RESEARCH ARTICLE

STUDY OF THE SENSITIVITY TO CARBAPENEMS IN THE *ESCHERICHIA COLI* ISOLATED FROM CHILDHOOD DIARRHEA AT THE PAUL MOUKAMBI HOSPITAL CENTER OF KOULA-MOUTOU

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ABSTRACT

Background: Carbapenems remain the last therapeutic alternative, so the occurrence of carbapenem-resistant enterobacteria is a public health problem. This study aims to determine the sensitivity of *Escherichia coli* to carbapenems. **Methods:** The susceptibility to carbapenems of *Escherichia coli* isolated from childhood diarrhea at the Paul Moukambi Regional Hospital Center was evaluated by the diffusion method in an agar medium in accordance with the recommendations of the Antibiogram Committee of the French Society (CA-SFM) and by the determination of Minimal Inhibitory Concentrations (MICs) of imipenem according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Confirmation was made by the Carbapenem Inactivation Method (CIM) test. The genotypic characterization was carried out by PCR by the search for CTX-M genes of group 1 and OXA. **Results:** The antibiogram shows resistance levels of 95%, 85%, 75% and 70% respectively for doripenem, ertapenem, imipenem and meropenem. The MIC results show that 35% of the strains are resistant to imipenem according to EUCAST while 85% are according to CLSI. The screening of the 20 strains by the CIM test reveals that 65% of strains produce carbapenemases. Molecular data reveal a prevalence of 25% of strains carrying carbapenem resistance genes, including 15% for the blaCTX-M gene and 10% for the blaOXA gene. **Conclusion:** The carbapenem resistance of *Escherichia coli* clinical isolates has been demonstrated for the first time in a rural area in Gabon.

Key words: Diarrhea, Escherichiacoli, carbapenems, Gabon, resistance.

INTRODUCTION

The emergence and the rapid spread of multidrug-resistant enterobacteriales a serious public health problem because this type of bacteria is responsible of many infections worldwide (Falagas, Karageorgopoulos and Nordmann, 2011). *Klebsiella pneumoniae* (*K. pneumoniae*), *Escherichia coli* (*E. coli*) and *Enterobacter*spp are the most common bacterial infections (Boucher, Albot and Bradley, 2009) frequently isolated from nosocomial infections and community. *E. coli* in particular is one of the bacterial species presenting a variety of strains such as intestinal commensals, intestinal pathogens and extra intestinal pathogens causing urinary tract infections, sepsis and meningitis (Mcnally *et al*., 2013). Moreover, *Enterobacteria ceae* are responsible of serious infections in humans and animals (Liu, Thungrat and Boothe, 2016), and mostly isolated from infant diarrhea (eg. enterophatogenic *E. coli*) (Moyo *et al*., 2011; Lamberti *et al*., 2014). In the same way, the appearance of resistance in *Enterobacteriaceae*, particularly related to the production of extended-spectrum β-lactamases (ESBL) of the TEM, SHV, CTX-M (1) type poses a real threat

(Paterson and Bonomo, 2005) and mostly predominant This resistance in *E. coli* isolates (Costa *et al*., 2009; Wasyl *et al*., 2012). With the recent therapeutic failures due to the inefficiency of third-generation of cephalosporins because of the production of ESBLs and cephalosporinases by bacteria, carbapenems treatment has been increasingly recommended to enterobacterial infections. In addition, carbapenems resistance in *E. coli* has been reported worldwide (Candan and Aksöz, 2017). As a result, carbapenem-resistant Enterobacteriaceae (CRE) associated to few alternative therapies are of particular concern (Nordmann, 2010; Liang *et al*., 2017). Resistance to carbapenems by Gram-negative bacilli in general, and especially to enterobacteria by the production of oxacillinase car bapenemases, OXA-48, has been reported for the first time in 1990 in Japan. Furthermore, the international distribution of KPC-type carbapenemase reveals the absence of data on the presence of carbapenem-resistant enterobacteria in the sub-Saharan region (Nordmann, 2010). Moreover, a study carried out between 2012-2013 in Belgium on enterobacteria producing imported carbapenemases underlines the presence of carbapenemase-producing *Enterobacteriaceae* in North African countries (Morocco, Algeria, Tunisia, Libya, Egypt) and West (Senegal) (Huang *et al*., 2018). However, the diffusion of KPC enzymes have endemic foci well identified both in the United States and in South American countries, but

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also in Europe including Greece and Italy (Poirel, Dortet and Nordmann, 2013). In Gabon, the status of *E. coli* resistance in general and *E. coli* isolated from infant diarrhea is poorly documented. Moreover, its effect in carbapenem resistance is unknown despite the fact that in several cities, prescription of carbapenems is mostly increasing.

