Characterization of the enzyme aac(3)-id in a clinical isolate of Salmonella enterica serovar Haifa causing traveler's diarrhea

Roberto Cabrera a, Joaquim Ruiz b, Javier Sánchez-Céspedes a, Pilar Goñi c, Rafael Gómez-Lus c, M. Teresa Jiménez de Anta a, Joaquim Gascón b and Jordi Vila a,*

a Servicio de Microbiología, IDIBAPS, Hospital Clínico, Barcelona, Spain
b Centro de Salud Internacional, IDIBAPS, Universidad de Barcelona, Hospital Clínico, Barcelona, Spain
c Departamento de Microbiología, Medicina Preventiva y Salud Pública, Facultad de Medicina, Universidad de Zaragoza, Spain

ABSTRACT

Introduction: The objective of this investigation was to identify the mechanism of decreased susceptibility to gentamicin in a Salmonella clinical isolate, leading to the detection of an aminoglycoside acetyltransferase gene found in a class 1 integron.

Methods: A multidrug-resistant Salmonella strain was recovered from feces of a traveler to Egypt. The antimicrobial susceptibility test to 12 antimicrobial agents was performed with the Kirby-Bauer method. The presence of class 1 integron was determined by PCR. The amplified product was recovered and sequenced in order to establish the genes carried. In addition, susceptibility to gentamicin C1a, gentamicin C1, sisomicin, neomycin, dibekacin, kanamycin, tobramycin, amikacin, netilmicin, apramycin, dactimycin, spectinomycin, streptomycin, lividomycin and butirosin, was established. The Champion™ PET101 Directional TOPO® Expression Kit was used to clone and express the aac(3)-I gene.

Results: The isolate was identified as Salmonella enterica serovar Haifa, showing resistance to nalidixic acid, tetracycline and decreased susceptibility to gentamicin. One integron with a size circa 1,500 bp, encoding an aac(3)-Id plus aadA7 genes was observed. The analysis of the susceptibility to different aminoglycosides in the E. coli TOP10F strain transformed with the vector carrying aac(3)-Id gene showed resistance to gentamicin C1a, gentamicin C1, and dactimycin, in accordance with the presence of this enzyme but, was susceptible to sisomicin. The homology of the amino acid and nucleotide sequences with the AAC(3)-Id enzyme was of 100%.

Conclusion: The presence of the AAC(3)-Id enzyme was described for the first time in a Salmonella clinical isolate, leading to the detection of a aminoglycoside acetyltransferase gene found in a class 1 integron.

Palabras clave:
- Aminoglicoside acetyltransferase
- Integron
- Salmonella
- Traveler’s diarrhea

Caracterización del enzima AAC(3)-Id en un aislamiento clínico de Salmonella Haifa causante de diarrea del viajero

RESUMEN

Introducción: el objetivo de este estudio fue identificar el mecanismo de sensibilidad disminuida a gentamicina en un aislamiento clínico de Salmonella, lo que nos condujo a la detección de un gen que codifica una acetyltransferasa modificante de aminoglucósidos localizada en un integron tipo 1.

Métodos: la cepa multiresistente de Salmonella fue aislada de las heces de un viajero a Egipto. La susceptibilidad a 12 agentes antimicrobianos se determinó mediante Kirby-Bauer. La presencia de integron clase 1 se realizó mediante PCR. El producto de PCR amplificado del integron fue recuperado y secuenciado para conocer los genes que contenía dicho integron. Además se determinó la susceptibilidad a gentamicina C1a, gentamicina C1, sisomicina, neomicina, dibekacin, kanamicina, tobramicina, amikacina, netilmicina, apramicina, dactimicina, espectinomicina, estreptomicina, lividomicina y butirosina. El kit de expresión Champion™ PET101 Directional TOPO® fue utilizado para clonar y expresar el gen aac(3)-I.

