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Antimicrobial resistance in extended spectrum β-lactamases (ESBL)-producing *Escherichia coli* isolated from human urinary tract infections in Ndjamena, Chad

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The prevalence of antibiotic resistance among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* has increased markedly in recent years. The purpose of this work was to investigate the occurrence and the antibiotic susceptibility of ESBL-producing *E. coli* in the urinary tract of the patients in Chad. Clinical strains of *E. coli* were isolated onto CLED agar and tested for ESBL production by using the double disk synergy test. Susceptibility to antibiotics was tested according to the guidelines of Clinical Laboratory Standards institute. Out of 283 cultures tested, 57 (20.14%) were positives for urinary tract infections of which 31 (54.5%) were identified as *E. coli* resistant to third generation cephalosporins. All these *E. coli* isolates expressed various level of resistance to antibiotics tested. Among these strains, 77.41 were detected to be ESBL-producers and 22.58% were non-producers. Moreover, resistance to the third generation cephalosporins was associated with significant cross resistance of 62, 38, 79, 70 and 95% to gentamicin, amikacin, nalidixic acid, ciprofloxacin and trimethoprim-sulfamethoxazole, respectively. However, cefoxitin and imipenem were found to still be efficient against the ESBL producers. These findings indicate that the trend was towards increased spread of ESBL-producing *E. coli* that could restrict the choice of antibiotic for the treatment of urinary tract infections in Chad.

Key words: *Escherichia coli*, resistance, antibiotic, extended-spectrum β-lactamase (ESBL), Chad.

INTRODUCTION

Escherichia coli is the primary pathogen of urinary tract infections (UTI), responsible for 75 – 95% of cases of morbidity in the community (Falagas et al., 2008). The emergence of extended-spectrum β-lactamase (ESBL)-producing *E. coli* restricted the choice of antibiotics for treatment of UTI, because β-lactamase resistance is often associated with cross resistance to other families of antibiotics (Messai et al., 2006). In Europe, according to the High Council of Public Health, ESBL-producing *E. coli* were 10% in Italy and Greece; 20% in Romania and

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Bulgaria; 28% in Turkey. In France, the rate of resistance was 75% to tobramycin, 35% to gentamicin, 25% to amikacin and 70% to ciprofloxacin (HCSP, 2010). In Africa, the resistance phenotype in *E. coli* has been reported in Nigeria, Cameroon, Benin, Algeria and Morocco (Gangoue et al., 2006; Ahoyo et al., 2007).

Whilst there is limited information concerning antimicrobial resistance in Africa, there are no data related to the subject in Chad. Therefore, this work constitutes the first attempts to evaluate antibiotic resistance.

MATERIALS AND METHODS

Study types and population

This prospective study was carried out from the 1st December 2012 to the 30th June 2013 at the "Hôpital de la Mere et de l'Enfant" of N'Djamena in Chad. It is a reference hospital for the treatment of the children from 0 - 14 years and women. This hospital has a capacity of 295 beds and 598 employees. In this study, the demographic variables noted were the age of patients and their provenance (inpatient or outpatient).

Specimen collection and isolation of *E. coli*

The urine samples from patients were collected in sterile disposable bottles and appropriately labelled. The specimens were transported immediately to the microbiology laboratory for bacteriological analysis. The samples were seeded onto Cystiene Lactose Electrolyte Deficient Agar and incubated at 35 - 37°C for 18 - 24 h. The colonies which grew on the CLED agar were suspected to be Gram-negative bacilli and were sub-cultured on Mueller Hinton agar for purification. Isolates were identified by Gram strains, indole production, Methyl Red, Voges Proskauer and citrate tests, and then confirmed by API 20 E identification system (bioMerieux).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing of the isolated organisms was performed by the disk diffusion technique according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2011). The following antimicrobial agents were tested: amoxicillin/clavulanic acid (20/10 µg), cefoxitin (30 µg), cefotaxim (30 µg), ceftazidim (30 µg), imipenem (10 µg), aztreonam (30 µg), gentamicin (10 µg), amikacin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg). Data were reported as sensitive, intermediate or resistant. The antibiotic potency of the disks was standardized against the reference strains *E. coli* ATCC 25922.

