Plasmid-mediated quinolone resistance in
Aeromonas allosaccharophila recovered from a Swiss lake

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Objectives: To search for plasmid-mediated qnr genes among waterborne environmental Aeromonas spp. recovered from Switzerland.

Methods: Isolates presenting MICs of nalidixic acid or ciprofloxacin ≥1 mg/L were screened for qnr genes by a multiplex PCR approach followed by sequencing. Plasmids were transferred by transformation, and further analysis of the genetic structures surrounding the qnrS2 gene was carried out by PCR and sequencing.

Results: A qnrS2 gene was identified from a single Aeromonas allosaccharophila isolate (Lugano lake, Lugano), as part of a mobile insertion cassette located on a broad host range IncU-type plasmid. This plasmid co-harboured a class 1 integron containing the aac(6')-Ib-cr, blaOXA-1, catB3 and arr-3 gene cassettes.

Conclusions: These findings strengthen further the role of Aeromonas spp. as a reservoir of antimicrobial resistance determinants in the environment.

Keywords: A. allosaccharophila, QnrS2, environment

Introduction

Quinolones are broad-spectrum antimicrobial agents widely used in both human and veterinary medicine and therefore found as residues in the environment.¹ Resistance to quinolones is mainly due to chromosomally encoded mechanisms such as mutations in the target of quinolones, i.e. DNA gyrase and topoisomerase IV, or impermeability mechanisms due to either porin loss or quinolone extrusion by overexpression of efflux pumps.² Plasmid-mediated transferable quinolone resistance (PMQR) determinants have been identified more recently, being of three types: the Qnr-type pentapeptide proteins (QnrA, QnrB, QnrC and QnrS) protecting DNA gyrase from binding to quinolones; the AAC(6')-Ib-cr aminoglycoside acetyltransferase possessing two specific amino acid substitutions enabling acetylation of ciprofloxacin and norfloxacin; and the QepA protein, an efflux pump able to extrude norfloxacin, ciprofloxacin and enrofloxacin.³–⁶

Although Qnr determinants have been identified quite exclusively in Enterobacteriaceae, a plasmid-borne qnrS2 gene was identified recently from environmental Aeromonas strains from the Seine river in Paris, France.⁷ The aim of this study was to further evaluate the spread of qnr genes (qnrC sequence is not available) among Aeromonas spp. strains with a decreased susceptibility to nalidixic acid or ciprofloxacin, recovered from rivers and lakes in the Swiss Alps.

Methods

Water samples were collected between 2002 and 2005 from the rivers Ticino and Vedeggio and from the lakes Cadagno and Lugano, located in the southern part of Switzerland. Water samples were plated out, and growing bacteria were identified by conventional biochemical methods (API-20NE system, bioMérieux,
Results and discussion

A single isolate, A34, was positive for the qnrS gene, whereas qnrA and qnrB were not identified. Isolate A34 was from a water sample originating from the Lugano lake, 2005. Sequencing identified the qnrS2 gene, identical to that reported for Aeromonas punctata and Aeromonas media isolates from the Seine river in France and in a non-Typhi Salmonella clinical isolate from the USA. Genotypic identification of isolate A34, according to the results of sequencing of the gyrB gene, identified Aeromonas allosaccharophila, which is a fish pathogen. Isolate A34 had reduced susceptibility to all quinolones and fluoroquinolones tested, and was resistant to amoxicillin and ticarcillin at high level, to sulphonamides, kanamycin and tobramycin (Table 1). Sequencing of the quinolone resistance determining regions (QRDRs) of the gyrA and parC genes of strain A34 indicated that the GyrA and ParC amino acid sequences were identical to those from the reference strain Aeromonas sobria CIP7433, except for a Val-168→Ile substitution in GyrA, located outside the QRDR, and therefore considered as wild-type.

