella pneumoniae*

Of the 60 strains of *E. coli,* only 2 strains were identified as sensitive to all antibiotic tested (3.33%) and 58 (96.66%) were resistant with various phenotypes to β-lactamase. Phenotypes detected were the low-level penicillinase (38.33%), high-level penicillinase (3.33%), low-level cephalosporinase (5%), high-level cephalosporinase (16.7%) and extended spectrum β-lactamase (ESBL) (33.33%). For *K. pneumoniae* tested (n = 23), the phenotype also were low-level penicillinase (30.43%), low-level cephalosporinase (8.7%), high-level cephalosporinase (4.3%) and ESBL (56.52%). Figure 1 show the different β-lactamase phenotypes detected.

Table 1: Rate of different *Enterobacteriaceae* isolated.

Bacteria	Number (n)	Percentage (%)
Escherichia coli	60	64.52
Klebsiella pneumonia	23	24.73
Enterobacter cloacae	2	2.15
Proteus mirabilis	3	3.23
Citrobacter freundii	2	2.15
Morganella morganii	2	2.15
Salmonella **sp**	1	1.08
Total	93	100

Table 2: Antibiotic resistance rates of *E. coli* and *K. pneumoniae*.

Antibiotics	*E. coli* (n = 60)		*K. pneumoniae* (n = 23)	
	n (% R)	P value	n (% R)	P value
AMX	58 (96.66)	0.035	23 (100)	NS
AMC	18 (30)	0.813	12 (52.17)	< 0.001
CEF	35 (58.33)	< 0.001	16 (69.56)	0.002
CFZ	30 (50)	< 0.001	15 (65.21)	< 0.001
PIP	13 (21.7)	0.006	11 (47.82)	< 0.001
TIC	30 (50)	< 0.001	12 (52.17)	< 0.001
FOX	8 (13.33)	0.039	7 (30.4)	0.005
CRO	20 (33.3)	0.001	13 (56.52)	< 0.001
CTX	20 (33.3)	0.001	13 (56.52)	< 0.001
CAZ	20 (33.3)	0.001	13 (56.52)	< 0.001
ATM	20 (33.3)	0.001	13 (56.52)	< 0.001
FEP	20 (33.3)	0.001	13 (56.52)	< 0.001
IMP	0 (0%)	NS	0 (0%)	NS
FOS	5 (8.33)	0.112	3 (13.04)	0.103
CN	17 (28.33)	0.001	9 (39.13)	0.001
AMK	9 (15)	0.027	5 (21.73)	0.027
TOB	15 (25)	0.002	9 (39.13)	0.001
NA	19 (31.66)	0.001	10 (43.47)	0.001
CIP	16 (26.66)	0.001	8 (34.78)	0.002
OFX	15 (25)	0.002	8 (34.78)	0.002
SXT	39 (65)	< 0.001	22 (95.65)	NS