Resultados: el aislamiento fue identificado como Salmonella enterica serovaridad Haifa, el cual presentaba resistencia al ácido nalidixico, tetraciclina y sensibilidad disminuida a gentamicina. Se observó la presencia de un integron tipo 1 con un tamaño de 1500 bp en el que se encontraron dos genes (aac(3)-Id y aadA7). El análisis de la sensibilidad a diferentes aminoglucósidos de la cepa de E. coli TOP10F transformada con el vector que contenía el gen aac(3)-Id demostró resistencia a gentamicina C1a, gentamicina C1, y dactimicina, de un integron tipo 1 con un tamaño de 1,500 bp en el que se encontraron dos genes (aac(3)-Id y aadA7).

* Autor para correspondencia.
Correo electrónico: jvila@ub.edu (J. Vila).

0213-005X/see front matter © 2008 Elsevier España, S.L. Todos los derechos reservados.
Introduction

Traveler’s diarrhea (TD) is a frequent health problem among travelers to developing countries. This illness may be due to a large variety of microorganisms, among these, *Salmonella* is one of the most frequent following diarrheogenic *Escherichia coli* and *Shigella* spp.[2,3]. Diarrhea associated with *Salmonella* spp. is usually self-limited and does not require antibiotic therapy. However, in specific cases, due to both the severity or the duration of the symptoms, antibiotic treatment is required. Unfortunately, antimicrobial resistance levels among diarrheagenic pathogens have increased in recent years, and *Salmonella* spp. is not an exception.[4,5]

Acquisition of resistance may be related to two different mechanisms: 1. Transferable, such as plasmids or transposons, and 2. Non transferable, usually associated with chromosomal point mutations.[5-7]. The gastrointestinal environment serves as a reservoir for integron-bearing strains and since integrons are carried on plasmids and transposons, antibiotic selective pressure can potentate the dissemination of antibiotic resistance genes through these genetic elements.[8,9]

To date, nine classes of integrons have been described.[10]. Of these, the most relevant at a clinical level are those belonging to classes 1 and 2. The integrons of these two aforementioned classes usually carry gene-cassettes encoding for antibiotic resistance mechanisms. Among these gene-cassettes, the aminoglycoside-modifying encoding genes are considered the most prevalent.[6] The aim of this work was to investigate the mechanism of decreased susceptibility to gentamicin in a clinical isolate of *Salmonella enterica* serotype Haifa.

Methods

**Bacterial isolate**

A *Salmonella* isolate recovered from feces of a traveler with diarrhea was identified by different typing methods, including biochemical tests and serotyping using somatic and flagella antiserum.[11]

**Antimicrobial susceptibility**

A preliminary antimicrobial susceptibility test was performed, using an agar diffusion method with commercially available disks (Becton Dickinson) to the following antibiotics: ampicillin, amoxicillin plus clavulanic acid, nalidixic acid, tetracycline, trimethoprim/sulphametoxazole, chloramphenicol, gentamicin, amikacin, imipenem, norfloxacin, ciprofloxacin and ceftazidime. Interpretation of results was performed according to the Clinical Laboratory Standards Institute (CLSI) guidelines. *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.[4,5]

To assess an activity pattern, susceptibility to gentamicin C1a, gentamicin C7, sisomicin, neomycin, dibekacin, kanamycin, tobramycin, amikacin, netilmicin, apramycin, dactimycin, spectinomycin, streptomycin, lividomycin and butirosin, the disk diffusion method on Mueller-Hinton agar was used. These disks were stained with ethidium bromide.

**DNA amplification and cloning of the aac(3)-I gene**

The Champion™ pET101 Directional TOPO® Expression Kit (Invitrogen, USA) was used to clone and express the aac(3)-I gene, following the manufacturer guidelines. Briefly, the entire AAC(3)I encoding gene was amplified using the forward primer AAC3IF: 5'-CAC CGT GTC AGT CGA AAT CAT C-3' and the reverse primer AAC3IR: 5'-GGC ATG ATT TTT ACT CTG C-3' using an agar diffusion method with commercially available disks. The amplification product was resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide (0.5 mg/L).

