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General review

Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum β-lactamase (ESBL)

Épidémiologie des entérobactéries productrices de β-lactamases à spectre élargi (BLSE) de type CTX-M

H. Lahlaoui [a], A. Ben Haj Khalifa [b,*], M. Ben Moussa [a]

[a] *Laboratoire de microbiologie, hôpital militaire de Tunis, Tunis, Tunisia*
[b] *Laboratoire de microbiologie, hôpital Tahar Sfar, Mahdia 5100, Tunisia*

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Abstract

Over the past 20 years, some Enterobacteriaceae mainly *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* have demonstrated acquisition of plasmids secreting ESBL. CTX-M type ESBL have been isolated worldwide and their incidence has increased dramatically and is still increasing leading to a major therapeutic issue. Currently more than 150 allelic variants of CTX-M have been identified. These enzymes are classified in five major phylogenetic groups based on their gene sequences: CTX-M -1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25; two additional groups have been reported recently: CTX-M-74 and CTX-M-75. The important dissemination of these enzymes has led to an increased use of carbapenems. Their global community and nosocomial dissemination is often associated with a virulent *E. coli* clone ST131 producing CTX-M-15.

Keywords: Extended-spectrum-β-lactamase; CTX-M; Enterobacteriaceae

Résumé

Depuis les 20 dernières années, les espèces des entérobactéries principalement *Escherichia coli*, *Klebsiella pneumoniae et P. mirabilis* ont démontré des particularités d'acquisition de plasmides exprimant les β-lactamases à spectre élargi (BLSE). Des BLSE de type CTX-M ont été isolés un peu partout dans le monde et leur fréquence a connu une augmentation spectaculaire ce qui est devenu un problème thérapeutique majeur puisqu'elle ne cesse de croître. Actuellement, on compte plus de 150 variants alléliques de CTX-M. Ces enzymes sont classées en cinq groupes phylogéniques majeurs en se basant sur leurs séquences génétiques : CTX-M-1 ; CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 et récemment deux autres groupes supplémentaires ont été rapportés : CTX-M-74 et CTX-M-75. Cette dissémination importante de ces enzymes a conduit à une utilisation croissante des carbapénèmes. Leur dissémination mondiale communautaire et nosocomiale est très souvent associée chez *E. coli* à un clone virulent ST131 producteur de CTX-M-15.

Mots clés : β-lactamase à spectre élargi ; CTX-M ; Entérobactéries

1. Introduction

CTX-M enzymes were discovered in 1989 [1] but they did not become predominant over the other ESBL enzymes until the first decade of the XXI century during which an extraordinary spread of these enzymes was observed [2] both in hospital and in community settings [3].

* Corresponding author.
E-mail address: aniswalid_tn2003@yahoo.fr (A. Ben Haj Khalifa).

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Since the early 2000s, *Escherichia coli* has become the enterobacteriaceae the most concerned by the emergence of ESBL; the spread of which was triggered by the massive use of broad spectrum cephalosporins in hospital settings, hand-borne cross transmission as well in hospital as in community settings.

The prevalence of ESBL producing *E. coli* has been increasing in almost all European countries, but with rates still ≤ 5% in a dozen countries (France, Scandinavian countries, Switzerland, Holland, Belgium, Poland, etc.), or > 10% in Italy and Greece, and even > 20% in Rumania, Portugal, Bulgaria, and Turkey [4]. The prevalence of ESBL producing *E. coli* is 12.1% in Africa [5]. The authors of multicentric studies carried out in Tunisia reported that 6% of *E. coli* isolated in 2007, in various hospital centers, were resistant to 3GC [6].

We had for objective to describe the epidemiological evolution of some enterobacteriaceae strains, mainly *E. coli, Klebsiella pneumoniae, and P. mirabilis* all producing CTX-M type ESBL, and to try to explain this.

2. Phylogeny

Contrary to other ESBL, the CTX-M family is a complex and non-homogeneous group of enzymes. The alignment of amino acid sequences of various CTX-M variants allowed classifying these enzymes in five groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. 2 additional groups CTX-M-74 and CTX-M-75 were identified recently [7]. The phylogenetic analysis suggests that CTX-M result from the mobilization of *Kluyvera* spp. chromosome *bla* genes when they were integrated in mobile genetic elements [2]. These original mobilized genes *bla* $_{\text{CTX-Ms}}$ affect cefotaxime more than ceftazidime. Nevertheless, and from an evolutive point of view, CTX-M tardily diverged by point mutations probably because of the pressure of selection of antibiotics. Once the *Kluyvera* spp. *Bla* $_{\text{CTX-M}}$ *genes* were mobilized and included in mobile genetic elements, the hydrolytic activity against ceftazidime was enhanced and new variants were described [7,8].