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 *E. coli* strains as for standard disk diffusion test. Antibiotic disks containing aztreonam and expanded-spectrum cephalosporins were then 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).

Figure 1. Strain of *E. coli* showing positive DDST when swabbed on Mueller Hinton Agar and incubated with cefotaxim (CTX), ceftazidim (CAZ), ceftriaxone (CRO), aztreonam (ATM) applied 30 mm from the amoxicillin/clavulanic acid (AMC).

Statistical analysis

Laboratory results and data collected on the patients were performed using Microsoftt Excel (2010) and analyzed through Statistical Package for Social Sciences (SPSS) version19. Data Analysis of ESBL productions and resistances to antibiotics was made by Person chi-square for the comparison of two quantitative variables. The differences were considered significant when $p < 0.05$.

Ethical consideration

This study was authorized by the head office of hospital of Mother and Child of N'Djamena. The research was carried out on the samples received by the laboratory for clinical diagnoses. The results were given back to the doctors for the patients' treatment.

RESULTS

Prevalence of ESBL-producing strains

Figure 1 shows characteristic distortion of the inhibition zones obtained between third generation cephalosporins (CTX, CAZ and CRO), aztreonam (ATM) and amoxicillin /clavulanic acid (AMC) distant to 30 mm.

Out of 283 samples tested, only 57 cultures were positive for UTI infections. Among the 57 isolates, 31 (54.5%) were identified as *E. coli*, of which 24/31 isolates (77.41%) were ESBL producers and 22.58% ESBL non-producers. The variation of prevalence of ESBL-

Table 1. Number and rate of ESBL-producers and ESBL non-producers by age group.

Age of patient	Sample	*Escherichia coli* isolated		
		Isolates	ESBL $_{Producers}$ (%)	ESBL $_{Non\ producers}$ (%)
0 - 24	97	5	2 (8.33)	3 (42.85)
25 - 44	186	26	22 (91.66)	4 (57.14)
Total	283	31	24 (100)	7 (100)

Table 2. Resistance of ESBL producing *E. coli* to antibiotics tested.

Antibiotics	*E. coli* isolated from urines (n = 31)		
	ESBL $_{Non\ producer}$ (%) (n=7)	ESBL $_{Producer}$ (%)(n=24)	
	R (%)	R (%)	*P*
AMC	1(14)	14(58)	0.040
FOX	0(0)	1(4)	0.583
CRO/CTX	1(14)	24(100)	0.000
CAZ	1(14)	22(92)	0.000
ATM	0(0)	22(92)	0.000
IMP	0 (0)	0 (0)	-
CN	1(14)	15(62)	0.025
AK	0(0)	9(38)	0.054
NA	1(14)	19(79)	0.002
CIP	2(29)	17(70)	0.008
SXT	2(29)	21(95)	0.000

AMC (amoxicillin+clavulanic acid); FOX (cefoxitin); CRO (ceftriaxon); CTX (cefotaxim); CAZ (ceftazidim); ATM (Aztreonam); IPM (Imipénème); CN (Gentamicin) AK (amikacin); NA (nalidixic acid); CIP (ciprofloxacin); SXT (trimethoprim/sulfamethoxazole); R (%): Resistance (percentage), *P*< 0.05 was considered statistically significant. ESBL $_{Producer}$: ESBL positive to DDST; ESBL $_{Non\ producer}$: ESBL negative to DDST.

producers according to age is presented in Table 1. Of the 24 ESBL-producing *E. coli*, 2 (8.33%) were between the age range of 6-24 years and 22 (91.66%) 25 - 44 years. These results show that adults are often affected by ESBL-producing bacteria than children. ($p<0.05$)

Antibiotic susceptibility

The resistance to antimicrobials tested for ESBL non-producers and ESBL producers is presented in Table 2. The resistance rate of ESBL-producers was 58% to amoxicilline + clavulanic and 4% to cefoxitin. Moreover, all ESBL-producers were resistant to third-generation cephalosporins (CRO, CTX) (100%). None of the strains was practically resistant to Imipenem. The co-resistance to gentamicin, amikacin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole was 62, 38, 79, 70 and 95%, respectively.