Hence here, this study aimed to determine phenotypic and genotypic characteristics of *E. coli* strains isolated from infant diarrhea on carbapenems at the Paul Moukambi Hospital Center in Koula-Moutou.

MATERIALS AND METHODS

Presentation of the study framework: A prospective study was conducted at the Paul Moukambi Regional Hospital Center in Koula-Moutou (Gabon) between November 2016 to March 2017 in a total of 36 fecal samples of both sexes (15 females, 21 males) with diarrhea and aged from 0 to 5 years.. We determined some inclusion criteria such as, children with acute diarrhea, hospitalized, outpatients or in consultation. We also specifically added the absence of any treatment or an antibiotic therapy took less than. 24 hours. Finally, it is important to specify here that before to collect fecal samples from children a consent form has been signed by their parents.

Analyses of samples: Fecal samples were collected by rectal swabbing and immediately we performed optical microscopy observations by identifying amoeba cysts and helminths eggs (KAOP) on the one hand and on the other hand we assessed the morphology and the abundance of bacteria. Bacterial isolation was done by stool staining on Hektoen, *Salmonella-Shigella*, Methyl Blue Eosin and Drigalski agar plates and incubated at 37°C for 18-24 hours. The biochemical identifications by Api 20E systems (Biomérieux, Marcy l'Etoile, France) were made from pure colonies. The results were interpreted using the ApiwebTM version 4.0 software. Only the strains duly identified as *E. coli* were the subject of this study.

Evaluation of the susceptibility of *E. coli* to carbapenems
Antibiogram: Evaluation of the susceptibility of *E. coli* to carbapenems was performed using the diffusion method of Mueller Hinton (MH) agar media. The antibiotics used were doripenem (DOR), ertapenem (ETP), meropenem (MEM) and imipenem (IPM) with concentrations of 10 μg each (Bio-Rad, Marnes-la-Coquette, France). Inocula standardized to 0.5 MacFarland were prepared from 18-24 hour pure colonies in sterile saline (0.9% NaCl). The interpretation of the results of the activity was based on the standards of the Antibiogram Committee of the French Society (CASFM) recommendations 2017 v.1.0.

Determination of Minimal Inhibitory Concentrations (MIC): The MIC of imipenem was determined to evaluate the sensitivity of *E. coli* to carbapenems (confirmation). Thus, the micro-dilution technique in the heart-brain broth was performed. A geometric dilution range of reason 2 of the antibiotic was made from an antibiotic solution of $128 \mu g.ml^{-1}$. The interpretation of the MIC results was based on the critical points standardized by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI), Recommendation 2014 version 4.0 presented in Table 1.

Table 1.Critical carbapenem concentrations according to EUCAST and CLSI standards

	MIC (mg/L)			
	EUCAST		CLSI	
	Sensitive (≤)	Resistant (≥)	Sensitive (≤)	Resistant (≥)
Doripenem	2	8	1	4
Ertapenem	0,5	1	0,5	2
Imipenem	2	8	1	4
Meropenem	2	8	1	4

Phenotypic characterization of carbapenemase-producing *E. coli* strains and confirmation of carbapenemase production

Type of carbapenemase produced by isolated *E. coli*: The phenotypic categorization of any carbapenemase-producing strains was determined by reference to the various critical MIC concentrations. For any strain of enterobacteria suspected to be carbapenemase-producing, the carbapenemase type is estimated according to the Nordmann guidelines (Nordmann, 2010; Nordmann *et al.*, 2012) Presented in Table 2.

