Introduction of OXA-48-producing Enterobacteriaceae to Israeli hospitals by medical tourism

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Objectives: The carbapenemase OXA-48 has been reported from different Mediterranean countries. It is mostly encoded on a single plasmid in various Enterobacteriaceae species. We characterized the epidemiological and molecular features of OXA-48-producing Enterobacteriaceae (OPE) in Israel.

Methods: Epidemiological investigation was conducted by the National Center for Infection Control. Genotyping was performed using multilocus sequence typing. The blaOXA-48-carrying plasmids were investigated using S1 endonuclease and restriction fragment length polymorphism (RFLP). Conjugation efficiency of the blaOXA-48-carrying plasmids was studied in a filter mating experiment.

Results: Since 2007, four OPE-infected patients were identified, all non-Israeli (two Palestinian, one Jordanian and one Georgian). Three had prior hospitalization; two in Jordan and one in Georgia. The blaOXA-48 gene was detected in three Escherichia coli strains belonging to different clonal complexes, one Klebsiella oxytoca and one Klebsiella pneumoniae sequence type 101, as previously reported from Tunisia and Spain. In all isolates, the blaOXA-48 gene was located inside Tn1999.2 and was carried on a 60 kb plasmid with an identical RFLP pattern. The plasmid was able to conjugate from Klebsiella spp. to E. coli, and had a conjugation efficiency up to ≏10000 times higher than that of pKpQIL.

Conclusions: OPE, introduced mainly by medical tourism, are an emerging threat to patients from affected Mediterranean countries. The blaOXA-48-carrying plasmid demonstrated remarkable conjugation efficiency, which is probably important in the success of its dissemination.

Keywords: conjugation, plasmids, carbapenemases

Introduction

OXA-48 is one of the few Ambler class D, carbapenem-hydrolysing β-lactamases that have been identified in Enterobacteriaceae and is the most commonly reported. First identified in Turkey in 2001,5 OXA-48-producing Enterobacteriaceae (OPE) have since been reported from several countries in the Middle East, North Africa and Europe.1,6 The blaOXA-48 gene has been detected in a variety of Enterobacteriaceae species and clones, but a common feature in almost all isolates is the location of the gene inside the Tn1999 transposon within a 70 kb plasmid.1,6

Since 2006, resistance to carbapenems in Enterobacteriaceae in Israel has been caused mainly by a single clone of KPC-producing Klebsiella pneumoniae, believed to have been imported from the USA in late 2005.5 Here we describe the epidemiology and molecular characteristics of OPE infection identified in Israel since 2007.6

Methods

Patients and isolates

Patients were identified following detection of blaOXA-48-harbouring Enterobacteriaceae as part of ongoing activity of the Israeli National Center for Infection Control. Rectal surveillance cultures were done as previously described.7 Patients' data were collected as part of the epidemiological investigation. The study was approved by the Ethics Committee of the Tel-Aviv Medical Center, Tel-Aviv, Israel.

Phenotypic and molecular characterization of isolates

Identification and antimicrobial susceptibility testing was performed using the VITEK-2 system (bioMérieux, Marcy l’Etoile, France). Ertapenem, imipenem and meropenem MICs were verified by Etest (AB Biodisk, Solna, Sweden). Phenotypic characterization comprised the extended-spectrum β-lactamase (ESBL) double-disc test, the modified Hodge test (MHT) and the boronic acid, EDTA and dipicolinic acid assays.8 Isolates were...
He was diagnosed with cholangiocarcinoma and underwent fully treated with ceftazidime and colistin. The second patient, a Jordanian woman referred to our hospital for oncological care, was diagnosed with an OXA-48-producing E. coli bacteraemia, success-fully treated with meropenem and colistin. On the day after transfer he developed K. oxytoca sepsis and was treated successfully with meropenem and colistin. In Patients 3 and 4, OPE were detected in rectal culture as part of either routine admission surveillance (Patient 3) or contact investigation (Patient 4). Following the detection of the OXA-48-producing E. coli in patient 3, the entire ward in the facility (40 patients) was surveyed for the presence of OPE by rectal swabs, yielding negative results. Patient 4 was hospitalized for 3 days for control of hypertension and was surveyed as part of an unrelated contact investigation. She had never been hospital-ized outside of Israel, but had made frequent visits to Jordan.

