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Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in outpatients among Mongo communities, Chad

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Abstract

In recent years, the world has seen a surge in extended-spectrum β-lactamase-producing-bacteria. However, data on the dissemination of ESBL-producing *Enterobacteriaceae* in the community is not available in Chad. This study aims to determine the prevalence and antibiotic susceptibility pattern of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in fecal carriage from outpatients. From September 2017 to February 2018, 102 stools samples collected were sent at IRED. All stool samples were seeded onto Mac Conkey agar plates supplemented with cefotaxim (CTX, 2µg/mL), subjected to standard bacteriological method for isolation and characterization. Susceptibility to antibiotics was tested according to Kirby Bauer disk methods in respect to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). Out of the 102 samples investigated, 53 isolates were identified as *Escherichia coli* strains (84.9%) and *K. pneumoniae* (15.1%).Moreover, among the 53 strains, 33 (62.3%) belonged to extended spectrum β-lactamase producing (ESBL) group. The maximum resistance were observed with amoxicillin and clavulanic acid (82, 22%-87, 5%), nalidixic acid (93, 33%-100%), ciprofloxacin (71, 11%-75%), and gentamicin (80.00%-87.5%). *K. pneumonia* resistance to fosfomycin was significant (P = 0.011) than *E. coli*. Mostly isolates tested were sensitive to imipenem. This result shows the high rate of ESBLs-producing isolates among outpatients in the community of Mongo. Surveillance of antimicrobial resistance needs to be implemented in Chad to tailor interventions targeted at stopping the dissemination of ESBL producing *Enterobacteriaceae*.

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Introduction

During the past few decades, ever-increasing use of antibiotic agents has led to selective pressure in favor of bacteria that have acquired resistance enzymes (Levy, 2004). Resistance to β-lactam antimicrobial drugs among gram-negative bacilli is mainly the result of extended-spectrum β-lactamase (ESBL), a major group of enzymes. ESBL-producing *Enterobacteriaceae* have been widely reported in many countries (Villegas *et al.*, 2011). These enzymes are typically plasmid-mediated and have the ability to hydrolyze all penicillin, third-generation cephalosporins and monobactams. They are not active against cephamycine or carbapeneme and are highly susceptible in vitro to inhibition by β-lactamase inhibitors, such as clavulanic acid (Thenmozhi *et al*., 2014). The resistances to β-lactam antimicrobial drugs are often associated with resistances of other classes of antibiotics such as aminoglycosides, quinolones, sulfamides and others. The infections caused by these strains are very difficult to treat. *Escherichia coli* and *Klebsiella pneumoniae* are two bacteria often detected in biological samples (Canton *et al*., 2008). Dissemination of bacteria under human, capacity of these bacteria to produce ESBL has been shown among inpatients as well as among those in the community (Bou *et al*., 2002).

The low hygiene measures are the first risk factors of bacterial transmission (Ali *et al*., 2017). Studies from different countries report varying prevalence of ESBL-producing *Enterobacteriaceae* in the communities (Livermore *et al*., 2012; Riaz *et al*., 2012; Ahmed *et al*., 2014).

In Chad, few studies on the prevalence of bacteria resistance to antibiotics have been reported in N'Djamena city by Yandai *et al*. (2014), Ndoutamia *et al.* (2015), Bessimbaye *et al*. (2015) and Linefiene *et al*. (2017). Data of antimicrobial resistant in rural communities is not available. The current study aims to determine the prevalence and antibiotic susceptibility pattern of ESBL producing *Escherichia coli* and *Klebsiella pneumoniaae* in fecal carriage from outpatients in the rural community of Chad.

Materials and methods

Samples collection

This prospective study was conducted at IRED in Chad, from September 2017 to February 2018. Stools samples given to outpatients presented at Mongo Hospital of Mongo for the parasitology diagnostic, 102 stools samples were collected in sterile disposable bottles and appropriately labeled at Mongo Hospital, province of Guera/Chad. These samples were taken with a swab and introduced into Cary Blair as transport medium. All samples collected were transported immediately at 4°C to the bacteriology laboratory of IRED in N'Djamena.

Bacteria isolation and identification

Stool samples were seeded onto MacConkey agar plates (Liofilchem, Italy) supplemented with cefotaxim (CTX, 2µg/mL) and incubated at 37°C for 18-24 hours. All bacteria developing on MacConkey agar were suspected to be gram-negative bacilli and were sub-cultured on Mueller Hinton agar (Liofilchem, Italy) for the purification. Isolates were identified by their characteristic appearance, gram strains, mobility, biochemical reactions (lysine decarboxylase, carbohydrate fermentation, indole production, methyl red, voges proskauer, citrate) using 20E identification system (Biomerieux, Marcy l'étoile, France).

Screening and confirming the presence of ESBL

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 cefotaxim (30µg), ceftazidim (30µg), cefepim (30µg), and aztreonam (30µg) disks were placed 30mm (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 20mm (center to center).

