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CTX-M-15 Extended-Spectrum-B-Lactamase among Clinical Isolates of Enterobacteriaceae in Abidjan, Côte d'Ivoire

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Abstract
This study aimed the investigation of *bla*$_{CTX-M-15}$ gene into 73 enterobacteria collected by the *Observatoire de la Résistance des Microorganismes aux Anti-Infecteux en Côte d'Ivoire* (ORMICI) in Abidjan. The identification of the species was carried out by standard microbiological methods and confirmed by Maldi-Tof. The antibiotic susceptibility was performed by Vitek-2 and the phenotypic characterization of ESBLs was carried out by double synergy test. The presence of *bla*$_{CTX-M-15}$ genes was carried out by PCR followed by sequencing. 56.2% of the strains were ESBL producers. The ESBLs were resistant to cefotaxime (57.5%), ceftazidime (57.5%) and cefepime (54.8%). High resistance to gentamicin (60.3%), ciprofloxacin (60.3%), colistin (79.4%) and cotrimoxazol (60%) was also observed. Carbapenem were the most effective on these strains. 95.1% of ESBLs harbored the *bla*$_{CTX-M-15}$ gene. Our study confirms predominance of CTX-M-15 among Enterobacteriaceae throughout the world.

Keywords: β-lactamases, CTX-M-15, enterobacteriaceae, abidjan, Côte d'Ivoire

1. Introduction
CTX-M type β-lactamases constitute a new group of enzymes encoded by transferable plasmids. It is a rapidly growing ESBL family [1]. CTX-M belong to class A of Ambler and to the group 2be of Bush, Jacoby and Medeiros. They were described for the first time in early 1990 and are currently the predominant ESBL in Enterobacteriaceae and in several parts of the world like Asia, Africa and South America [2]. The CTX-M enzymes can be subdivided into five phylogenetic groups based on their amino acid sequences. These are CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 [3]. Among the different types of CTX-M ESBL, the CTX-M-15 belonging to the CTX-M-1 group are currently the most widespread worldwide [4]. CTX-M-15 is derived from CTX-M-3 by the substitution of an amino acid (Asp240Gly) thus increasing its catalytic activity against ceftazidime which is not a usual substrate for the enzymes of this β-lactamase family. The *bla*$_{CTX-M-15}$ gene was recently responsible for international epidemics of Escherichia coli and Klebsiella pneumoniae through their respective clones ST11 and ST131 [5]. Horizontal transfer of this gene by conjugative plasmids and diffusion of these clones contributed to the prevalence increasing [6]. The objective of this study was to demonstrate the presence of CTX-M-15 in Enterobacteriaceae isolated from various infections in Abidjan.

2. Materiel and methods
2.1 Bacterial strains
The strains were collected by the surveillance group for antibiotic resistance in Côte d'Ivoire (ORMICI). These are 73 infectious strains isolated from various biologicals products. Species identification was performed by standard microbiology methods. Complete identification was obtained with MALDI-TOF technology according manufacturer's recommendations after seeding on Columbia agar with fresh sheep blood and Mac Conkey agar (bioMérieux, France) at 37 °C during 18 hours. The *E. coli* ATCC 25922 was used as controle.

2.2 Antibiotic susceptibillity test
Antibiotic susceptibillity test was carried out with Vitek-2 according manufacturer's recommendations. The AST-N236 card containing the following antibiotics: temocillin, ampicillin, amoxicillin + clavulanic acid, ticarcillin + clavulanic acid, cefuroxime, cefotaxime, ceftazidime, cefepime, ertapenem, the meropenem, the Amikacin, gentamicin, ciprofloxacin, tigecycline, fosfomycin, nitrofurantoin, colistin and cotrimoxazole was used for the determination of MICs.

2.3 ESBL detection
The double synergy test [7] was used for detection of ESBL-producing strains. The disks of cefotaxime (30 µg), ceftazidime (30 µg), céfépime (30 µg) and meropenem (10 µg) were placed around an amoxicillin/clavulanic acid disk (20/10 µg) on Mueller Hinton agar (BioMérieux, France). The distance between the discs, center to center was 20 mm. This test was performed when the strain was categorized intermediate or resistant to third generation cephalosporins

2.4 Plasmid DNA extraction
Bacterial strains were seeded on Luria Bertunia agar (LBA) and in Luria Bertunia broth (LBB) (Becton Dickinson and

Conda). GeneJET plasmid kit (Thermo Scientific) was used for plasmid DNA extraction according to the manufacturer's recommandations. The DNA was stored at -20 °C.