The plasmid harbouring the QnrS2 determinant was extracted by the Kieser technique and transferred into the Escherichia coli TOP10 recipient strain by electroporation, as described previously. The E. coli transformant had reduced susceptibility to quinolones, tobramycin and kanamycin, whereas it was fully resistant to rifampicin, sulphonamides, amoxicillin and ticarcillin (Table 1). Further analysis of this E. coli transformant identified a single ca. 80 kb plasmid, p34, hybridizing with an internal probe for a qnrS-like gene (data not shown). It was determined to be of the IncU incompatibility group by using specific primers, as described previously. Interestingly, the qnrS2 genes identified from Aeromonas spp. strains from the Seine river in France were also found on the same broad host range IncU-type plasmid. PCR assay using primers designed to anneal to the 5′- and 3′-conserved sequences of class 1 integrons resulted in a single ampiclon of ca. 3.3 kb. Sequencing identified four gene cassettes, namely aac(6′)-Ib-cr, blaOXA-1, catB3 and arr-3, encoding an aminoglycoside acetyltransferase, an oxacillinase conferring resistance to penicillins and reduced susceptibility to cepefime and cefpirome, an acetyltransferase conferring resistance to chloramphenicol and an ADP-ribosylating transference conferring resistance to rifampicin, respectively. Interestingly, the aac(6′)-Ib-cr gene encoding another PMQR determinant was found in association with the qnrS2 gene on the same plasmid. Resistance determinant AAC(6′)-Ib-cr affects kanamycin, tobramycin, netilmicin and amikacin, in this decreasing order. However, once expressed from the natural plasmid p34 in an E. coli background, the AAC(6′)-Ib-cr-mediated resistance to aminoglycosides was of a low level (if any) (Table 1), suggesting that additional resistance mechanisms to aminoglycosides were present in Aeromonas allosaccharophila A34. Surprisingly, E. coli TOP10 (p34) and the A34 isolate were of wild-type susceptibility to chloramphenicol (Table 1), suggesting that the catB3 gene was probably not expressed in both the donor and the transformant strains.

The class 1 integron content identified on the qnrS2-carrying plasmid revealed its perfect identity with other integrons such as In37, previously described from different qnrA1-positive enterobacterial isolates from Shanghai, and also from qnrB10-positive Enterobacter cloacae and Klebsiella pneumoniae isolates from Argentina, or qnrB4-positive K. pneumoniae from France. However, these In37-like integrons were associated with an ISCR1 element located at their 3′-end, which was not present on plasmid p34.

PCR mapping and sequencing of the qnrS2 gene environment showed that it was part of a mobile insertion cassette, the insertion of which had disrupted an mprR gene encoding a putative zinc-metalloprotease (MpR), in association with a structure identical to that found on the qnrS2-positive IncU-type plasmids from A. media and A. punctata (Figure 1). Recently, a plasmid-mediated QnrS2 was identified from another Aeromonas sp. strain, Aeromonas veronii from Spain. However, lack of plasmid characterization and of sequence determination of the surrounding sequence of the qnrS2 gene in A. veronii prevents further comparison.

Our study identified two PMQR determinants, qnrS2 and aac(6′)-Ib-cr, along with four different antimicrobial resistance markers, on a single plasmid from A. allosaccharophila recovered from an aquatic environment in Switzerland. The presence of those quinolone resistance determinants from a strain with reduced susceptibility, but still susceptible to quinolones,
suggests that these genes may spread silently. In addition, the fact that the same mobile insertion cassette-associated qnrS2 structure has been found in different Aeromonas species from aquatic environments from distantly related geographical areas may indicate that these PMQR determinants are widespread, at least in Europe. Our findings strengthen the possible role of Aeromonas spp. and of mobile insertion cassette-type structures as vehicles for the dissemination of quinolone resistance markers. They may be the link between the progenitor of QnrS proteins (Vibrioceae) and enterobacterial clinical species such as Salmonella.

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Transparency declarations

None to declare.

References