Antibacterial susceptibility testing

Antibiotic susceptibility test was performed by Bauer *et al*., (1966) disk diffusion method in respect to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). Pure culture of *E. coli* and *K. pneumoniae* was inoculed on Muller-Hinton agar plate (Liofilchem, Italy) with a depth of 4mm. Bio-Rad antibiotic disk used were: amoxicillin/clavulanic acid (20/10µg), cefoxitin (30µg), cefotaxim (30µg), ceftazidim (30µg), imipenem (10µg), aztreonam (30µg), gentamicin (10µg), amikacin (30µg), ciprofloxacin (5µg), ofloxacin (5µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg) and fosfomycine (10µg). Isolates were classified as susceptible or resistant according to EUCAST (2014). In the analyses intermediary resistant and resistant isolates were classified as non-susceptible. *Escherichia coli* American Type Culture Collection (ATCC25922) were used as quality control strains.

Statistical analysis

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.

Results

Prevalence of E. coli and *K. pneumoniae*

In total, 102 patients had a fecal sample taken and analyzed. Out of these samples, 64 (62, 74%) bacteria were suspected to be gram-negative bacilli and all resistant to cefotaxim using for screening. Among these bacteria,53 (51, 96%) *Enterobacteriaceae* were identified as *E. coli* (n = 45) and *K. pneumoniae* (n = 8).

This result indicated that the major group was *E. coli* (84.9%) than *K. pneumoniae* (15.1%). The prevalence was high in all age groups (table 1).

Table 1. Carriage prevalence of *E.coli* and *K. pneumoniae* according to age.

Age (years)	Patients (n=102)	*E. coli* (n=45)	*K .pneumoniae* (n=8)	Total (n=53)	Rate %
0 -14	23	9	1	10	43,48
15 - 29	12	2	0	2	16,67
30 - 44	32	15	3	18	56,25
45 - 59	23	11	3	14	60,87
60 and more	12	8	1	9	75,00
Total	102	45	8	53	51,96

Table 2. Antimicrobial susceptibility of *E. coli* and *K. Pneumoniae.*

Antibiotics	*E. coli* (n = 45)		*K. pneumoniae* (n = 8)		
	S (%)	R (%)	S (%)	R (%)	P value
AMC	8 (17,78)	37 (82,22)	1 (12,5)	7 (87,5)	0,714
IMP	44 (97,78)	1 (2,22)	7 (87,5)	1 (12,5)	0,160
FOS	42 (93,33)	3 (6,67)	5 (62,5)	3 (37,5)	0,011
CN	9 (20,00)	36 (80,00)	1 (12,5)	7 (87,5)	0,617
AK	24 (53,33)	21 (46,67)	5 (62,5)	3 (37,5)	0,631
NA	3 (6,67)	42 (93,33)	0 (0)	8 (100)	0,452
CIP	13 (28,89)	32 (71,11)	2 (25)	6 (75)	0,822
SXT	5 (11,11)	40 (88,89)	0 (0)	8 (100)	0,322

AMC: amoxicilline + acide clavulanique, IMP: Imipeneme, FOS: fosfomycine, CN: Gentamicine, AK: Amikacine, NA: Nalidixic acid, CIP: ciprofloxacine, SXT: Trimethoprim-sulfamethoxazole., S: sensitive, R: resistance.

Phenotype of ESBL detected

Out of the 53 isolates studied, 33 (62.3%) strains were confirmed ESBL producers (ESBL pop) by double-disk tests. However, 20 (37.7%) strains were tested negative and considered as ESBL no producers (ESBL neg). For the 33 ESBL-producing strains, 28 (45%) were *E. coli* strains and 5(62, 5%) were *K. pneumoniae* (Fig. 1).

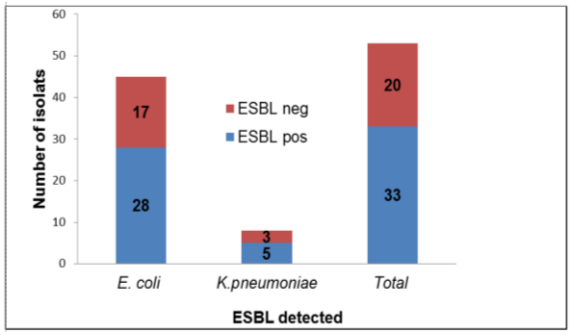

Fig. 1. Carriage prevalence of ESBL- producing *E. coli* and *K. pneumoniae.*Absolute numbers are presented within bars.