2.5 Gene Detection and Amplification

A simplex PCR was performed for detection of CTX-M-3, CTX-M-15 and CTX-M-22 using the following primers: CTXM_for_GTTACAATGTGTGAGAAGCAG and CTXM_rev_CCGTTTCCGCTATTACAAAC [8]. Amplification was carried out in a final reaction volume of 25 µl consisting of 12.5 µl of Master Mix (Thermo Scientific), 1 µl of the primer mixture (Fermentas), 9.5 µL of milliQ water and 2 µL of DNA using a FlexCycler block thermocycler (Analytik Jena). Amplification program comprises an initial denaturation of 7 min at 94 °C followed by a repetition of 35 cycles. Each cycle consists of a denaturation of 50 seconds at 94 °C, a hybridization of primers at 52 °C for 40 s and an elongation of 30 s at 72 °C, followed by a final extension at 72°C for 7 min.

2.6 Visualization of Amplification Products

Visualization of amplification products was done by electrophoresis on 2% agarose gel (Conda) containing Midori Green (Nippon Genetics) under a voltage of 100 V for one hour. DNA Ladder 100 bp (Thermo Scientific) was used as a molecular weight marker and visualization of amplicons was done by transillumination (UV ray).

2.7 Sequencing

Amplification products were purified using the GeneJet PCR purification kit (Thermo Scientific) according the manufacturer's recommendations. All samples were sequenced at the GIGA Technology platform at the University of Liège (Belgium). BigDye® Terminator version 3.1 was used and the sequences were analyzed using ABI PRISM (Applied Biosystems).

3. Results

3.1 Bacterial strains and susceptibility to antibiotics

Among the 73 strains of enterobacteriaceae, the following species were identified: *E. coli* (17 strains), *K. pneumoniae* (33 strains), *P. mirabilis* (11 strains), *E. cloacae* (6 strains) and *M. morganii* (6 strains). Antibiotic resistance was higher with colistin (79.4%) and cotrimoxazole (63%). For aminoglycosides, resistance was higher to gentamicin (60.3%) and lowest to amikacin (2.7%). Concerning fluoroquinolones mainly represented by ciprofloxacin, resistance rate was also high (60.3%). For the β-lactams, enterobacteriaceae strains showed an ampicillin resistance of 80.8%. The last generations cephalosporins were also less effective with resistance rate of 68.5% (cefuroxime), 57.5% (cefotaxime and ceftazidime) and 54.8% (cefepime). All strains were susceptible to meropenem. However, one strain of *E. cloacae* was intermediate to ertapenem (Table 1).

Table 1: Sensitivity of strains to β-lactams

Antibiotic family	Resistance rate (%)
	R
Ampicillin	80,8
Amoxicillin / clavulanic acid	68,5
Cefuroxime	68,5
Piperacillin / tazobactam	57,5
Cefotaxime	57,5
Ceftazidime	57,5
Cefepime	54,8
Temocillin	16,4
Ertapenem	1,4
Meropenem	0

R: resistant

3.2 Characterization of EBLSE Strains

The prevalence of ESBL in the 73 strains was 56.2% (41/73). This prevalence was 65.8% for *K. pneumoniae*, 24.4% for *E. coli*, 7.3% for *E. cloacae* and 2.4% for *M. morganii*. However, no ESBL was detected in *P. mirabilis*. All ESBLEs strains were resistant to cefotaxime, ceftazidime and cefepime. However, higher sensitivity was observed with meropenem (100%) and ertapenem (97.6%) (Table 2). Among the 41 ESBL strains, 39 (95.1%) harbored the $bla_{\text{CTX-M-15}}$ gene. This gene was present in 10 *E. coli* (24.4%), 25 *K. pneumoniae* (61%), 3 *E. cloacae* (7.3%) and one *M. morganii* (2.4%). No strain carried the $bla_{\text{CTX-M-3}}$ and $bla_{\text{CTX-M-22}}$ genes

Table 2: Sensitivity of Enterobacteriaceae to β-lactams

Strains	AM		AMC		CXM		CTX		CAZ		FEP		ETP		MEM		$bla_{\text{CTX-M}}$ genes
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
E. coli (N=10)	0	10	0	10	0	10	0	10	0	10	0	10	10	0	10	0	CTX-M-15 (10 strains)
K. pneumoniae (N=27)	0	27	1	26	0	27	0	27	0	27	1	26	27	0	27	0	CTX-M-15 (25 strains)
E. cloacae (N=3)	0	3	0	3	0	3	0	3	0	3	0	3	2	1	3	0	CTX-M-15 (3 strains)
M. morganii (N=1)	0	1	0	1	0	1	0	1	0	1	0	1	1	0	1	0	CTX-M-15 (1 strain)
Total	0	41	1	40	0	41	0	41	0	41	1	40	40	1	41	0	39

S: Susceptible, R: Resistant, AM: Ampicilin, AMC: Amoxicillin/clavulanic acid, CXM: Cefuroxime, CTX: Cefotaxime, CAZ: Ceftazidime, FEP: Cefepime, ETP: Ertapenem, MEM: Meropenem.