**Results and discussion**

A *Salmonella enterica* serovar Haifa was isolated from feces of a traveler with diarrhea returning from Egypt. This strain showed resistance to nalidixic acid, tetracycline and decreased susceptibility to gentamicin, while remaining susceptible to ampicillin, amoxicillin plus clavulanic acid, ceftazidime, cotrimoxazole,
chloramphenicol, amikacin, imipenem, norfloxacin, and ciprofloxacin.

The isolate was investigated for the presence of class 1 integrons. One amplicon of circa 1500 bp was detected. The sequence of this amplicon revealed the association of the integron with aac(3)-Id plus ant(3') (also named aadA7) aminoglycoside resistance genes (Figure 1). The detected aac(3)-Id nucleotide sequence showed amino acid and nucleotide homologies of 100% both with the aac(3)-Id and aac(3)-Ig genes located in different integrons in Salmonella enterica serovars Newport and Kentucky15,16, as well as in Vibrio fluvialis17 (GeneBank accession: AY458224, AY463797 and AB114632). Meanwhile, the homology with aac(3)-Ia, aac(3)-Ib and aac(3)-Ic was lower. The ant(3') did not show differences with other nucleotide sequences previously reported.

The Salmonella enterica serovar Haifa isolate showed a resistance pattern partially consistent with the presence of an A/C(3)-IId plus an ANT(3') aminoglycoside nucleotidyltransferase, with resistance or decreased susceptibility to gentamicin C1α, gentamicin C1β, daptomycin, and spectinomycin, but susceptible to sisomicin an aminoglycoside also considered a substrate of the A/C(3)-IId-type enzymes. (Table 1)18. In order to establish the exact role of the aac(3)-IId in the aminoglycoside resistance pattern detected, the gene was cloned in an expression vector. In the presence of IPTG, the transforming strain showed resistance to gentamicin C1α, gentamicin C1β, daptomycin and spectinomycin, but susceptible to sisomicin and an aminoglycoside also considered a substrate of the A/C(3)-IId-type enzymes. (Table 1)19. Therefore, our results suggest the chromosomal location of the integron.

The phenotypic characteristics of the strains analyzed (decreased susceptibility to gentamicin and susceptibility to sisomicin) suggested the presence of a new aminoglycoside acetyltransferase gene. Nevertheless, the genetic study show the presence of AAC(3)-Id enzyme, before described in Salmonella enterica serovar Newport. The phenotype (decreased susceptibility to gentamicin and susceptibility to sisomicin) shown by the strain may be explained by a post-translational change in the conformation of the enzyme. However, structural studies would be needed to show this hypothesis.

A Salmonella enterica serovar Haifa carrying a aac(3)-Id gene was identified in a class 1 integron for the first time. This result shows the potential of integrons to carry and spread resistance genes.

Acknowledgments

This study was funded by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III – FEDER, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008), FIS 04/0068, and grant 2005 SGR 0444 from the Departament d' Universitats, Recerca i Societat de la informació de la Generalitat de Catalunya, Spain to J.V. and by the DGA/Group of Ecology of Bacterial resistance, Spain. R.C. has a fellowship from Fundación Carolina and BBVA, Spain. J.R. research is supported by project CP05/0130.

References


Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Gm C1a</th>
<th>GmC1</th>
<th>Sisomicin</th>
<th>Daptomycin</th>
<th>Streptomycin</th>
<th>Spectinomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli ATCC 27912</td>
<td>27*1</td>
<td>26</td>
<td>25</td>
<td>18</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>BL2E</td>
<td>28</td>
<td>29</td>
<td>32</td>
<td>19</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>S. Haifa</td>
<td>13</td>
<td>15</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AAC(3)-Idb</td>
<td>0</td>
<td>0</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

* Diameter of the inhibition zone in mm.

- Table 1
- Antimicrobial susceptibility of S. Haifa

* Diameter of the inhibition zone in mm.

# BL2E E. coli strain transformed with the vector carrying the aac(3)-Id gene.