Table 2. Variability of carbapenem resistance in clinical strains of enterobacteria expressing acquired carbapenemases

Type of carbapenemases	MIC (mg/L)		
	Imipenem	Meropenem	Ertapenem
KPC	0,5 à >32	0,5 à >32	0,5 à >32
MBL (IMP/VIM/NDM)	0,5 à >32	0.5 à >64	0.38 à >32
OXA-48/OXA-181	0,25 à 64	0.38 à 64	0.38 à >32

Confirmation test: Carbapenem inactivation method Modified (CIMm) test: The Modified CIM Test (CIMm) was used for phenotypic detection and confirmation of carbapenemase production of the 20 *E. coli* strains of this study as described by Pierce *et al.* (2017). The *E. coli* strain JFY-0117 in this study is a strain of *E. coli* wild whose antibiotic profile was determined by Vitek® (BioMérieux) and by ATB (BioMérieux). This strain showed sensitivity to all antibiotic molecules contained in the gallery. Thus, 10 μl of suspension of each strain of *E. coli* were resuspended in 2 ml brain-heart broth. Then, a disk of meropenem (10 μg) was introduced into the tubes and the various preparations incubated at 35°C±2°C for 4h15 minutes. After this incubation time, the meropenem disks were deposited on Mueller Hinton plates previously inoculated with a 0.5 MacFarland inoculum of the indicator strain. The dishes were incubated at 35°C± 2°C for 18-24 hours. A strain is declared to produce carbapenemases for any inhibition diameter induced around the disc between 6 and 15 mm. For values ≥ 19 mm, the test is negative. For values between 16-18 mm, the test is indeterminate.

Caractérisation génotypique de la résistance: Detection of the resistance genes of *E. coli* isolates was performed by the conventional PCR amplification method. It was carried out in a thermal cycler (Bio-RAD, T100TM, USA) for 30 cycles. The different genes sought were *blaCTX-M* group 1 (Ben Achour *et al.*, 2009) and *blaOXA* and whose primer sequences were described by Mosquito (Mosquito*et al.*, 2013). The reaction volume was 25 μl, the various parameters and conditions of which were as described by Yala *et al.*, (2016). The amplicons obtained were separated by electrophoresis on a 1.6% (w / v) agarose gel and revealed under an ultraviolet transilluminator.

Statistical analyzes: The statistical analyzes of the data were carried out using the R version 3.2.2 software. The test used is the analysis of anova variances at one factor at the significance level of 5%. This test was followed by the Tukey parity test.

RESULTS

Evaluation of the sensitivity of *E. coli* strains Antibiogram: The frequency of *E. coli* isolated from diarrheal feces is 26% (20/76) compared to the total isolated bacterial population. The phenotypic carbapenem profile of these 20 strains is reported in Table 3. The analysis of the results shows that among the twenty (20) *E. coli* strains tested, only *E. coli* strain 13 has no inhibition diameter in the presence of the four (4) antibiotics. The largest inhibition diameters recorded are of the order of 23.69 ± 0.8 mm, 23.16 ± 1.2 mm, 22.67 ± 0.0 mm and 19.51 ± 0.5 mm respectively for ertapenem, doripenem, imipenem and meropenem. Statistical analysis shows that there is a very significant difference between registered antibiotic diameters ($p= 2.05 \times 10^{-5}$). In addition, the Tukey parity test notes that a significant difference is observed between the effect of ertapenem and doripenem ($p= 0.00245$), imipenem ($p= 0.00127$), and meropenem ($p = 0.001$).

Similarly, no significance is observed at the 0.05 threshold between meropenem and doripenem and between imipenem and meropenem with respectively p =0.61 and p = 0.71 and between imipenem and doripenem (p= 0.99). Of the three (3) of the 4 antibiotics tested, the 20 strains of *E. coli* have the same profile. Indeed, they are either resistant or intermediate to ertapenem, doripenem and meropenem. On the other hand, these strains have the three phenotypes with respect to imipenem. Also, these strains have a high level of resistance to 4 antibiotics. The highest rate of resistance was observed against doripenem (95.0%) followed by ertapenem (85.0%), imipenem (75.0%) and finally meropenem (70.0%).

