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SHORT REPORT

Occurrence of a Rare Multidrug Resistant *Escherichia coli* Coharboring *bla*$_{CTX-M-32}$ and *bla*$_{CTX-M-2}$ Genes in a Bovine

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In this study, a rare strain of *Escherichia coli* co-harboring *bla*$_{CTX-M-32}$ and *bla*$_{CTX-M-2}$ genes was characterized by whole genome sequencing. *Escherichia coli* strain was recovered from a bovine cecal sample collected at slaughter. The strain was resistant to third and fourth generation cephalosporins, fluoroquinolones, aminoglycosides, tetracycline and sulfamethoxazole, which was in concordance with the genotype: *bla*$_{CTX-M-32}$ and *bla*$_{CTX-M-2}$, *aac(3)*-Via and *tet(A)* and *sul1* genes, respectively. The *E. coli* strain was characterized as pathogenic, and belongs to the ST226 (CC226), serotype O141ab/ac:H5, phylogenetic group A and fumC27/fimH41 types. No virulence genes were found in the genome. According to the plasmid analysis, IncFIB, IncHI2 and p0111 were present. The analysis of the contigs revealed that the *bla*$_{CTX-M-32}$ was located in the chromosome, upstream IS*5*-like/IS*Kpn26* and IS*1380*-like/IS*Ec9* sequences, whereas *bla*$_{CTX-M-2}$ was in the p0111 plasmid, within an integron class 1, and flanked by *Orf3*/qacEΔ1 and IS*91*/IS*CR1*. To our knowledge, this is the first report of a multidrug resistant *Escherichia coli* strain co-harboring two CTX-M enzymes in a healthy bovine from Portugal. This finding allows a better understanding on the dissemination of these resistance mechanism among bacteria of animal origin.

Correction added on August 12, 2021 after first online publication of February 16, 2021: The article category has been changed to Short Report. The text ‘‘Letter to Editor’’ is removed from the title and the abstract has been included to reflect this change.

Introduction

The emergence of extended-spectrum β-lactamases (ESBLs)-producing Enterobacteriaceae represents a public health concern. Over 10 families associated with ESBLs have been documented, being cefotaximase enzymes of the CTX-M family the most prevalent and distributed worldwide.[1]

In Europe, CTX-M-1, -14, and -15 are the most frequently reported enzymes in Enterobacteriaceae isolated from food-producing animals and food products.[2] The CTX-M-32 enzyme has been widely found in Europe as well, whereas CTX-M-2 is typically reported in South American countries.[3] Of note, CTX-M-32 enzyme exhibits enhanced catalytic efficiency against ceftazidime.[4] The coexistence of β-lactamases in the same bacteria is not uncommon, but they are usually of different families. The simultaneous presence of both CTX-Ms in the same habitat is thought to be a requirement for recombination, and the maintenance of both CTX-Ms in the strain can also be seen as a bacterial strategy to enhance antibiotic resistance.[3]

In this study, we describe a rare strain of *Escherichia coli* coharboring *bla*$_{CTX-M-32}$ and *bla*$_{CTX-M-2}$ genes, recovered from a bovine cecal sample collected at slaughter, under the scope of monitoring and reporting antimicrobial resistance in zoonotic and commensal bacteria (CD652/2013). The antimicrobial susceptibility profile of the strain was determined through minimum inhibitory concentrations (MICs) using commercially available 96-well microplates (Sensititre®; Trek Diagnostic Systems, UK); interpretation of results was done according to European Committee on Antimicrobial Susceptibility Testing epidemiological breakpoints. Resistance mechanisms associated with ESBLs were evaluated by PCR followed by Sanger sequencing, as previously described.[5]

Whole genome sequencing was performed by Illumina Hi-Seq technology (NovaSeq 6000 S2 PE150 XP sequencing mode; Eurofins Genomics Europe Sequencing GmbH, Germany). The data for this study have been deposited in the European Nucleotide Archive at European Institute of Bioinformatics under accession number PRJEB40129. Illumina platform generated 5,866,558 reads. FastQC was used to check raw data quality. Reads were trimmed using BBDuk, from the BBTools package, and 97.2% of reads remained in the data set for further analysis by bioinformatics tools. The assembly using SPAdes 3.12.0 (with default settings) originated 121 contigs (longer than 500 bp and a N50 of 114,128 bp) and a

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Table 1. Phenotypic and Genomic Characterization of *Escherichia coli* Strain

Phenotype	*MIC (mg/L)*	*WGS analysis*	*WGS tools*
Ampicilin	>64	$bla_{CTX-M-32}$; $bla_{CTX-M-2}$	ResFinder 3.2
Cefepime	>32		
Cefotaxime	>64		
Cefoxitin	8		
Ceftazidime	8		
Ciprofloxacin	0.25	—	
Nalidixic acid	16		
Gentamicin	32	*aac(3)*-VIa	
Tetracycline	>64	*tet(A)*	
Sulfamethoxazole	>1024	*sul1*	
Plasmid replicons	IncFIB; IncHI2; p0111		PlasmidFinder 2.1
MLST	ST226		MLST 2.0
Serotype	O141:H5		SerotypeFinder 2.0
Virulence genes	—		VirulenceFinder 2.0
fumC/fimH type	*fumC*27/*fimH*41		*CHTyper 1.0*
Pathogenicity	Yes (92.9%)		PathogenFinder 1.1
Phylogenetic group	A		Clermont Typing

"—" not found.

