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ARTÍCULO ORIGINAL

ß-lactamases produced by amoxicillin-clavulanate-resistant enterobacteria isolated in Buenos Aires, Argentina: A new *bla*$_{TEM}$ gene

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Abstract
Resistance to β-lactam/β-lactamase inhibitors in enterobacteria is a growing problem that has not been intensively studied in Argentina.
In the present work, 54/843 enterobacteria collected in a teaching hospital of Buenos Aires city were ampicillin-sulbactam-resistant isolates remaining susceptible to second- and third-generation cephalosporins. The enzymatic mechanisms present in the isolates, which were also amoxicillin-clavulanic acid (AMC)-resistant (18/54) were herein analyzed. Sequencing revealed two different variants of *bla*$_{TEM-1}$, being *bla*$_{TEM-1b}$ the most frequently detected allele (10 *Escherichia coli*, 3 *Klebsiella pneumoniae*, 2 *Proteus mirabilis* and 1 *Raoultella terrigena*) followed by *bla*$_{TEM-1a}$ (1 *K. pneumoniae*). Amoxicillin-clavulanate resistance seems to be mainly associated with TEM-1 overproduction (mostly in *E. coli*) or co-expressed with OXA-2-like and/or SHV β-lactamases (*K. pneumoniae* and *P. mirabilis*).
A new *bla*$_{TEM}$ variant (TEM-163) was described in an *E. coli* strain having an AMC MIC value of 16/8 μg/ml. TEM-163 contains Arg$_{275}$Gln and His$_{289}$Leu amino acid substitutions. On the basis of the high specific activity and low IC$_{50}$ for clavulanic acid observed, the resistance pattern seems to be due to overproduction of the new variant of broad spectrum β-lactamase rather than to an inhibitor-resistant TEM (IRT)-like behavior.

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PALABRAS CLAVE
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ß-lactamasas producidas por enterobacterias resistentes a amoxicilina-ácido clavulánico aisladas en Buenos Aires, Argentina: un nuevo gen bla_{TEM}

Resumen
La resistencia a la combinación de β-lactámico/inhibidor de β-lactamasa en enterobacterias es un problema creciente que no ha sido estudiado intensamente en Argentina.
En el presente trabajo, 54/843 enterobacterias recolectadas en un hospital universitario de la ciudad de Buenos Aires fueron resistentes a ampicilina-sulbactama, pero se mantuvieron sensibles a las cefalosporinas de segunda y tercera generación. Se analizaron los mecanismos enzimáticos presentes en los aislamientos que también fueron resistentes a amoxicilina-ácido clavulánico (AMC) (18/54).
La secuenciación reveló dos variantes diferentes de bla_{TEM-1}, donde bla_{TEM-1b} es el alelo más frecuentemente detectado (10 *Escherichia coli*, 3 *Klebsiella pneumoniae*, 2 *Proteus mirabilis* y 1 *Raoultella terrigena*), seguidos por bla_{TEM-1a} (1 *K. pneumoniae*). La resistencia a AMC parece estar asociada principalmente con la hiperproducción de TEM-1 (sobre todo en *E. coli*) o con la coexpresión con β-lactamasas tipo OXA-2 y/o SHV (*K. pneumoniae* y *P. mirabilis*).
Se describió una nueva variante de bla_{TEM} (TEM-163) en un aislamiento de *E. coli* que presentó una CIM frente a AMC de 16/8 μg/ml. La enzima TEM-163 contiene dos sustituciones de aminoácidos respecto de TEM-1, $Arg_{275}Gln$ y $His_{289}Leu$. Teniendo en cuenta la alta actividad específica observada y la baja IC_{50} para el ácido clavulánico, el patrón de resistencia de este aislamiento parece obedecer a la hiperproducción de la nueva variante de la β-lactamasa de amplio espectro, en lugar de vincularse con un comportamiento similar al de una TEM resistente a inhibidores (IRT).

Introduction

Amoxicillin-clavulanate (AMC) is one of the most frequently prescribed antibiotic combinations in many countries, especially in ambulatory patients. For this reason, even if it is still a low occurrence event, resistance to β-lactam/β-lactamase inhibitors among enterobacteria clinical isolates is an emerging worldwide problem. Different enzymatic mechanisms are associated with AMC resistance: hyperproduction of chromosome-encoded class C β-lactamases, acquired plasmid-encoded cephalosporinases (AmpC type), hyperproduction of plasmid-mediated class A β-lactamases (such as TEM-1 and SHV-1 enzymes), production of class D oxacillinases and inhibitor-resistant TEM (IRTs or CMTs) and SHV mutants[6,14,19,23] (whose β-lactamase activities are poorly inhibited by clavulanate). In addition to enzymatic mechanisms, a decrease in the expression or absence of outer membrane proteins might also be involved, i.e., a deficiency in the OmpF and/or OmpC production has been associated to this phenotype in *Escherichia coli*[4].

IRTs were frequently described as plasmid-encoded enzymes derived from TEM-1 or TEM-2 β-lactamases and detected mainly from European isolates. Substitutions of one or more amino-acid residues at positions 69, 244, 275 and 276 render structural changes in β-lactamases that affect affinity for inhibitors, but produce only slight modifications in the isoelectric point (pI) or activity on other β-lactam compounds. Other substitutions also found in IRT β-lactamases do not seem to be involved in the IRT phenotype[9].

Overproduction of both TEM-1 and SHV-1, or production of an IRT may increase resistance to amoxycillin, ticarcillin, amoxicillin-clavulanic acid and, frequently to piperacillin and ticarcillin-clavulanic acid, while only slightly affecting susceptibility to narrow spectrum cephalosporins, cephamycins, extended-spectrum cephalosporins, and, in most cases, to piperacillin-tazobactam[3].

Over the last years, an increase in the rate of resistance to AMC has been noted among *E. coli* isolates in our country. According to the "Sistema Informático de Resistencia (SIR)" susceptibility to ampicillin-sulbactam in *E. coli* has been decreasing from 62%[22] (years 2004-2005) to 50% (years 2006-2008; M. Radice, personal communication). In spite of the high use of this association, there is little information available about the prevalence of AMC resistance mechanisms in *Enterobacteriaceae*.

The aim of this study was to investigate the enzymatic mechanisms of amoxicillin-clavulanate-resistant enterobacteria lacking inducible chromosomal *ampC* genes isolated from a teaching hospital of Buenos Aires city.

Materials and methods

Microorganisms

Isolates recovered from different clinical specimens that fulfilled a screening criteria (*Enterobacteriaceae* resistant to ampicillin-sulbactam but remaining susceptible to cefoxitin, ceftazidime and cefotaxime by a disk diffusion method) were studied. Isolates were collected within a 5-month period (May to September 2008) from patients attending the Hospital de Clínicas that belongs to UBA (University of