Determination of the minimum inhibitory concentration (MIC): The different minimum inhibitory concentrations obtained by microdilution for the 20 *E. coli* strains in this study for imipenem are shown in Figure 1. The results of Figure 1 reveal that the MIC range is between 4 ± 0.0 mg.L^{-1} and 32 ± 0.0 mg.L^{-1}. However, no strain has a MIC value of 16 mg.L^{-1}. Thirty percent (30%) of *E. coli* have a MIC of about 8 mg.L^{-1} and 25% of strains have a MIC of 12 mg.L^{-1}. 20% and 15% of the isolates have MIC values of 6 mg.L^{-1} and 4 mg.L^{-1}, respectively, in this study.

Table 3. Susceptibility of strains to carbapenems

	ETP (10)	MEM (10)	DOR (10)	IPM (10)
E. coli$_1$	$21{,}78 \pm 0{,}4^R$	$19{,}19 \pm 0{,}5^I$	$19{,}94 \pm 1{,}7^R$	$21{,}12 \pm 1{,}4^I$
E. coli$_2$	$23{,}44 \pm 0{,}3^I$	$18{,}96 \pm 0{,}0^I$	$20{,}23 \pm 0{,}2^R$	$20{,}89 \pm 0{,}3^I$
E. coli$_3$	$20{,}57 \pm 1{,}5^R$	$15{,}31 \pm 0{,}7^R$	$18{,}73 \pm 1{,}0^R$	$22{,}22 \pm 0{,}1^S$
E. coli$_4$	$22{,}72 \pm 0{,}5^I$	$17{,}87 \pm 2{,}0^I$	$14{,}08 \pm 0{,}7^R$	$18{,}74 \pm 1{,}9^I$
E. coli$_5$	$21{,}69 \pm 0{,}7^R$	$12{,}86 \pm 0{,}0^R$	$11{,}95 \pm 0{,}0^R$	$10{,}47 \pm 0{,}1^R$
E. coli$_6$	$23{,}69 \pm 0{,}8^I$	$15{,}91 \pm 1{,}6^R$	$19{,}59 \pm 0{,}6^R$	$14{,}85 \pm 0{,}7^R$
E. coli$_7$	$17{,}15 \pm 1{,}8^R$	$16{,}00 \pm 1{,}0^R$	$14{,}81 \pm 1{,}1^R$	$15{,}08 \pm 1{,}9^R$
E. coli$_8$	$20{,}43 \pm 2{,}2^R$	$19{,}51 \pm 0{,}5^I$	$18{,}75 \pm 0{,}4^R$	$18{,}72 \pm 1{,}1^R$
E. coli$_9$	$16{,}62 \pm 1{,}6^R$	$18{,}75 \pm 0{,}2^I$	$18{,}19 \pm 0{,}2^R$	$13{,}31 \pm 1{,}1^R$
E. coli$_{10}$	$20{,}86 \pm 0{,}2^R$	$15{,}10 \pm 0{,}4^R$	$23{,}16 \pm 1{,}2^I$	$22{,}67 \pm 0{,}0^S$
E. coli$_{11}$	$14{,}24 \pm 0{,}2^R$	$12{,}57 \pm 0{,}5^R$	$12{,}19 \pm 0{,}2^R$	$14{,}50 \pm 0{,}2^R$
E. coli$_{12}$	$14{,}08 \pm 0{,}6^R$	$13{,}94 \pm 1{,}1^R$	$14{,}28 \pm 0{,}7^R$	$11{,}24 \pm 0{,}3^R$
E. coli$_{13}$	$0{,}00 \pm 0{,}0^R$	$0{,}00 \pm 0{,}0^R$	$0{,}00 \pm 0{,}0^R$	$0{,}00 \pm 0{,}0^R$
E. coli$_{14}$	$15{,}27 \pm 0{,}1^R$	$15{,}05 \pm 0{,}2^R$	$14{,}98 \pm 0{,}2^R$	$13{,}26 \pm 0{,}3^R$
E. coli$_{15}$	$15{,}45 \pm 0{,}5^R$	$15{,}15 \pm 0{,}3^R$	$14{,}57 \pm 0{,}6^R$	$13{,}86 \pm 0{,}8^R$
E. coli$_{16}$	$16{,}54 \pm 0{,}2^R$	$15{,}56 \pm 0{,}3^R$	$16{,}56 \pm 0{,}2^R$	$13{,}77 \pm 0{,}4^R$
E. coli$_{17}$	$16{,}36 \pm 1{,}0^R$	$15{,}46 \pm 1{,}0^R$	$16{,}17 \pm 2{,}4^R$	$14{,}44 \pm 0{,}7^R$
E. coli$_{18}$	$15{,}59 \pm 0{,}2^R$	$14{,}83 \pm 0{,}8^R$	$15{,}05 \pm 0{,}7^R$	$12{,}68 \pm 0{,}4^R$
E. coli$_{19}$	$14{,}55 \pm 2{,}4^R$	$14{,}84 \pm 1{,}7^R$	$17{,}41 \pm 2{,}4^R$	$11{,}98 \pm 0{,}1^R$
E. coli$_{20}$	$19{,}13 \pm 0{,}4^R$	$14{,}79 \pm 0{,}5^R$	$18{,}18 \pm 0{,}2^R$	$16{,}19 \pm 0{,}4^R$
% de résistance	17 (85,0%)	14 (70,0%)	19 (95,0%)	15 (75,0%)