DISCUSSION

In the present study, 31 out of 57 isolates were identified as *E. coli* of which 24 (77. 41) are ESBL-producers and 7 (22. 58) non-producers. Thus, the overall prevalence of ESBL producers was 8. 4% (24/283).

Observations of a high proportion of ESBL-producers among strains of *E. coli* analysed are disturbing because ESBL resistance is often associated with cross resistance to other families of antibiotic. However, these findings were in agreement with previously published results that show simultaneous resistance to both β-lactam and antibiotic of other groups.

Indeed, surveillance data showed that resistance in *E. coli* is consistently highest for antimicrobial agents that have been used for long time in human and veterinary medicine (NARMS, 2010). Moreover, *E. coli* is sometimes used as a sentinel for monitoring antimicrobial drug resistance in faecal bacteria because it is found more frequently in wide range of hosts, acquire resistance easily (Erb et al., 2007), and it is a reliable indicator of resistance in Salmonellae (Chijioke and Christian, 2013). It was also shown that *E. coli* strains can efficiently exchange genetic material with pathogen such as *Salmonella, Shigella, Yersina* and *Vibrio* species as well as pathogenic *E. coli*. This could explain the ease with which resistance develop in *E. coli*. The data generated in this study sound a warning because the indiscriminate use of antibiotics along with poor hygiene and infection

control are highly prevalent in Chad and others developing countries.

A recent study reported a prevalence of 1.3% of ESBL-producing *E. coli* in Morocco (Barguigua et al., 2011). A similar study conducted In Nigeria showed a prevalence of 26.4% ESBL-producers in Ebonyi State (Iroha et al., 2009) and 15% in Kano in North West Nigeria (Yusuf et al., 2013). It was 14.3% in Yaoundé (Cameroon).

As far as the other continents are concerned, the prevalence of ESBL-producing isolates of *E. coli* were 0.7% in Bosnia and Herzegovina (Uzunovic-Kamberovic et al., 2006), 9.2% in Korea, 10.3% in Arabia, 13.3% in Lebanon and 17% in Turkey (Ananthan and Subha, 2005). Other data have shown that ESBL-producing *E. coli* are found to be the highest, 60% in India (Hsueh et al., 2011) and 57.8% in Israel (Colodner et al., 2004), followed by Hong Kong (48%) and Singapore (33%) (Hsueh et al., 2011).

In vitro antimicrobial susceptibility revealed a rate of resistance of 100% to cephalosporins (CRO, CTX). This rate is much higher than the ones reported in Nigeria, where the rate of resistance in *E. coli* to ceftriaxon was 74.5%. However, our results were similar with the data obtained in Spain (Colodner et al., 2004). In other work, human *E. coli* isolates recovered in 1997 showed resistance to ceftiofur and ceftriazone. The same isolates were also resistant to other antimicrobial drugs. Moreover, studies showing decreased susceptibilities to ceftiofur and ceftriaxone showed carriage of *blacmy* allele that conferred resistance to cephalothin, ampicillin and amoxicillin/clavulanic acid in *Salmonella* (Shaohua et al., 2005).

The co-resistance to thrimetoprim-sulfamethoxazole was the most common co-resistance phenotype (95%) followed by resistance to Nalidic acid (79%), ciproflaxin (70%), gentamicin (62%), amoxicillin (58%) and amikacin (38%). However, cefoxitin and imipenem were the most effective antibiotics tested.

