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Research Article

Prevalence of Esbl-Producing Enterobacteriaceae Strains Isolated to the University Hospital Center of N'djamena and their Sensitivity to Antibiotics

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Abstract

Introduction: The resistance of Enterobacteriaceae to antibiotics is experiencing a worrying worldwide evolution with a growing impact in the community and medical sector. The difficulties encountered in hospitals in treating certain infections resulting from multi-resistant bacteria are a source of great concern in human health. The aim of this study was to determine the prevalence, diversity and antibiotic resistance of Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae in the hospital setting.

Materials and Methods: A total of 830 specimens from hospitalized and outpatients including 341 stools, 257 urine and 232 pus were collected between August 2017 and February 2019. Enterobacteriaceae were identified using the Api 20E gallery and the VITEK® 2TM 15 compact automaton. *E. coli* and *Salmonella* serotyping were performed by serum agglutination using the Latex Test Kil and the Kauffmann-White Kit, respectively. Sensitivity tests according to the Antibiogram Committee of the French Society of Microbiology and double disc synergy to detect ESBLs have been carried out.

Results: Out of the 830 specimens, 181 (21%) strains were isolated at the N'Djamena National Reference University Hospital between August 2017 and February 2019. 56 strains produced ESBLs, i.e. a prevalence of 30.9% (56/181). Escherichia coli was more common (74%), followed by *Klebsiella pneumoniae* (6.6%), Proteus spp (14.9%), Salmonella (2.8%) and *Shigella* (1.7%) (p<0,05). These germs were isolated from urine at 47%, pus at 38% and stool at 15%. Resistance with E. coli was respectively 60.8% and 50% for imipenem and amoxicillin + clavulanic acid. Regarding *Klebsiella pneumoniae*, these rates were 70% and 55% respectively. Salmonella showed resistance to ciprofloxacin (80%), gentamicin (80%) and ceftriaxone (60%). *E. coli*, *Klebsiella pneumoniae* have been the most implicated with strong resistance to the majority of antibiotics.

Conclusion: This study highlighted the diversity of enterobacteria causing diarrhea and the emergence of multi-resistant bacteria. It helps to update knowledge for better patient care.

Keywords: *Enterobacteriaceae;* Multiresistant; ESBLs; Hospitol; Tchad

Introduction

The resistance of *Enterobacteriaceae* to antibiotics is experiencing a worrying worldwide evolution with a growing impact in the community and medical sector. The number of *Enterobacteriaceae* producing Extended-Spectrum Beta-Lactamases (ESBL) has recently increased worldwide [1]. The resistance of *Enterobacteriaceae* to antibiotics is observed all over the world, but to varying degrees depending on the country and the services, depending on prescription habits and hygiene practices. It is experiencing a worrying global evolution with a growing impact in the community environment [2,3].

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The emergence of these resistant germs has upset not only the epidemiological profile of infections but also the resulting treatment regimens [4]. This situation gives rise to a great deal of reflection which should lead to preventive measures in order to control the repercussions on public health. The characterization of multi-resistant germs in the hospital is one of the quality indicators for healthcare establishments. The aim of this study was to determine the prevalence, diversity and antibiotic resistance of extended-spectrum beta-lactamase -producing (ESBLs) *Enterobacteriaceae* in hospitals in Chad.

Materials and Methods

Sample Collection

A total of 830 clinical samples (urine, pus and stool) were collected in sterile universal vials taken from patients sent to the Bacteriology laboratory of the national reference of N'djamena university hospital center. All samples were immediately transported to the laboratory for processing.

Isolation and Identification of *Enterobacteriaceae* Strains

The bacterial strains were isolated at the national reference of N'djamena university hospital center following the methods in force in the center. After the Gram staining of the suspicious colonies, a subculture on Muller Hinton (MH) was carried out and the identification of the strains is carried out by the API 20E gallery (Bio Mérieux, France) and the VITEK® 2TM 15 compact automatons.

The study of the sensitivity to antibiotics of the strains of enterobacteria isolated was carried out on antibiogram by the method of diffusion of the discs in agar medium. The interpretation of the strain profiles was made according to the recommendations of the antibiogram committee of the French society of microbiology. The search for extended spectrum ß-lactamase (ESBL) class A secretion was carried out by the double disc synergy test (synergy between an Amoxicillin + Clavulanic acid (AMC) disc and the 3rd grade Cephalosporin discs (C3G). Serotyping of *E. coli*, Shigella and *Salmonella* spp strains was performed by serum agglutination using the Latex Test Kil, Research Shigella sera, BIO-RAD and the Kauffmann-White Kit respectively for serogroups.

The isolated strains were stored at -20°C in brain heart broth (BCC) (BIO-RAD) with 20% glycerol following the guidelines of the National Committee on Characterizations of Clinical Laboratory Standards and Centers for Disease Control for future research.