Microbiological and molecular characteristics of OPE strains and blaOXA-48-carrying plasmids
All isolates yielded a positive MHT and a negative ESBL double-disc test (except 6537) and negative boronic acid, EDTA and dipicolinic acid assays. PCR was positive for the blaOXA-48 gene in all isolates. All isolates and their respective transformants (T), except E. coli 6537, were susceptible to ceftriaxone and ceftazidime but had elevated MICs of ertapenem and imipenem (Table 2). Isolate E. coli 6537, but not 6537T, tested positive for the blaOXA-48 gene. The MICs of imipenem and imipenem for all the isolates and their corresponding transformants were similar, with the exception of those for E. coli 1736, which showed decreased OmpC expression.

Conjugation studies
blaOXA-48-carrying plasmids from all isolates were able to undergo both intra- and inter-species self-conjugation. Intra-

<table>
<thead>
<tr>
<th>Species (strain number)</th>
<th>Patient number</th>
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</thead>
<tbody>
<tr>
<td>E. coli (1736)</td>
<td>1</td>
</tr>
<tr>
<td>K. oxytoca (3439)</td>
<td>2</td>
</tr>
<tr>
<td>E. coli (6537)</td>
<td>3</td>
</tr>
<tr>
<td>E. coli (4360A), K. pneumoniae (4360B)</td>
<td>4</td>
</tr>
</tbody>
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species conjugation of the \( \text{bl} \text{a}_{\text{OXA-48}} \) -carrying plasmid (isolates 6537 and 4360A) was highly efficient, with a conjugation frequency of \( 5.5 \times 10^{-1} \) and \( 8.4 \times 10^{-2} \), respectively, compared with only \( 1 \times 10^{-5} \) for the pKpQIL plasmid (isolate 2112).

### Discussion

This report highlights the role of medical tourism as a main route for the introduction of OPE to Israel; three of the four patients (Patients 1–3) were hospitalized outside of Israel (two in Jordan and one in Georgia) prior to the detection of an OPE infection. In the fourth patient, the acquisition could not be traced to healthcare exposure, raising the possibility of community acquisition, possibly during one of her frequent trips to Jordan. Interestingly, she was a carrier of OXA-48-producing \( \text{K. pneumoniae} \) ST101, as previously reported from Tunisia\(^4\) and Spain.\(^1\)\(^8\)

Our study demonstrates the presence of the \( \text{bl} \text{a}_{\text{OXA-48}} \) gene inside Tn1999.2, in an identical 60 kb plasmid in various \( \text{E. coli} \) and \( \text{Klebsiella} \) spp. isolates. The \( \text{rep} \text{P} \) gene, detected in the previously described 70 kb plasmid,\(^1\) was not detected in our plasmid. A recent report from Tunisia identified two types of 70 kb, \( \text{bl} \text{a}_{\text{OXA-48}} \) -carrying plasmids, one presenting the IncA/C group and the other undetermined.\(^1\)\(^9\) Hence, it appears that \( \text{bl} \text{a}_{\text{OXA-48}} \) -carrying plasmids are not identical, and may vary in their Inc group.

Unlike \( \text{bl} \text{a}_{\text{KPC-3}} \), which has spread mainly in a single epidemic \( \text{K. pneumoniae} \) clone (ST258),\(^5\) the spread of the \( \text{bl} \text{a}_{\text{OXA-48}} \) gene is likely to be facilitated by the transfer of plasmids.\(^1\) The conjugation experiments performed have demonstrated that (i) the \( \text{bl} \text{a}_{\text{OXA-48}} \) -carrying plasmid identified here is capable of interspecies conjugation, as previously described,\(^1\) and (ii) its conjugation efficiency is \( \sim10,000 \) times higher than that of pKpQIL, the \( \text{bl} \text{a}_{\text{KPC-3}} \) -carrying plasmid. These differences in conjugation frequencies may help to improve our understanding of the different ways in which different carbapenemases spread. As knowledge regarding the prevalence of resistant organisms in certain countries may be lacking, admission screening for resistant organisms of all patients who have received medical care in foreign countries may be prudent.

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### Transparency declarations

None to declare.

### Supplementary data

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

### References