Co-resistance to different antibiotics within the same isolate, as detected in this study has also been reported in other countries. The ESBL producers were resistant to different antibiotics families including the β-lactams, fluoroquinolons, aminoglycosides and trimethoprim /sulfamethoxazole. The resistance to amino-glycosides was also significant with gentamicin (62%) and the amikacin (38%). Similar rate of resistant to gentamicin were reported in Nigeria, 80% in UNTN and 87% in ESUTH (Iroha et al., 2009). For the amikacin, 25% of resistances have been reported in Israel (Bishara et al., 2005).

As far as quinolone are concerned, high level resistance were observed with nalidixic acid (79%) and ciprofloxacin (70%). In Soudan, these rates were 72% to nalidixic acid and 58.4% to ciprofloxacin (Ibrahim et al., 2012). The rate of resistance to ciprofloxacin can be compared to the data obtained in Israel, Spain, London and Nigeria which were 72.05, 77, 91.3 and 80.9%, respectively (Aruna and Mobashshera, 2012; Iroha et al., 2009; Melzer and Petersen, 2012; Tamayo et al., 2007).

Trimethoprim-sulfamethoxazole resistance was among the highest in our study (95%). Similar rates have been reported in several studies: 91% in Nigeria, 88.3% in Soudan (Ibrahim et al., 2012) 81% in Pakistan (Ullah et al., 2009) and 82% in India (Supriya et al., 2004). In France, resistance to trimethoprim-sulfamethoxazole in *E. coli* varies from 50 to 80% (Goldstein, 2006). The combination of trimethoprime/sulfametoxazole is extensively used in Chad owing to its antimicrobial spectrum of activity and its low cost (Goldstein, 2006). In addition, ESBL-production is usually associated with resistance to non β-lactam antibiotic such as aminoglycosides, fluroquinolones and trimethoprim/sulfamethoxazole (Ibrahim et al., 2012). It is most likely that the selective pressure generated by overuse could explain the relatively high prevalence of resistance in *E. coli*.

ESBL-producing *E. coli* shows simultaneous resistance to both β-lactam and antibiotic of other group are defined as multidrug resistant strain (Chan-Tompkins, 2011). It was shown that resistant genes for β-lactams are often located in mobile genetic elements such as plasmids and integrons, whereby the horizontal transfer of these genes is possible not only in bacteria of the same species but also between bacteria of different species (Bush et al., 2008; Dominika et al., 2014). This characteristic location of genes responsible for resistance could explain the high prevalence of ESBL-producers among strains of *E. coli* observed in this work

Interestingly, our data indicated that infection with ESBL-producing *E. coli* was significantly higher in adults than in children. Similar results have been reported (Jahad et al., 2005). Other work using multivariate analysis (Johnson and Wichern, 2007) demonstrated that age over 60 years was found to be an independent risk factor for infection of ESBL-producing bacteria. The explanation behind these results is not clear, but it is likely that factors such as immunity status and host-microbe interactions need to be taken into account.

Due to the study design, our investigations have certain limitations because the work concerns only one of the five hospitals in N'djamena. Therefore, the data collected cannot be considered representative of N'djamena population. Also, patients' information was limited because we have no data for prior antimicrobial drug exposure. These could bring to the possibility of selection bias.

Conclusion

Despite these limitations, our study provides foundational information for resistance development in Chad. Indeed, our data demonstrated a high prevalence of ESBL-producing *E. coli* resistant to β-lactams, quinolones, aminoglycosides and sulfonamides. This is a clear

indication of a high trend increased multi-drug resistance in *E. coli* in Chad. Further characterization of these strains will help to throw light on the underlying molecular mechanisms of resistance. However, cefoxitin and imipenem were found to be the most effective antibiotics tested that can be used in treating infections caused by *E. coli* and other related bacteria in Chad. The future usefulness of these drugs will depend on the rational and judicious use of antimicrobial agents.

Conflict of interests

The authors did not declare any conflict of interest.

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