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Sir,

Currently, CTX-M-type enzymes are the most common group of extended-spectrum β-lactamases (ESBLs) not belonging to the TEM and SHV families.

Here, we report the phenotypic and molecular characterization of two Portuguese strains of *Escherichia coli* harbouring CTX-M-15. Both strains were isolated in 2004. One strain (INSRA5753), isolated at Hospital Garcia de Orta in Lisbon, was from the urine of a 78-year-old woman who was living in a residential care home, using a catheter. The other strain (INSRA5754) was isolated from the blood of a 90-year-old woman 1 day after hospitalization in Vila Real. These two hospitals are about 400 km apart. The strains were detected as ESBL-producers in the hospitals, using the VITEK 1 system and ATB G-5, respectively.

In the Antibiotic Resistance Unit, at the National Institute of Health in Lisbon, β-lactamase production was confirmed using the Etest ESBL screen method. Using PCR, fragments indicating the presence of the *bla*<sub>TEM</sub> gene, the *bla*<sub>OXA</sub> gene, the *bla*<sub>CTX</sub> gene and the ubiquitous *ampC* gene were amplified from both strains.

The amplification products from *bla*<sub>TEM</sub>, *bla*<sub>OXA</sub> and *bla*<sub>CTX</sub> genes from both strains were then purified and sequenced. For sequencing of the *bla*<sub>CTX</sub>-M-15 gene, we used primers specific for the consensus of CTX-M group I: CTX-M-15F, 5’-AGAA-TAACCATCCATGGTT-3’ and CTX-M-15R, 5’-ACCCTCG-GTACGATTAGT-3’. Sequencing confirmed three resistance genes in both strains, namely *bla*<sub>TEM</sub><sub>1B</sub>, *bla*<sub>OXA</sub><sub>30</sub> and *bla*<sub>CTX</sub>-M-15. Sequence analysis using CTX-M-15 primers and ISEcp1, IS26 or IS903r primers indicated the presence of an ISEcp1-like element in both strains although neither had IS903, thus differing from the mobile elements of the *bla*<sub>CTX</sub>-M-15 gene detected previously in *Klebsiella pneumoniae*. The IS26 element was also not present in our strains. These findings suggest that CTX-M-15 enzymes in these two species in Portugal might have emerged in multiple places by plasmid acquisition of *bla*<sub>CTX</sub>-M-15 genes, with different elements implicated in the dissemination of these β-lactamase genes. This was corroborated by the PFGE analysis, which showed different clonal origins for each isolate (data not shown).

The transconjugant C600-URA5753 was a CTX-M-15-positive-transconjugant from *E. coli* INSRA5753 derived using C600 as the recipient. The transconjugant C600-URA5754 co-expressed CTX-M-15, OXA-30 and TEM-1 enzymes; indeed, none of these enzymes was transferable alone from *E. coli* INSRA5754 strain to *E. coli* C600 (Table 1), suggesting that the *bla*<sub>CTX</sub>-M-15 gene was carried in only one transferable plasmid with *bla*<sub>TEM</sub><sub>1B</sub> and *bla*<sub>OXA</sub><sub>30</sub>. MICs of various β-lactams, alone or in combination with β-lactamase inhibitors (Table 1), were determined using an agar dilution method, and MICs of other antimicrobial agents were determined using a broth microdilution method. The clinical isolates and the transconjugants showed a higher level of resistance to cefotaxime, ceftriaxone and aztreonam than to ceftazidime, which is characteristic of CTX-M producers. Isoelectric focusing revealed that both the clinical strain and transconjugant C600-URA5754 produced β-lactamases with pIs of 5.4 (TEM-1), 7.5 (OXA-30) and 8.9 (CTX-M-15). Transconjugant C600-URA5753 produced a β-lactamase with a pI of 8.9.

**References**


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**CTX-M-15, OXA-30 and TEM-1-producing *Escherichia coli* in two Portuguese regions**

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Keywords: extended-spectrum cephalosporins, antimicrobial resistance, β-lactamases, Enterobacteriaceae, Portugal

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Transparency declarations

None to declare.
To our knowledge the combination of CTX-M-15, TEM-1 and OXA-30 β-lactamases, here reported, was described previously only in strains from Senegal. However, the combination of CTX-M-15 and OXA-1 (similar to OXA-30) has been described in the UK, Canada and India. The spread of resistance to CTX-M-15 and OXA-1 (similar to OXA-30) has been described with dissemination either from or to the community, which is of high concern.

The coexistence of strains co-expressing the same TEM, OXA and β-lactamases, here reported, was described previously in strains isolated in two hospitals, transconjugants and recipients. However, the combination of CTX-M-15, TEM-1 and OXA-30-β-lactamases among clinical isolates of *E. coli* isolated from dogs in Portugal. J Antimicrob Chemother 2002; 49: 477–82.

To our knowledge the combination of CTX-M-15, TEM-1 and OXA-30 β-lactamases, here reported, was described previously only in strains from Senegal. However, the combination of CTX-M-15 and OXA-1 (similar to OXA-30) has been described in the UK, Canada and India. The spread of resistance to extended-spectrum cephalosporins, their extensive use, and the coexistence of strains co-expressing the same TEM, OXA and CTX-M enzymes in distant regions of Portugal may impede the use of β-lactams in other regions of the country. These cases involving hospital and community environments are consistent with dissemination either from or to the community, which is of high concern.