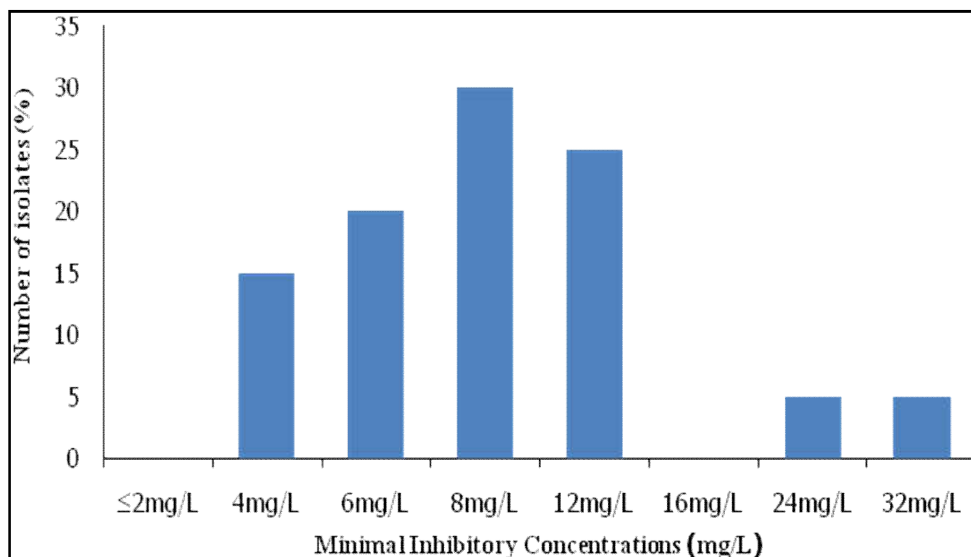**phenotype**:**R :** resistant **; S :** sensitive ; **I :** intermediate

Figure 1.Values of minimal inhibitory concentrations of *E. coli* to imipenem

The interpretation of the different corresponding phenotypes and the comparison of the results of the two phenotypic methods are shown in Table 4. After analyzing the results, and comparing the MIC values obtained in this work with the data in Table 2, it appears that 35% of *E. coli* are "resistant" to imipenem according to EUCAST standards, while 85% are "resistant" according to CLSI guidelines (Table 4). Also, the comparison between the antibiogram method and the MIC method reveals that the imipenem resistance rates obtained according to the CLSI (85%) and the antibiogram (75%) are high and similar. On the other hand, they are higher than that obtained by the EUCAST standards (35%).

Categorization of strains carbapenemase types and confirmation of carbapenemase production by the CIMm test

Categorization of carbapenemase types: The determination of MICs was also performed for the probable detection of carbapenemases in case of phenotype of sensitivity decreased at least to one of carbapenem.The results in Figure 1 highlight that *E. coli* isolates are likely carbapenemase producers. Comparison of the MIC values of reference and those obtained during this study reveals that the 20 strains of *E. coli* are suspected to produce carbapenemases of KPC, MBL and OXA-48 type (Table 5).