MIC, minimum inhibitory concentration; MLST, multi locus sequence type; WGS, whole genome sequencing.

predicted genome size of 4,975,618 bp with 50.3% guanine cytosine content. Data analysis was performed using tools available at the Center for Genomic Epidemiology website, The Comprehensive Antibiotic Resistance Database and ClermonTyping tool, as described on Table 1. The strain was resistant to third and fourth generation cephalosporins, fluoroquinolones, aminoglicosides, tetracycline, and sulfamethoxazole, which was in concordance with the genotype: $bla_{CTX-M-32}$ and $bla_{CTX-M-2}$, *aac(3)*-Via, *tet(A)* and *sul1* genes, respectively (Table 1).

Although the strain evidenced decreased susceptibility to ciprofloxacin, no plasmid-mediated quinolone resistance genes or chromosomal point mutations was identified. However, several efflux pumps were present in the genome, including *acrA*, *acrB*, *emrB*, and *emrR*, which may explain the nonsusceptible phenotype. Despite not having any virulence genes, this strain was predicted by PathogenFinder as having 92.9% probability of being a human pathogen. Beyond the virulence genes, this tool predicts pathogenicity based on genomic features associated with both pathogenicity and nonpathogenicity, as well as plasmids, secretion systems, antibiotic resistance genes, adherence factors, secretion systems, among others.

In addition, PLACNETw was used to predict and reconstruct plasmids and the location of resistance genes within the contigs. The $bla_{CTX-M-32}$ was located in the chromosome, and $bla_{CTX-M-2}$ in the p0111 plasmid (Supplementary Fig. 1). Contigs containing the bla_{CTX-M} genes were further analyzed with Artemis, ISFinder, and Basic Local Alignment Search Tool to identify genetic platforms. The analysis revealed that $bla_{CTX-M-32}$ is located upstream IS*5*-like/IS*Kpn26* and IS*1380*-like/IS*Ec9* sequences, whereas $bla_{CTX-M-2}$ is present within a class 1 integron and flanked by transposable elements, namely *Orf3*/qacEΔ1 and IS*91*/IS*CR1* (Supplementary Fig. 1).

CTX-M enzymes are plasmid-mediated acquired cefotaximases related to the chromosome-encoded genes of *Kluyvera* spp. and, in their original context, the presence of a specific genetic platform is required for the gene expression and consequently the increase of MIC values.[1,3] According to Zhao and Hu,[1] an aspartate aminotransferase-encoding gene is commonly found upstream the variant when the bla_{CTX-M} gene is located in the chromosome, but in plasmid-borne genes, this genetic context can be replaced by mobile genetic elements such as IS*Ecp1* or IS*CR1*, important for the mobilization and dissemination of CTX-M genes. These observations are in accordance with the genetic platform found in our study (Supplementary Fig. 1). In addition, the hypothesis of $bla_{CTX-M-32}$ being located in the chromosome is reinforced by the presence of a pyridoxal phosphate-dependent aminotransferase gene upstream this variant.

To our knowledge, this is the first report of a rare multidrug resistant *E. coli* strain, recovered from a bovine in Portugal, coharboring two CTX-M-enzymes, namely CTX-M-32 and CTX-M-2. This finding highlights the dissemination of ESBL-producing bacteria and reinforces the importance of an active surveillance of antimicrobial resistance mechanisms in zoonotic and commensal bacteria.

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Disclosure Statement

The authors declare that no competing interests exist.

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Supplementary Material

Supplementary Figure S1

References

1. Zhao, W.H., and Z.Q. Hu. 2013. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. Crit. Rev. Microbiol. 39:79–101.
2. Silva, N., I. Carvalho, C. Currie, M. Sousa, G. Igrejas, and P. Poeta. 2019. Extended-spectrum-β-lactamase and carbapenemase-producing Enterobacteriaceae in food-producing animals in Europe. In: J.-L. Capelo-Martínez and G. Igrejas (ed.), Antibiotic Drug Resistance. John Wiley & Sons, Inc, New Jersey, pp. 261–273.
3. Cantón, R., J.M. González-Alba, and J.C. Galán. 2012. CTX-M enzymes: origin and diffusion. Front. Microbiol. 3:110.
4. Cartelle, M., M. del Mar Tomas, F. Molina, R. Moure, R. Villanueva, and G. Bou. 2004. High-level resistance to ceftazidime conferred by a novel enzyme, CTX-M-32, derived from CTX-M-1 through a single Asp240-Gly substitution. Antimicrob. Agents Chemother. 48:2308–2313.
5. Dallenne, C., A. Da Costa, D. Decré, C. Favier, and G. Arlet. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. 65:490–495.

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