Each group of CTX-M enzymes is usually related to the chromosomal *bla* genes present in various *Kluyvera* species, which belong to the normal human intestinal microbiome, and is considered as a saprophyte and an opportunistic pathogen [9]. Furthermore, these groups were sometimes isolated in humans and associated to various infections, concerning mainly the urinary tract, the skin, and soft tissues [10] and are also present in the environment in water, ground, and food products of animal origin [9].

The chromosomal *bla Kluč* gene, identified in *Kluyvera cryocrescens*, was considered as the ancestor of the CTX-M-1 group [11]; the gene *Klua* de *Kluyvera ascorbata* was at the origin of the CTX-M-2 group; and three different chromosomal *bla* genes of *Kluyvera georgiana* were at the origin of groups CTX-M-8, CTX-M-9, and CTX-M-25 respectively [8]. CTX-M-74 and CTX-M-75 were characterized in a survey carried out in Brazil and with the change of a single amino acid compared to CTX-M-2 [8].

3. The *bla* $_{\text{CTX-M}}$ gene environment

The relationship of various *bla* $_{\text{CTX-M}}$ genes with specific genetic elements surrounding these genes including insertion sequences (IS), integrons, and transposons was described. The IS also participate in the over-expression of these genes and some of them are adjacent to integrons structures which are also integrated in the transposition units. These supra-structures are often included in conjugate plasmids. Furthermore, they can act as evolutive units and individually as selection units [12]. Some plasmids harboring *bla* $_{\text{CTX-M}}$ genes can also harbor other genes of resistance, including those encoding AmpC β-lactamase, carbapenemase, plasmid mediated quinolone resistance genes (PMQR), or methylase affecting aminoglycosides. All these genes could also help maintain *bla* $_{\text{CTX-M}}$ genes because of the co-selection process.

The *bla* $_{\text{CTX-M-10}}$ gene was associated to phage related sequences, which stresses the involvement of bacteriophages in the potential mobilization of this gene [13]. A new structure called integron mobilization unit (IMU) included in a 7 kb plasmid was recently associated to the mobilization of *bla* genes [14].

The *bla* $_{\text{CTX-M-15}}$ gene was proven to be mainly associated to plasmids of the FII group [15]. They are found mainly in enterobacteriaceae and were recently named "plasmids of resistance responsible for outbreak" because of their capacity to acquire genes of resistance and to transfer among bacteria [15]. Furthermore, a wide range of plasmids and replicon such as Incn, IncI1, and IncL/M have also been implicated in the diffusion and spread of CTX-M enzymes.

Many authors have studied the implication of various clones in the spread of CTX-M enzymes associated to *K. pneumoniae.* This is the cases of CC11, which was demonstrated to be widely distributed in Asia, associated to various CTX-M enzymes, including CTX-M-14 and CTX-M-15 [15,16]. In Hungary, this CC and also ST15 and ST147 were believed to be responsible for the spread of CTX-M-15 *K. pneumoniae* resistant to ciprofloxacin [17].

It is well known that chromosomal *bla* $_{\text{KLU}}$ genes are weakly expressed in wild strains and need a strong promoter upstream so as to increase the MICs of various antibiotics and consequently may be considered as phenotypically resistant. The IS provide this promoter in strains of enterobacteriaceae resistant to cefotaxime; this is the cases for *Ecp* 1 and IS *CR* 1 while IS26 was detected only in a few isolates [18].

From this several mobilization events may have occurred. Ceftazidime could have been one of the main factors of selection having contributed to the diversification of CTX-M [19].

The building of mutants carrying combinations of these mutations revealed that the simultaneous presence of cefotaxime and ceftazidime in the environment modelized the exponential evolution and diversification of the CTX-M enzyme [20].

Recombination events also boost evolution. These events have also been described in *bla* $_{\text{CTX-M}}$ genes. For example, the CTX-M-64 enzyme results from a recombination between genes