Discrimination between Native and Tn 6010-Associated oqxAB in Klebsiella spp., Raoultella spp., and other Enterobacteriaceae by Using a Two-Step Strategy

Thomas Guillard, Anne-Laure Lebreil, Lars Hestbjerg Hansen, Aymric Kissierli, Sibel Berger, Alain Lozniewski, Corentine Alauzet, Christophe De Champs

To cite this version:


HAL Id: hal-01481982
https://hal.univ-lorraine.fr/hal-01481982
Submitted on 3 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
We developed a two-step PCR-based strategy to detect genes encoding OqxAB, allowing a specific assignment of Tn6010-associated oqxAB in Enterobacteriaceae. Chromosomal location in this setup was confirmed by hybridization with I-CeuI-restricted genomes. This approach led us to find that Klebsiella sp. and Raoultella sp. reference strains chromosomally carried oqxAB.

Four plasmid-mediated quinolone resistance (PMQR) mechanisms have been described in Enterobacteriaceae so far: Qnr, AAC(6’)-Ib-cr, QepA, and OqxAB (1–4). OqxAB belongs to the resistance-nodulation-cell-division (RND) family multidrug efflux pump, and OqxA and OqxB have shown high homology with the AcrAB pump from Escherichia coli (4). It has been shown that OqxAB-encoding genes may be located in Enterobacteriaceae on the conjugative plasmid pOLA52, within a composite transposon Tn6010 flanked by IS26 (5), but also natively on the chromosome in Klebsiella pneumoniae without association to a transposon (6, 7).

As for other PMQR, OqxAB alone confers low-level resistance to fluoroquinolones (8). Although the increased MIC of ciprofloxacin remains below the critical concentration, categorizing the enterobacterial isolate as susceptible, it has been shown that PMQR pose a challenge to antimicrobial treatments (9). Moreover, differentiation between plasmid-borne and chromosomal locations of these genes has a stake in preventing the spread of such resistance markers (10). All together, these data underscore the importance of setting up molecular detection assays. The aim of this study was to develop a reliable PCR-based method to detect not only oqxA and oqxAB but also any associations with IS26, allowing the detection of the transposon-associated oqxAB (Fig. 1A). The mobile version of oqxAB, which is embedded in Tn6010, has been almost exclusively reported on plasmids (10–13). To our best knowledge, only two Salmonella isolates carrying a chromosomally encoded OqxAB associated with IS26 have been reported so far (14). It is noteworthy that the transposon-borne version is associated with boosted expression because of the lack of rArA that normally downregulates oqXR and the presence of several plasmid copies of the plasmid-borne transposon version (7).

First, we carried out a multiplex conventional PCR to detect oqxA, oqxB, and an amplification product covering IS26 and oqxA. DNA extractions were all performed using the NucliSens easyMAG (bioMérieux, Marcy l’Étoile, France), according to the manufacturer’s recommendations. Multiplex PCRs were run using the MyCycler thermocycler (Bio-Rad, Marnes-la-Coquette, France). Amplicons of 222 bp, 127 bp, and 746 bp targeting oqxA, oqxB, and the region spanning the IS26 right end and oqxA 5’ terminus, respectively (Fig. 1A), were generated in a 50-µl PCR that contained 5 µl of target DNA, 5 µl of PCR buffer, 0.5 µl of MgCl2 (25 mM), 0.5 µl of deoxyribonucleoside triphosphate (dNTP) (25 mM), 25 or 100 pmol of each primer (Table 1), 0.5 µl of Taq polymerase (5 U/µl), and 26.5 µl of sterile water. The PCRs were run using the following temperature-time profile: 95°C for 3 min, 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. PCR products of the expected size were verified by electrophoresis in a 2% agarose gel. The control strains used for setting up the multiplex PCR were E. coli CSH26/pOLA52 (4), a clinical isolate of K. pneumoniae (KP10887) that we previously detected as positive for oqxAB (personal data), and E. coli CIP 54.82T as a negative control. An IS26-oqxA fragment specific to Tn6010 was amplified only in the plasmid-borne control E. coli CSH26/pOLA52 (Fig. 1B). The hybridization of I-CeuI pulsed-field gel electrophoresis (PFGE) profiles using an oqxB probe showed a chromosomal location only for K. pneumoniae KP10887 (15).

Second, we succeeded in setting up a simplex real-time PCR targeting each amplicon described above. These assays were carried out with the LightCycler 480 (Roche Molecular Diagnostics, Germany) using the same primers as described above (Table 1), with a PCR mix as follows: 2 µl of target DNA, 10 µl of 1× Kapa SYBR Fast quantitative PCR (qPCR) master mix (Kapa Biosystems, Wilmington, MA, USA), 0.4 µl of each primer, and 7.2 µl of sterile water. The PCR thermocycling program was as follows: 95°C for 7 min, followed by 40 cycles of 95°C for 10 s, 52°C for 30 s, and then melting resolution from 60°C to 98°C. As shown in Fig. 1B.