Confirmation test of carbapenemase by Carpapenem Inactivation modify Method (CIMm): The results of this confirmation test are shown in Table 6. The results of phenotypic carbapenemase production screening indicate that 65% (13/20) of *E.coli* isolated from carbapenem-resistant infantile diarrhea feces produce at least one carbapenemase. However, 35% (7/20) of strains are carbapenemase negative and no strain has an indeterminate phenotype.

Caractérisation moléculaire de la résistance: The detection of some genes responsible of resistance was performed by the PCR amplification method. The results show that 25% (5/20) carry the carbapenem resistance genes. The *blaCTX*-M and *blaOXA* genes frequencies have been detectedat 15% (3/20) and 10% (2/20), respectively.

They also reveal that 75% (15/20) of *E. coli* strains are not characterized (Table 7).

DISCUSSION

This prospective study proposed to evaluate the sensitivity to carbapenems of *E. coli* isolated from diarrhea feces in children (0-5 years). These results are the first relative information on susceptibility statistics for the last class of broad-spectrum antibiotics still effective in Gabon. However, these results can not be considered exhaustive given the limitations of this study. These data were collected only within a rural locality in a target population for a short time. Overall, this study reveals strong carbapenem antibiotic resistance in *E. coli* diarrheal isolates. The antibiogram shows a resistance rate of about 95% (19/20) to doripenem (DOR), 85% (17/20) to ertapenem (ETP), 75% (15/20) imipenem (IMP) and 70% (14/20) with meropenem (MEM). Similar results were obtained by Baran and Aksu in 2016 in Turkey, which recorded resistance rates of 84.62% and 76.92% respectively for ETP, IPM and MEM over 13 *E. coli* isolates (Baran and Aksu, 2016). In contrast, a recent study in Nigeria found low levels of resistance to ETP (30%) and IMP (0.0%) in 50 strains of *E. coli* isolated at the University College Hospital (Oladipo *et al*., 2018). The same study showed that this resistance was high in *Pseudomonas aeruginosa* 87.2% for ETP.

This strong resistance observed in this study could be explained by the consequence of the massive use of broad-spectrum antibiotics, Abbas *et al*., 2012; Sbiti *et al*., 2017). Moreover, the disadvantageous socio-economic and health context prevailing in developing countries (DCs). But also, many other factors such as, precariousness, deplorable hygienic conditions, the lack of qualified and competitive human resources in key areas, the absence of national networks for monitoring resistance, and especially the panel and quality of antibiotics in circulation and, the lack of regulation in their marketing could be participated to explain the occurrence and the rapid spread of resistance in developing countries, particularly in Gabon (Ouedraogo *et al*., 2017).

Table 4. Phenotypes of *E. coli* strains tested

	Minimal inhibitory concentrations				Antibiogram CASFM	
	EUCAST		CLSI			
	n (%) R	n (%) I	n (%) R	n (%) I	n (%) R	n (%) I
Imipenem	7 (35)	13 (65)	17 (85)	3 (15)	15 (75)	3 (15)

Table 5. Comparison of the MIC profiles obtained

Type of carbapenemases	MIC (mg/L)	
	Imipenem (Values reference)	Imipenem (study)
KPC	0,5 à >32	4 - 32
MBL (IMP/VIM/NDM)	0,5 à >32	
OXA-48/OXA-181	0,25 à 64	

Table 6. Results of the modified CIM test

Interpretation	Diameter values (mm)	staffings (%)
Positive carbapenemases	6-15	13 (65)
Undetermined	16-18	0 (0,0)
Negative carbapenemases	≥19	7 (35)

Table 7. Distribution of the different isolated genotypes

	Genes of resistance: n (%)			Total n (%)	
	bla$_{CTX-M}$	bla$_{OXA}$	bla$_{CTX-M/OXA}$	Positive	Not characterized
E. coli	3 (15)	2 (10)	0 (0)	5 (25)	15 (75)