n = number; % R = resistance rate; % S = sensitivity rate, AC = clavulanic acid
Exact P-values were determined by the X^2 test. P-value < 0.05 was considered statistically significant, NS: Non-significant.
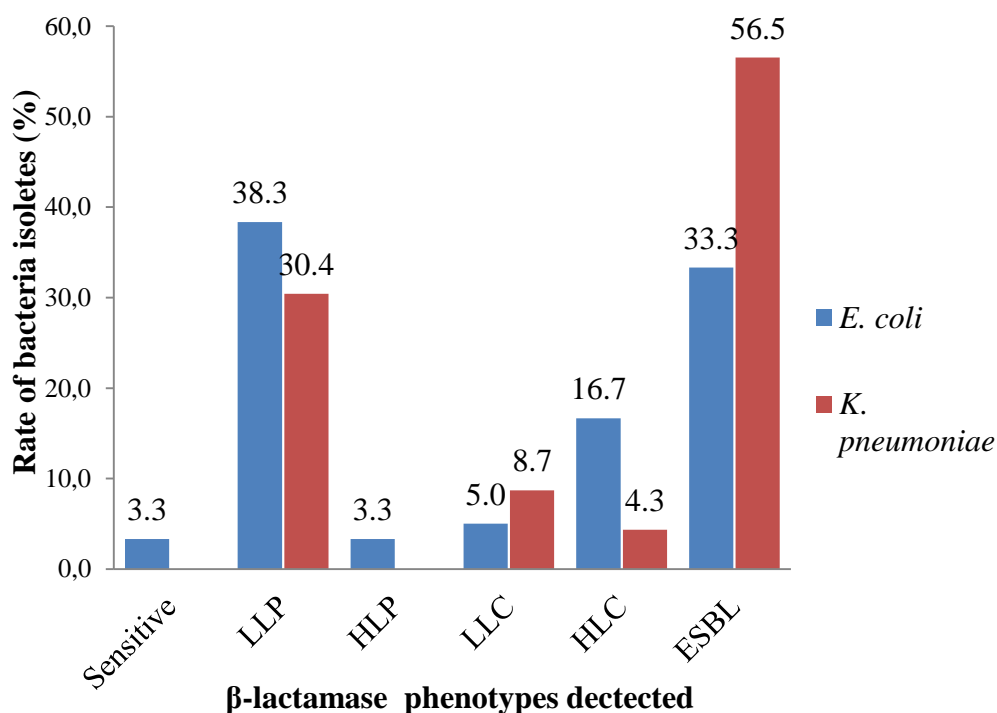
Figure 1: Different β-lactamase phenotype among *E. coli* and *K. pneumoniae*.
LLP: low-level penicillinase. HLP: high-level penicillinase. LLC: low-level cephalosporinase. HLC: high-level cephalosporinase. ESBL: extended spectrum β-lactamase.

DISCUSSION

The present study reveals that *E. coli* and *K. pneumoniae* are two major *Enterobacteriaceae* responsible for urinary tract infections in Chad. These bacteria are usually commensals of the human and animal digestive tract. The poor hygiene and asepsis conditions make these bacteria often involved in various human pathologies, including urinary tract infections, septicemia of urinary origin, adenitis, deep abscesses, neonatal meningitis and other infections. This result corroborates the work of Ebongue et al. (2015) in Cameroon, Raji et al. (2013) in Nigeria and Moutachakkir et al. (2015) in Morocco. Analysis of the susceptibility of isolates to β-lactams showed variable resistance levels. The resistance of *E. coli* to amoxicilline was very high. On the other hand, the resistance of *K. pneumoniae* to amoxicilline is natural because of the presence of a gene coding for chromosomal penicillinase of the SHV-1 type. This so-called low level penicillinase inactivates amoxicillin, ticarcilline and in some cases piperacilline. It is sensitive to clavulanic acid. Sensitivity tests performed on amoxicillin + clavulanic acid showed significant resistance of the isolates to this molecule. This bacterial resistance to clavulanic acid could be related to the production of high-level penicillinase by isolated organisms. These resistance rates are comparable to those of Ferjani et al. (2009) in Tunisia.

As regards cefalotin and cefazolin, considerable resistance has been noted. These antibiotics are frequently inhibited by various bacterial enzymes. There are currently enteric bacterial strains carrying penicillinase varieties: TEM, SHV and OXA. TEM type enzymes are present in some *E. coli* strains. These enzymes are sometimes able to inactivate cefalotine and cefazoline. The frequently cited mechanism of resistance is

the production of low-level, high-level cephalosporinase that render cefalotine and cefazoline ineffective, respectively.

Resistance rates greater than 30% have been observed with third-generation cephalosporin (CRO, CTX, and CAZ). These rates were far higher with *K. pneumoniae* than *Escherichia coli*. The resistance mechanisms highlighted are mainly the production of the ESBL. This enzyme already existed in *Kluvyera* spp. It was subsequently detected in *K. pneumoniae*. The identified resistance carrier is a plasmid capable of spreading in *E. coli* and other *Enterobacteriaceae*. In the present study, the causes associated with high levels of ESBL could be the excessive consumption of ceftriaxone and cefotaxime found in referral and peripheral hospitals. This excessive use and often based on a bundle of clinical argument, without bacteriological examination. This practice could contribute to the selection of multi-resistant strains. The results of this study are comparable to those of Nadmi et al. (2010), Ahmed et al. (2012), Ndoutamia et al. (2014), Sumera et al. (2014) and Dissinviel et al. (2017). Isolated strains in hospitalized patients were more resistant than those detected in outpatients. This is probably due to the fact that the hospital environment can favor the endemicity of certain strains when the hygiene measures are not effective. Similar study reported that *E. coli* strains isolated in hospitals seem more endemic in long-stay facilities (Ruppé, 2010). Patient records were not consulted to identify the most exposed services to ESBL-producing bacteria. However, this result is similar to that of Mkaouar et al. (2008) who report considerable rates of *K. pneumoniae* isolated in the intensive care unit (48%), the hemato-oncology department (27%) and the pediatric ward (25%). in Tunisia