4. Discussion

This study was carried out on isolates of Enterobacteriaceae selected in the collection of the Observatory of Resistance of Micro-Organisms to Anti-Infective in Côte d'Ivoire (ORMICI) to make the inventory about CTX-M-15. Most Enterobacteriaceae are opportunistic pathogens responsible of infections in hospital and community settings. The results of our study bring to evidence an alarming and worrying situation of the presence of ESBL in Côte d'Ivoire with a rate of 56.2%. A rate well above the rate of a previous study on ESBLEs prevalence (9%) in the country [9]. This high level of ESBLEs could be the consequence of improper use of antibiotics both in community and in hospitals.

BlaCTX-M-15 gene was the only CTX-M type β-lactamase detected at 95.1% (39/41 strains). This gene has already been highlighted in *K. pneumoniae* in Côte d'Ivoire [10]. However, this is the first time that β-lactamase is identified in other species of Enterobacteriaceae. Our result confirms that CTX-M-15 is currently the type of β-lactamase CTX-M most common and most widespread worldwide. Some studies in several countries such as France [11], United States [4] and India [12] have shown that $bla_{\text{CTX-M-15}}$ gene is predominant in Enterobacteriaceae.

In Africa, a high prevalence of CTX-M-15 has also been observed in some countries such as Central African Republic [13], Nigeria [14], Algeria [15], Egypt [16], Tanzania [17] and Cameroon [18]. Similarly, studies on the molecular epidemiology of ESBL in companion animals have shown that CTX-M-15 is the β-lactamase commonly encountered in the United States [19], United Kingdom [20] and in Germany [21].

These studies show the ubiquitous character of CTX-M-15 which, according to some authors, invades pratically all human and animal compartments and the environment around the world [22, 23].

The antibiotic susceptibility test showed that all strains were highly resistant to cefotaxime but also to ceftazidime and cefepime. The resistance to ceftazidime confirms perfectly the presence of CTX-M-15 which is not an usual substrate for the enzymes of the CTX-M type [4]. Also, high resistance to other families of antibiotics such as ciprofloxacin, gentamycin, cotrimoxazole and colistin have been observed.

This result corroborates those of previous studies that have demonstrated a high level of resistance to these antibiotics in Enterobacteriaceae bearing the $bla_{\text{CTX-M-15}}$ gene [14, 13, 24]. A situation attributed to coexpression of $bla_{\text{CTX-M-15}}$ with other resistance genes such as $bla_{\text{TEM-1}}$, $bla_{\text{CMY-2}}$, $bla_{\text{OXA-1}}$, aac (6 ')-Ib-cr and *qnr* [13, 24].

The presence of $bla_{\text{CTX-M-15}}$ gene is of particular interest because it has been shown in previous studies that this gene was associated mainly with the plasmids of incompatibility group FII [25, 26]. Indeed, these plasmids are present in Enterobacteriaceae and have the propensity to acquire resistance genes and transfer among them [27]. Furthermore, broad-spectrum host plasmids such as IncN, InclI and IncL/M are also involved in the dissemination and propagation of enzymes of this family [20]. The $bla_{\text{CTX-M-15}}$ gene is flanked upstream of the insertion sequence *ISEcp1* which is responsible for its expression and mobilization [28, 29].

Finally, there was no resistance to carbapenems. These antibiotics remain the most effective in the treatment of infections caused by Enterobactericeae resistant to third generation cephalosporins, within the limits of their use (hospital use and combination).

5. Conclusion

The high level of the $bla_{\text{CTX-M-15}}$ gene observed in enterobacteriaceae represents a real epidemic risk with the compromise of treatment of infections by broad spectrum cephalosporins. It would therefore be essential to set up a system for monitoring the consumption of antibiotics, in particular β-lactam antibiotics, and to strengthen surveillance of the resistance of bacteria to antibiotics.

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7. Conflict of interest

The authors declare that they have no conflict of interest.

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