Furthermore, this strong resistance is obviously explained by the establishment of a diversity of resistance mechanisms by bacteria, the most illustrated are the joint action of a high level of production of a chromosomal or plasmidic cephalosporinase or a broad-spectrum beta lactamase (ESBL) associated with a decrease in membrane permeability (Poirel, Dortet and Nordmann, 2013) due to a modification of OmpC, OmpD, OmpF and OmpKporins, and more particularly OmpC and OmpF in *E. coli* (Martinez-Martinez, 2008). This association obviously affects the minimum inhibitory concentration (MIC) and leads to carbapenem resistance by a mechanism that does not involve the production of carbapenemases. In addition, this strong resistance could be also attributed to the production of carbapenemases by *E. coli* isolates, supported by many studies in which the authors found that strains of *E. coli*, *Klebsiella pneumoniae* are the main enterobacteria producing carbapenemases in the same rank as *Acinetobacter baumannii* (Cantón *et al*., 2012; Khorsi *et al*., 2015).

It is not excluded that enterobacterial strains make simultaneous use of both mechanisms of resistance (mechanisms not involving the production of carbapenemases and that involving them). These results are in accordance with the global spread of carbapenem-resistant enterobacteria (CRE), particularly in Africa, although it is not widely available (Leski *et al*., 2013). The spread of this plague within the group of Gram-negative bacilli is attributed mainly to the species *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *E. coli* (Cantón *et al*., 2012; Poirel, Dortet and Nordmann, 2013; Khorsi *et al*., 2015). This situation would promote increased treatment failures for multi-drug resistant Gram-negative bacilli and jeopardize the latest therapeutic alternatives (Poirel, Dortet and Nordmann, 2013; Boivin *et al*., 2016). In order to control their transmission and dissemination, the Centers for Disease Control and Prevention (CDC) recommends the characterization of the type of resistance of CRE (Eser, 2017). The harmonization of the analysis of antibiogram results and minimum inhibitory concentrations by the National Antimicrobial Susceptibility Testing Committee (EUCAST / CASFM and CLSI) has established values of critical concentrations of carbapenemases of isolates producing primordial resistance mechanisms (Jehl, 2012). In this study, the MIC determination of imipenem revealed that 85% of these isolates are carbapenem-resistant *E. coli* (CREC) according to american CLSI guidelines. Whereas, a resistance lower than 35% is recorded according to European EUCAST / CASFM standards. This disparity is justified by the significant decrease in the sensitivity threshold of carbapenems by CLSI in order to improve the phenotypic detection of carbapenem-resistant enterobacteria (CRE) with moderately increased MICs (Nordmann, Dortet and Poirel, 2012).

The relevance of selecting presumptive strains able to reduced sensitivity to carbapenems is critical to the identification of carbapenemase-producing isolates. For this purpose, the MIC values of imipenem (4-32 mg.L^{-1}) of the CRECs in this study are included in the cut-off values for the carbapenemase-producing bacteria recommended by EUCAST (Nordmann *et al*., 2012). These results suggest that all isolates obtained from diarrheal feces in children under 5 years of age produce carbapenemase enzymes of the KPC, MBL and OXA type (Nordmann *et al*., 2012). However, the modified Carbapenem Inactivation Method (CIMm) enzymatic hydrolysis test confirms this production only for 65% (13/20) of the CREC strains. These results underline the inadequacy of the precision

of the phenotypic tests which do not make it possible to distinguish the various mechanisms of resistance related to the variability of their profile. In addition, methods based on the hydrolytic activity of carbapenems are more specific because they prove the carbapenemase activity despite the lack of characterization and allows to take into account the new variants of these enzymes (Pierce *et al.,* 2017). Of the 20 CREC isolates detected phenotypically, 5 (25%) carry the carbapenem resistance genes. This low prevalence is linked to the non-specificity of the genes sought. Indeed, the β-lactamase genes that were targeted belong to the group of extended-spectrum β-lactamases (CTX-M) and class D carbapenemases, oxacillinases (OXA) (Nordmann, 2010). The high representativity of the CREC secretory strains of ESBL-CTXM (15%) in this study is explained by the clonal expansion of CTX-M *E. coli*, which now constitute the majority of ESBLs whatever the region of the world (Cantón *et al*., 2012; Ouedraogo *et al*., 2017). However, it has been shown that ESBLs have hydrolytic activity on all β-lactams with the exception of carbapenems (Nordmann, 2010; Ruppé, Woerther and Barbier, 2015). The resistance of these strains is justified by the combination of resistance mechanisms. Indeed, one of the resistance mechanisms of CREs is the association of the production of a broad-spectrum β-lactamase (ESBL) with a decrease in the membrane permeability of the bacterial strain (Zuji *et al*., 2014), but also with the hyperproduction of a cephalosporinase (AmpC) combined with the loss of a porin (Abbas *et al*., 2012). The phenotypically determined carbapenemase resistance is confirmed by PCR in this study, as 10% of the CREC carry the OXA carbapenemase gene, which is found in the main carbapenemases isolated from enterobacteria (Poirel, Dortet and Nordmann, 2013). This rate is significantly lower than those of Dortet *et al*., (2017), of which 85.6% of the carbapenemases were of the OXA-48 type, while those of Candan show that of the 50 strains of *E. coli*, 22% carry this gene (Candan and Aksöz, 2017). Strains of *E. coli* isolated from blood samples carrying the OXA-48 gene (19%) were reported in India (Khorsi *et al*., 2015).

