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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*, bla_{OXA-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,

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Marcy-l'Étoile, France). Susceptibility testing of isolates was performed using Etest strips containing nalidixic acid or ciprofloxacin. Isolates exhibiting nalidixic acid or ciprofloxacin MICs $\geq$1 mg/L were selected for further studies. Fifty isolates of *Aeromonas* spp. were screened for the presence of *qnrA*, *qnrB* and *qnrS* genes by multiplex PCR, as described previously.[8]

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.[7,9] Genotypic identification of isolate A34, according to the results of sequencing of the *gyrB* gene,[10] 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.[11]

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.[7] 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.[7] 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.[7] PCR assay using primers designed to anneal to the 5′- and 3′-conserved sequences of class 1 integrons resulted in a single amplicon of ca. 3.3 kb. Sequencing identified four gene cassettes, namely *aac(6′)-Ib-cr*, bla_{OXA-1}, *catB3* and *arr-3*, encoding an aminoglycoside acetyltransferase, an oxacillinase conferring resistance to penicillins and reduced susceptibility to cefepime and cefpirome, an acetyltransferase conferring resistance to chloramphenicol and an ADP-ribosylating transferase 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.[4] 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 *A. 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.

Table 1. MICs (mg/L) of antimicrobial agents for *A. allosaccharophila* 34, *E. coli* TOP10 harbouring natural plasmid p34 and *E. coli* reference strain TOP10

Antibiotic	*A. allosaccharophila* 34	*E. coli* TOP10 with plasmid p34	*E. coli* TOP10
Nalidixic acid	1.5	4	1
Norfloxacin	0.5	1	0.03
Ofloxacin	0.38	0.5	0.01
Ciprofloxacin	0.12	0.25	<0.01
Moxifloxacin	0.38	0.19	<0.01
Pefloxacin	0.25	0.75	0.02
Enrofloxacin	0.38	0.38	<0.01
Rifampicin	16	>256	16
Sulphonamides	>256	>256	16
Amikacin	64	2	2
Kanamycin	64	2	1
Tobramycin	32	2	0.38
Gentamicin	0.25	0.12	0.19
Chloramphenicol	4	2	2
Amoxicillin	>32	32	6
Ticarcillin	>256	>256	4
Ceftazidime	<0.06	<0.06	<0.06
Cefotaxime	<0.06	<0.06	<0.06
Cefepime	<0.06	0.12	<0.06
Imipenem	<0.06	<0.06	<0.06

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.[12] However, these In37-like integrons were associated with an IS*CR1* 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 *mpR* 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).[7,13,14] Mobile insertion cassette elements are peculiar DNA elements made of two inverted repeats bracketing a DNA sequence without coding sequence for any transposase.[13] Recently, a plasmid-mediated QnrS2 was identified from another *Aeromonas* sp. strain, *Aeromonas veronii* from Spain.[15] 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,

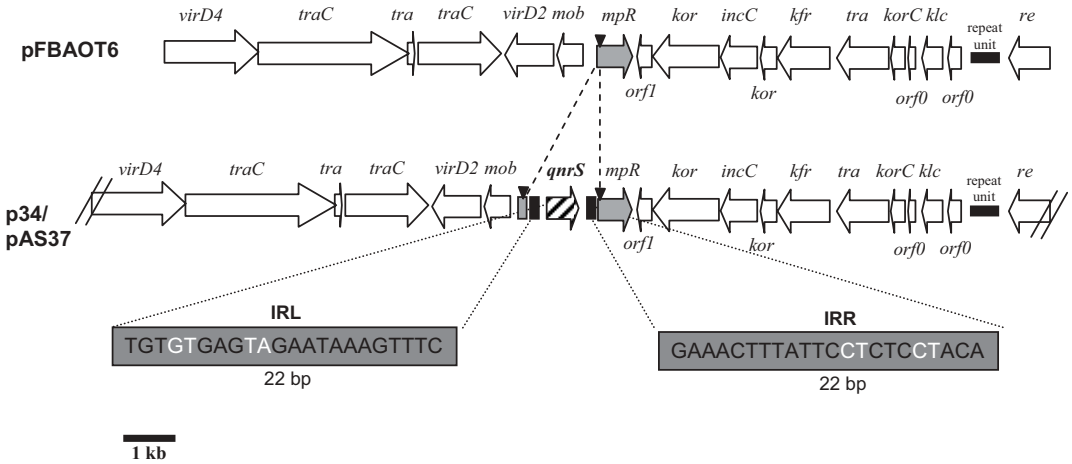

Figure 1. Genetic environment of the *qnrS2* gene in plasmid p34 from *A. allosacharophila* 34 and comparison with related plasmid structures. Plasmid pFBAOT6 is from *A. punctata* from the UK, whereas plasmid pAS37 is from *A. media* and *A. punctata* from France.[7,14] Open reading frames are indicated by horizontal arrows. The right and left inverted repeats (IRR and IRL) are indicated, and duplication sites (CCTCC) are represented by black triangles. The identified mobile insertion cassette element is bracketed by 22 bp IRL and IRR (black nucleotides are complementary, whereas white nucleotides are not).

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 (Vibrionaceae) and enterobacterial clinical species such as *Salmonella*.

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Transparency declarations

None to declare.

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