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### Transparency declarations

None to declare.

### References


Low incidence of severe liver toxicity in patients receiving antiretroviral combinations including atazanavir

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Keywords: HIV, hepatotoxicity, protease inhibitors, HAART

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Sir,

Hepatotoxicity is associated with all antiretroviral drugs currently used. A significant increase in plasma aminotransferase concentrations has been observed in 1–9% of patients in registration trials with different protease inhibitors (PIs).¹ In most cohort studies, higher rates of liver toxicity have been found, especially in hepatitis B virus (HBV)- or hepatitis C virus (HCV)-coinfected patients.² However, lower rates of severe hepatotoxicity are associated with all antiretroviral drugs currently used. A significant increase in plasma aminotransferase concentrations has been observed in 1–5% of patients taking atazanavir.

The aim of this study was to assess the incidence of severe liver events in patients taking antiretroviral drug combinations including atazanavir outside well-circumscribed clinical trials. From May 2003 to March 2005, 99 patients in three Spanish hospitals started a drug combination containing atazanavir. Two patients were antiretroviral-naive and the remainder had been treated previously. Of the 99 patients, 33 received unboosted atazanavir at a dose of 400 mg once daily, whereas the other subjects were given atazanavir 300 mg plus ritonavir 100 mg once daily. Patients were followed every 3 months after starting atazanavir for at least 6 months.

We considered either of the following conditions to be severe liver events: (i) decompensation of pre-existing liver cirrhosis or (ii) grade 3–4 HT, i.e. plasma aspartate aminotransferase or alanine aminotransferase values more than 5 times above the upper limit of normality when baseline levels were normal, or more than 3.5 times the baseline values when they were abnormal.

In the statistical analysis, continuous variables were compared using the Mann–Whitney U-test. The χ² test was used to compare categorical variables.

A total of 37 patients (37%) carried serum HCV antibodies, 9 (9%) were HBV surface antigen (HBsAg)-positive and 5 (5%) were coinfectected with both HCV and HBV. Ten subjects (10%) reported consuming more than 50 g of alcohol daily. Other antiretroviral drugs prescribed along with atazanavir were didanosine in 51 patients, tenofovir in 43, lamivudine in 38, abacavir in 21, zidovudine in 15, stavudine in 11, efavirenz in 5, enfuvirtide in 4, lopinavir-ritonavir in 3 and nevirapine in 1 patient. The median (Q1–Q3) follow-up on atazanavir was 49 (39–57) weeks.

Six months after starting atazanavir, 30 out of 37 patients (81%) with detectable plasma HIV viral load at baseline showed severe hypertransaminasaemia (HT) have been reported in other studies, particularly in patients taking ritonavir-boosted lopinavir.³

Atazanavir is a PI that can be used alone or boosted with ritonavir. Atazanavir is associated with a benign increase in unconjugated bilirubin, due to an inhibition of the hepatic enzyme UDP-glucuronosyltransferase.¹ In clinical trials,¹–⁷ a grade 3 or 4 increase in the plasma aminotransferase concentrations has been observed in 1–5% of patients taking atazanavir. However, data regarding atazanavir-related liver toxicity in clinical cohorts are very limited, particularly in settings where HCV- or HIV-coinfections are very prevalent.⁸

The baseline characteristics of patients who developed severe liver events and of those who did not are shown in Table 1.

Table 1. Baseline characteristics of patients who developed severe liver events and of those who did not

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Grade 3–4 HT or HD (n = 3)</th>
<th>No grade 3–4 HT or HD (n = 96)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years³</td>
<td>45 (34–52)</td>
<td>40 (36–45)</td>
<td>0.6</td>
</tr>
<tr>
<td>Male gender, no. (%)</td>
<td>1 (33)</td>
<td>67 (70)</td>
<td>0.2</td>
</tr>
<tr>
<td>AIDS, no. (%)</td>
<td>1 (33)</td>
<td>36 (38)</td>
<td>1</td>
</tr>
<tr>
<td>HCV seropositive, no. (%)</td>
<td>3 (100)</td>
<td>34 (35)</td>
<td>0.05</td>
</tr>
<tr>
<td>Undetectable HIV viral load, no. (%)</td>
<td>2 (67)</td>
<td>60 (63)</td>
<td>1</td>
</tr>
<tr>
<td>CD4+ count (cells/mm³)³</td>
<td>172 (93–327)</td>
<td>445 (270–699)</td>
<td>0.04</td>
</tr>
<tr>
<td>ALT (IU/mL)³</td>
<td>51 (29–58)</td>
<td>31 (21–45)</td>
<td>0.3</td>
</tr>
<tr>
<td>AST (IU/mL)³</td>
<td>43 (35–49)</td>
<td>31 (23–45)</td>
<td>0.2</td>
</tr>
<tr>
<td>TB (mg/dL)³</td>
<td>1 (0.8–1.4)</td>
<td>0.6 (0.4–1.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>GGT (IU/mL)³</td>
<td>67 (52–90)</td>
<td>43 (24–122)</td>
<td>0.4</td>
</tr>
<tr>
<td>Boosted ATV, no. (%)</td>
<td>1 (33)</td>
<td>65 (68)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

³Median (Q1–Q3). HT, hypertransaminasaemia; HD, hepatic decompensation; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; GGT, gamma-glutamyltransferase; ATV, atazanavir.