For cefoxitine, moderate resistance was observed for *E. coli* and *K. pneumoniae*. This antibiotic is a good marker of bacterial resistance through the impermeability mechanism. This could be explained by the use of other mechanisms of resistance. ESBL producing strains are sometimes sensitive to cefoxitine. However, only carbapeneme

(imipeneme) were effective on all strains tested. It is important to emphasize that these molecules have just been introduced in Chad. These antibiotics are protected and prescribed by a very small group of doctors. This very rational and rigorous management could explain the lack of resistance to imipeneme found in this study. This result confirms the work of Ndoutamia et al. (2015) in Chad. However, it differs from Nordmann et al. (2009) in France and Barguigua et al. (2013) in Morocco where resistance of *K. pneumoniae* to imipeneme was significant.

The efficiency test for other classes of antibiotics showed high resistance to nalidixic acid, gentamicine, tobramycine and sulfamethoxazole-trimethoprime. This situation could be the consequences of the pressure of selection due to inappropriate prescriptions and the misuse of broad-spectrum antibiotics in both community and hospital. In Chad, antibiotics can be acquired freely and over the counter. People uninformed about the harmful effects of antibiotics or poor use antibiotics sold on the street or on market stalls. In hospitals, antibiotic therapy is mainly probabilistic because of the limited number of bacteriology laboratories. All these different factors contribute to the emergence of multiresistant strains

Quinolone resistance may be related to the acquisition of resistance genes. According to Guessennd et al. (2008), three types of genes are involved in the resistance of *Enterobacteriae* to quinolones. These genes were "qnr" genes, genes encoding N-acetyl transferase, ACC- (6 ') - Ibcr and genes encoding the QepA efflux pump. The association of these genes with the resistance mechanisms to β-lactam and aminoglycoside may induce resistance to fluoroquinolone, including ciprofloxacin and ofloxacine. These resistance levels obtained are higher than those reported by Ndoutamia et al. (2015). Such a difference could be explained by the growth of bacterial resistance to quinolone during the last four years.

Concerning trimethoprim-sulfamethoxazole, resistance can be linked to

the existence of a gene capable of inducing resistance to different families of antibiotics. The *qnr* A, B and S genes are present in some *E. coli* and *K. pneumoniae*. These genes are responsible for plasmid resistance to trimethoprim-sulfamethoxazole, cephalosporin and aminoglycosides (Guessennd et al., 2008).

In contrast, fosfomycine was effective on most strains tested. This molecule is rarely prescribed in Chad. Its indication is much more restricted than quinolones, trimethoprim-sulfamethoxazole and aminoglycoside. This good bacterial sensitivity towards this antibiotic has also been reported by El Bouamri et al. (2014) in Morocco.

Conclusion

The importance of the resistance of *Escherichia coli* and *Klebsiella pneumoniae* observed in this study probably reflects the intensive prior use of antibiotics in medical settings. The strains were essentially resistant to old molecules of the quinolone family, trimethoprim-sulfamethoxazole and aminoglycoside. On the other hand, fosfomycine seems more effective probably due to its relatively recent introduction in Chad. This situation should encourage the rational and controlled use of antibiotics through increased awareness of stakeholders and the establishment of appropriate consultation and regulatory frameworks.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

FHY conceived the study, monitored the experimental and laboratory work, analyzed data, drafted and finalized the manuscript for publication. GN, BN and NB read and approved the final manuscript.

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