Data Management and Analysis

Data analysis was performed using Microsoft Excel 2016 and Statistical Software for the Social Sciences (SPSS™) version 20.0 (IBM, Armonk, NY, USA) and presented as percentage of baseline distribution. Data with a p-value less than 0.05 (95% CI) were considered significant.

Results and Discussion

During the study period, 830 samples were taken and cultured in the laboratory bacteriology unit of the national reference of N'djamena university hospital center, including 257 (31%) urine, 232 (28%) various pus and 341 (41%) stools.

Prevalence of Isolated *Enterobacteriaceae*

The analysis of the cases shows out of the 830 samples taken, 181 strains of *Enterobacteriaceae* were isolated and identified, i.e. a prevalence of 21.8%.

Table 1 shows the distribution of *Enterobacteriaceae* strains isolated according to the type of sample. The species *Escherichia coli* dominated the etiology of the infections with a rate of 74% (n=134) of all the *Enterobacteriaceae* isolated, followed by *Proteus* spp (14.9%), *Klebsiella* (6.6%), Salmonella (2.8%) and *Shigilla* (1.7%). These respective prevalence rates vary significantly ($p<0.05$) depending on the origin of the samples. In addition, *Escherichia coli* was observed in the urine, various pus and stool samples respectively with a proportion of 38.7%, 24.9% and 10.5%. *Proteus* spp and *Shigella dysenteriae* were only observed in urine (6.6%) and stool (2.2%) samples. While *Salmonella* spp was observed in urine (0.6%) and stool (1.7%) samples respectively [5] (WHO, 2018). In addition, these enterobacteriaceae were isolated from urine, various pus and stool with a proportion of 47%, 38% and 15% respectively. Similar *Enterobacteriaceae* prevalence levels in hospital settings were also observed by Nadlou et al. [6,7], respectively in the refugee camps and the National General Hospital of N'Djamena in Chad. However, our results are lower than those observed in Chad (62.9%) by ouchar. et al. (2019) [8]. This difference could be explained by the period and the number of study sites. Indeed ouchar et al. [8] had worked in three hospitals in the city of N'Djamena.

Prevalence of ESBL- *Enterobacteriaceae*

Our study revealed that among the 181 *Enterobacteriaceae isolates*, 56 (30.9%) were defined as class A ESBL producers based on the results of double-disc synergy tests with the image of "cork plug". champagne", comparable to the rates found in Togo by Toudji et al. (22.4) [7]. Levels of ESBL expression by higher *Enterobacteriaceae* have been observed in Chad by Ouachar et al. (2019) (45%) [8]. these results could be explained by the history of antibiotic consumption or hospitalization which are risk factors for ESBL carriage.

Table 2 shows that among the ESBL-producing isolates, *E. coli* was the dominant species with a carrier rate of 25.4%, followed by *Salmonella* spp with a rate of 3.9%. Finally, come *Klebsiella pneumoniae* and *Proteus* spp with a rate of 1.7% and 1.1% respectively each. Our results are lower than those observed in Togo by Toudji et al. (2017) [9] (E. coli (52.75%), *Klebsiella pneumoniae* (30.10%). These results could be explained by the types of practices and the environment of the study.

Table 1: Distribution of bacterial strains isolated.

Enterobacteriaceae	Origin			Percentage (n)
	Urines%	Pus divers%	Selles%	
E.coli	38.7(70)	24.9(45)	10.5(19)	74(n=134)
Proteus spp	2.2(4)	12.7(23)	0	14.9(n=27)
Klebsiella pneumoniae	6.6(12)	0	0	6.6(n=12)
Shigella dysenteriae	0	0	1.7(3)	1.7 (n=3)
Salmonella spp	0.6(1)	0	2.2(4)	2.8(n=5)
Total	47(85)	38(68)	15(26)	100(n=181)

Table 2: Prevalence of strains of ESBL- Enterobacteriaceae.

Isolates	BLSE -	BLSE+ (%)
Escherichia coli (n=134)	88	46(25.4)
Salmonella spp (n=5)	0	5(2.7)
Proteus spp (n=27)	25	2(1.1)
Klebsiella pneumoniae (n=11)	8	3(1.7)
Shigella dysenteriae (n=3)	3	0
Total	125	56(30.9)

ESBL: extended spectrum beta-lactamases.

Table 3: Distribution of strains of ESBL Enterobacteriaceae depending on the sample.