This difference would be interpreted by the non-specificity of the target gene used in this study. The search for the other most common carbapenemases, like the KPC types, and the metallo β-lactams (NDM, VIM) (Albiger *et al*., 2015; Eser, 2017), would have increased this prevalence. This could probably be used to characterize the 75% of the CREC isolates that have not been characterized. Moreover, these results corroborate with the wide diffusion of class D (OXA) carbapenemase-producing bacteria in the world, particularly in North Africa (Tunisia, Israel, Egypt, Morocco), with the OXA-48 group (Lahlaoui *et al*., 2012; Baran and Aksu, 2016; Huang *et al*., 2018) and the OXA-23, and OXA-24 and OXA-51 groups detected in *Acinetobacter baumannii* strains in Algeria (Khorsi *et al*., 2015). Also, sporadic cases of OXA carbapenemase variants in West Africa (Senegal, Nigeria, Sierra Leone) have been reported by several authors, (Moquet *et al*., 2011; Leski*et al*., 2013; Ouedraogo *et al*., 2017). This study highlights the resistance to carbapenems of *E. coli* isolated from diarrheal feces in children under 5 years old in Gabon by carrying the genes that govern the production of carbapenemases considered as epidemic mechanism. This situation is all the more worrying because these genes are generally localized in the mobile genetic elements and are able to diffuse within species of enterobacteria or even other Gram-negative bacilli. (Rodriguez-Villalobos *et al*., 2006; Fern, Guerra and Rodicio, 2018; Mahalingam *et al*., 2018). In addition, these carbapene

mase-producing seeds are also associated with other resistance determinisms of a different structural nature. From this association arise so-called multiresistant or panresistant strains at the origin of therapeutic impasses justifying the magnitude of the phenomenon (Poirel, Dortet and Nordmann, 2013). Also, these germs found in subjects whose immune system is fragile: children under 5, poses a problem of pediatric antibiotic and public health in Gabon.

Conclusion

This study shows the presence of *E. coli* strains highly resistant to carbapenems isolated in children 0-5 years old. It highlights the potential circulation of carbapenem-resistant enterobacteriain Gabon. Similarly, this study showed the importance of continuous National monitoring programming of multidrug resistance in our hospitals for the good management of antibiotics and therefore be able to control the spread of multidrug resistance. Clearly, this study showed also the need to use molecular methods for the identification and differentiation of carbapenemases. Moreover, molecular typing and sequencing of resistance genes in these CRECs are being considered to characterize circulating types of carbapenemases and to a large extent their epidemiological profile.

State of current knowledge on the subject: In Gabon, despite the high incidence of diarrhea among children under 5 years old, there is no available data about the resistance of bacterial agents to antibiotics (e.g. carbapenems). In addition, the characterization of carbapenemase produced by *E. coli* has not been shown.

Contribution of our study to the knowledge: This work provides the first data about the resistance associated to *E. coli* strains especially, the fact that some of these strains has been characterized to produce carbapenemases. Furthermore, with the high rates of resistance reported here, this study raises the great interest to perform this kind of study at the national level and the need to improve the biological management of patients.

Conflict of interest: The authors declare no conflict of interest.

Contribution of the authors

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