Antibiotic susceptibility

Result of Antimicrobial susceptibility showed the high resistance against amoxicillin + clavulanic acid (82.22%-87.5%), nalidixic acid (93, 33%-100%), trimethoprim-sulfamethoxazole (88.89%-100%), ciprofloxacin (71.11%-75%) and gentamicin (80.00% - 87.5%). A poor resistance was obtained with amikacin (46, 67%-37, 5%) and fosfomycine (6, 67%-37, 5%).Imipenem was very active on *E. coli* and *K. pneumoniae* tested. Two strains of *E. coli* and three of *K. pneumoniae* was resistant to fosfomycin. The resistance rates of *E. coli* compared to those of *K. pneumoniae* did not differ significantly for most antibiotics tested (P >0.05), except for fosfomycin (*P = 0.011*) (Table 2).

Discussion

This study reveals the high rate of bacteria resistant to antibiotic used for infections treatment in Chad. Mostly *E. coli and K. pneumoniae* tested were resistant to third-generation cephalosporin. ESBL-producing-bacteria were detected as mechanism of resistance essentially. However, mostly bacteria tested were sensitive to imipenem.

This high rate observed with β-lactam antimicrobial may be due to several factors and practices. In Mongo region the laboratory for the culture and antimicrobial test is not implanted. All antibiotic used for the disease treatment are guided by clinical and etiological arguments. Many people can buy the antibiotics without medical prescriptions and on advice of street vendors.

According to Ndoutamia *et al*. (2017) many people in Chad believe that antibiotics can kill viruses, heal flu or reduce fever. The inappropriate consumption of antibiotics and empirical treatment of diseases constitute risks in selection of multidrug-resistant strains within the commensal flora. These bacteria may accept the plasmids and these plasmids can be transferred readily under stress to other species (Bagre *et al*., 2015). In our findings, similar studies reports the rate ranged from 10 to 100% in West Africa (Ouedraogo *et al*., 2017), 32.6% of children under 5 in Guinea-Bissau (Isendahl *et al*., 2012), 10, 3% of hospitalized patients in Nigeria (Olowe *et al*., 2010), 63% at the orphanage and 100% of staff members in Mali (Tande *et al*., 2009). These different rates could be related to variations of health organization systems and differences between regions and targets.

In our study the rate was high in all age groups, also among the youngest where 43, 48% were carriers in the ages 0-14 years, and 75, 00% from 60 years and over. This indicates that colonization with ESBL-producing bacteria often occurs early in life and can increase with age in this population. For other antibiotics family other than β-lactam antimicrobial drugs, our findings found a maximum résistance to nalidixic acid and ciprofloxacin. In Chad, the ciprofloxacin and ofloxacine are often used for treatment of typhoid fever. Similar data about ciprofloxacin were presented by Ndoutamia *et al*., (2015), Bessimbaye *et al*., (2017).

According to Rodríguez-Baño *et al*., (2004), the use of fluoroquinolones constitutes a risk factor for the acceptance and transfer of the resistance gene of ESBL-producing germs. For Guessennd *et al*. (2008), three genes implicated to quinolone resistance were: "*QNR*" genes, genes encoding N-acetyltransferase, ACC- (6')-IBCR and genes encoding the QepA efflux pump.

As for other antibiotics classes, the resistance to gentamicin is greater than 80%, but less with amikacin (62, 5%). This difference is due most likely to the difference of mechanism of resistance which may vary from one antibiotic to another. Similar study reported a rate from 73.3% to 94.7% in Madagascar (Andriatahina *et al*., 2010). For our result, *E. coli* and *K. pneumoniae* were very resistant to trimethoprim-sulfamethoxazole. This antibiotic was often used in empirical treatment of different syndromes diseases. This rate was similar to 95% reported in N'Djamena (Ndoutamia *et al*., 2015), 98.6% in Sudan (Ibrahim *et al.,* 2013) and 91% in Nigeria (Iroma *et al*., 2009). According to Guessennd *et al*., (2008), the *qnr* A, B and S genes were present in *E. coli*. These genes were also responsible of plasmid resistance to trimethoprim-sulfamethoxazole, cefepim, cefoxitin and aminoglycosides. In contrast, fosfomycin was effective on most strains tested. The effectiveness of this molecule is linked with the fact that it is not available in peripheral environments. Its indication is much more restricted than quinolones, trimethoprim-sulfamethoxazole and aminoglycosides. This good bacterial sensitivity towards this antibiotic has also been reported by El Bouamri *et al*., (2014) in Morocco.

Conclusion

This study revealed a high rate of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in Mongo. The use of drugs such as amoxicillin,trimetoprim/sulfamethoxazole and nalidixic acid does not seem appropriate for empirical treatment because of emergence. Several factors would be involved, self-medication in the community and the empirical treatment to antibiotics. Thus, the risks of selection of multidrug-resistant strains within the commensal flora were therefore quite high and constituted a great threat to human and animal health. Further studies on the large scale on various clinical samples could help to learn more about the antibacterial drug resistance. At the molecular level, it is evident that much remains to be learnt about the control of expression of drug resistance genes.

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Conflict of interest

The authors declare that there is no conflict of interests.

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