Services Isolates	Urology	Gastroenterology	Infectious disease	Pus	External	Diabetology	ORL	EP	Total (%)
E. coli	31	4	6	4	2	1	0	0	46(82.1)
Proteus spp	0	0	0	2	0	0	0	0	2(3.6)
Klebsiella	2	0	0	0	0	0	0	0	2(3.6)
Salmonella spp	1	5	0	0	0	0	0	0	6(10.7)
Shigella	0	0	0		0	0	0	0	0
Total	33(58.9%)	9(16.1%)	6(10.7%)	5(8.9%)	2(3,6%)	1(1.8%)	0	0	56(100%)

ORL: Otorhinolaryngology, **EP:** Emergency Pavilion.

Table 4: Susceptibility to antibiotics of strains of ESBL-positive Enterobacteriaceae.

Antibiotics	*E. coli* (n= 46)		*Proteus* spp (n=2)		*K. pneumoniae* (n=3)		*Salmonella* spp (n=5)	
	R+I (%)	S (%)	R+I	S	R+I	S	R+I	S
Imipenem	28(60,8)	18(39,2)	0	2(100)	2(70)	1(30)	1(20)	4(80)
Amoxicillin +clavulanic acid	23(50)	23(50)	1(50)	1(50)	1(33,4)	2(66,6)	1(20)	4(80)
Ciprofloxacin	20(43,5)	31(56,5)	1(100)	0	2(33,4)	3(66,6)	4(80)	1(20)
Ceftriaxone	19(41)	27(59)	1(50)	1(50)	3(33,4)	4(66,6)	3(60)	2(40)
Gentamicin	25(54)	21(46)	2(100)	0	2(66,6)	1(33,4)	4(80)	1(20)

Table 3 shows that most *Enterobacteriaceae* were isolated in the urology department with a rate of 58.9% of which 55% are E.coli isolates, followed by the other departments respectively: Gastroenterology (16.1%), Infectious Diseases (10.7%), Emergency Pavilion (8.9%) and external consultation (3.6%). In addition, the prevalence of ESBLs observed in the urology department was significant compared to the other departments ($p<0.05\%$). This high *E. coli* carriage rate is explained by the fact that *E. coli* isolates are responsible for the majority of community urinary tract infections and are involved in a large number of nosocomial infections. This distribution of *Enterobacteriaceae* is consistent with that observed by Bessimbaye et al. (2015) [8] at the Bacteriology Department of the National Reference General Hospital of N'Djamena in Chad and Mohamed et al. (2017) in the laboratory microbiology department of Moulay Ismail hospital in Meknes, Morocco [10].

Prevalence of *Salmonella* Serotypes

Of the 181 enterobacteriaceae identified, six (5) Salmonella serotypes were identified, i.e. a prevalence of 2.7% (Paratyhi A, O: 4 (A) Hb (1), Paratyphi C, O: 7 (B) Hc (1), Salmonella enterica subsp Arizonae (1) and S. Enteritidis O: 9 (A) Hd (2). These *Salmonella* serovars of human origin were isolated from two (2) stool and urine samples in the following clinical situations: gastroenteritis and urinary tract infection. This observed carriage rate highlights an alarming public health problem knowing that the isolated serovars are responsible for typhoid fever (paratyphoid A and B) and theoretically the cause of systemic infections in people with a low immune status *S. Enteritidis*).

Susceptibility of Isolated Strains to Aantibiotics

Of the 56 (30.9%) strains of ESBL-producing *Enterobacteriaceae* isolated and identified. Table 4 illustrates the resistance profile of the most represented ESBL-producing *Enterobacteriaceae* in this study. These are *E. coli*, *Proteus* spp, *Klebsiella pneumoniae* (n=3) and *Salmonella* spp. All the strains were highly resistant up to 100% to beta-lactams. Imipenem (Carbapemene) on the contrary totally inhibited all strains of *Proteus* spp and 80% of strains of *Salmonella* spp. While the *E. coli* strains showed resistance from 41% to 60.8% and those of *Klebsiella pneumoniae* from 33.4% to 66.6 depending on the antibiotics. These levels of resistance expressed by all the isolates studied showed a significant difference at the threshold of $p<0.05$. These results could be explained by a massive and uncontrolled use of certain antibiotics, poor hygiene in hospitals and/or selection pressure on resistant enterobacteria; which has led to the emergence of resistant strains. Our results are similar to those obtained by Toudji et al. (2019) in Togo [9] and in Chad by Bessimbaye et al. (2015) [6].

Conclusion

Ultimately, the study conducted in the National Reference University Center of N'Djamena revealed the effective presence of *Enterobacteriaceae* producing broad-spectrum beta-lactamases with a prevalence of 30.9%. *E. coli* isolates were the most represented germs up to 82.1% of all ESBL-producing strains. These strains were highly resistant to beta-lactams as well as other antibiotics, which could no doubt complicate the treatment of patients. It is therefore essential to carry out a molecular characterization of these strains to assess the different genes involved in the production of beta-lactamases.

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