Address correspondence to Thomas Guillard, tguillard@chu-reims.fr.

Copyright © 2015, American Society for Microbiology. All Rights Reserved. doi:10.1128/AAC.00669-15.
distinct peaks were clearly distinguished for each amplicon except IS26-oqxA for *K. pneumoniae* KP10887, demonstrating the absence of a neighboring IS26. According to the prevalence of *oqxAB* (6, 16, 17), we proposed to combine our assays into a two-step strategy, with screening of *oqxAB* by detecting *oqxB* in real-time PCR and then determination of *oqxAB* location only on strains that were positive by multiplex PCR. This method was applied to (i) 25 *E. coli*, 25 *K. pneumoniae*, and 3 *Raoultella ornithinolytica* strains prospectively isolated from urine samples from patients hospitalized in our emergency room in December.

### TABLE 1 Primers used in this study

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer</th>
<th>Primer sequence (5′ to 3′)</th>
<th>$T_m$ (°C)</th>
<th>Concn (pmol/reaction)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS26-oqxA</td>
<td>IS26-oqxA_Fm2</td>
<td>GTTTTTCATTTTACAGGCAGCATA</td>
<td>60</td>
<td>100</td>
<td>746</td>
</tr>
<tr>
<td></td>
<td>IS26-oqxA_Rm</td>
<td>TTCACCTTAATCAGTGATCCACAGAC</td>
<td>60</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>oqxA</em></td>
<td><em>oqxA_Fm5</em></td>
<td>GCCAAAACRCCAGGCCAGYCT</td>
<td>60</td>
<td>25</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td><em>oqxA_Rm3</em></td>
<td>TCACCGSCGGCTGCCGCGCGCGCG</td>
<td>60</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><em>oqxB</em></td>
<td><em>oqxB_Fm3</em></td>
<td>GGCTGGGATTTTCAGGCAGATTTAC</td>
<td>60</td>
<td>25</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td><em>oqxB_Rm4</em></td>
<td>GCCGCGCATACGCGCGCGCGCGCG</td>
<td>60</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

*Degenerate residues are underlined. All primers were designed using Geneious 7.1 (Biomatters Ltd., Auckland, New Zealand).*

*These primer concentrations were used for the multiplex PCR, while all primers were used at 25 pmol/reaction in the simplex real-time PCR.*
ber 2014 and April 2015, (ii) 38 E. coli and 35 K. pneumoniae strains whose epidemiological links were studied previously (18), and (iii) the reference strains of the related genera Klebsiella and Raoultella (19) (Klebsiella oxytoca CIP 103434^T, K. pneumoniae subsp. ozaenae CIP 52.211^T, K. pneumoniae subsp. pneumoniae CIP 82.91^T, K. pneumoniae subsp. rhinoscleromatis CIP 52.220^T, Klebsiella michiganensis CIP 110787^T, Klebsiella singapurensis CIP 108642^T, Klebsiella variicola CIP108585^T, R. ornithinolytica CIP 103364^T, Raoultella planticola CIP 100751^T, and Raoultella terrigena CIP 80.7^T). For all the strains with no detection of IS26-oqxAB using our method, we confirmed the chromosomal location of oqxAB by amplifying a 1.3 kb-long fragment overlapping the upstream region of oqxA that matches with the chromosome and oqxA.

Among the E. coli clinical strains, we did not detect any oqxAB genes. However, all the clinical K. pneumoniae isolates (61/61) carried oqxAB without detection of Tn6010. Interestingly, all the Klebsiella sp. and Raoultella sp. reference strains carried oqxAB in their chromosome, indicating normal housekeeping functions in these specimens.

Norman et al. (5) have proposed that K. pneumoniae may be a reservoir for oqxAB and that an oqxAB-carrying plasmid, such as pOLA52, may have arisen by capture from the chromosome of K. pneumoniae recruited by IS26. Nonetheless, some studies failed to detect oqxAB in all the K. pneumoniae isolates studied (6, 16, 17). This raised the question of the chromosomal location of oqxAB in K. pneumoniae, which might be particular not only for capture from the chromosome but also for its ability to lose oqxAB. However, it is noteworthy that a lack of detection in previous studies might have resulted from inadequate primers.

The method we describe here led us to show that (i) all Klebsiella spp. (clinical, n = 61; reference, n = 7) and Raoultella spp. (reference, n = 3) strains harbored oqxAB as part of their native chromosome, and (ii) the degenerate primers used in the present study allowed the detection of oqxAB in all cases. Moreover, our findings highlight the hypothesis stated by Norman et al. (5) and also demonstrate the importance of discriminating native and Tn6010-associated oqxAB regarding the overexpression of the mobile version.

ACKNOWLEDGMENTS

We thank Janick Madoux from CHU Reims for her excellent technical assistance. We also thank Hélène Moret and Lydie Morcrette for their helpful discussion.

This work was supported by an annual grant from Université de Reims Champagne-Ardenne (EA 4687).

REFERENCES


