Short Communication

Plasmid-mediated qnrA1 in Klebsiella pneumoniae ST147 in Recife, Brazil

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doi:10.1016/j.ijid.2014.04.026

ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 22 April 2014
Accepted 26 April 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Quinolones
Resistance
qnrA1

SUMMARY

Objectives: Qnr-mediated quinolone resistance is increasingly detected worldwide, but few studies have been carried out so far in Brazil. The aim of this study was to test for qnr determinants in isolates of ciprofloxacin-resistant Klebsiella pneumoniae.

Methods: Fifteen ciprofloxacin-resistant isolates from urine cultures of hospitalized patients at a university hospital in North-East Brazil were investigated. Specific PCRs were performed for blaCTX-M and blaTEM, qnr, and class 1 integrons. Plasmid analyses and sequence type (ST) determination were performed, as described previously.

Results: The KP 930 isolate showed qnrA1 and blaTEM-1, together with dfrA12 and aadA2 in a class 1 integron. The qnr gene was located in a 133-kb plasmid. Multilocus sequence typing classified the isolate as ST147.

Conclusions: We identified the combination of qnr with ST147 in Brazil; this is a clone that has disseminated widely and successfully in Latin America. The purpose of describing Qnr-mediated quinolone resistance in North-East Brazil is to draw attention to the spread of this mechanism in the country.

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1. Introduction

The first case of plasmid-mediated resistance to quinolones was described in the USA in a strain of Klebsiella pneumoniae.1 The gene that was discovered, called qnr, encodes a pentapeptide, Qnr, which is responsible for protecting the enzyme DNA gyrase against inhibition by fluoroquinolones.2 Since it was first characterized, the qnr gene has been reported in Escherichia coli, K. pneumoniae, and other members of the Enterobacteriaceae family in several countries, including Brazil.3,4 Its rapid spread is of concern because it might affect the future clinical use of fluoroquinolones. This study describes the isolation and characterization of a strain of K. pneumoniae containing qnrA1 in North-East Brazil.

2. Materials and methods

In April 2010, a strain of Qnr-producing K. pneumoniae (KP 930) was obtained from a 41-year-old female patient who was HIV- and human T cell lymphotropic virus (HTLV)-positive. Species identification was performed by means of conventional biochemical tests, namely positive results for MacConkey growth, citrate (Simmons), urea hydrolysis, d-glucose acid/gas, sucrose fermentation, lactose fermentation, and esculin hydrolysis, and negative results for indole production, hydrogen sulfide production, ornithine decarboxylase, and motility (36 °C). Susceptibility tests were conducted by broth microdilution method.5

Specific PCRs were performed for blaCTX-M and blaTEM, qnr, and class 1 integron (intI1) and its variable region. The PCR products were sequenced with a 3500 Genetic Analyzer. The nucleotide sequences were evaluated through the BioEdit program and were submitted to online BLASTn analysis at GenBank (National Center

http://dx.doi.org/10.1016/j.ijid.2014.04.026
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for Biotechnology Information, NCBI). Plasmid extraction was performed by alkaline lysis miniprep protocol and the E. coli R861 and E. coli TOP 10 strains were used as controls. Transformation was performed with E. coli DH5α as the recipient strain.

The transformants were selected on LB (Luria-Bertani) agar plates containing 0.03 mg/ml of ciprofloxacin. The Inc group of the plasmid harboring the qnr gene was determined in accordance with Carattoli et al. Multilocus sequence typing (MLST) was performed in accordance with Diancourt et al. This work was approved by the Ethics Committee of the State University of Pernambuco, Brazil (reference number 111/10).

3. Results

The minimum inhibitory concentrations (MICs) for the clinical isolate of KP 930, transformant (T 930), and the recipient cells are shown in Table 1. We observed a 62-fold increase in ciprofloxacin MIC values of the transformant cells when compared with the recipient strain. The qnrA1 gene was identified in both the donor and transformed cells. In the search for beta-lactamase genes, only blaTEM-1 was detected.

The positive PCR for int1 indicated the isolate to be carrying class 1 integron, and sequencing of the variable region revealed the genes dfrA12 and adaA2, which confer resistance to trimethoprim and aminoglycosides, respectively.

The analysis of the plasmids showed that KP 930 contained the 133, 118, and 2.5 kb plasmids; the first of these was also present in the transformed cells, which indicated where the qnr gene was located. MLST analysis of the isolate of KP 930 revealed ST147.

4. Discussion

This work represents the first study of plasmid-mediated Qnr resistance to fluoroquinolones in North-East Brazil, although the qnrA1 allele that was found has already been described in this country. In addition, as far as we are aware, this is the first time that K. pneumoniae ST147 harboring the qnr gene has been detected in Latin America.

The presence of the qnr gene usually causes only a reduced sensitivity to ciprofloxacin. In this study, the resulting elevated MIC value of the transformed cells did not reach the range of resistance values, which is consistent with what occurs when the qnr gene is present alone. The high resistance levels observed in KP 930 were probably due to a combination of other mechanisms, mainly chromosomal mutations in the quinolone resistance determinant region (QDRR). Moreover, additional non-plasmid-mediated mechanisms could explain the higher MIC values in KP 930 for the other antimicrobials tested, despite the fact that they did not reach the resistance range.

The transformation experiments showed the presence of the qnr gene in a 133-kb plasmid belonging to the IncX group, which has often been associated with qnr genes, in particular qnrS1. However, no association with qnrA1 has been reported so far.

The K. pneumoniae ST147 identified in this study corresponds to an epidemic clone that is described as a blaCTX-M-15 producer and responsible for its dissemination. More recently, ST147 has also been related to the dissemination of qnr in Europe. Here we describe the association of qnr with ST147 in Brazil.

The results that are provided here raise the possibility that qnr genes might become widespread in Brazil and that they show the presence of K. pneumoniae ST147, a clone that has disseminated widely and successfully in Latin America.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Dr Ana Cristina Gales (Laboratório Alerta – UNIFESP), who kindly provided the qnr-positive control strains. We are also grateful to the team of the curators of the Institut Pasteur MLST Scheme (Paris, France) for the MLST (data) analysis. This work was supported by the following Brazilian Funding Agencies: CNPq, CAPES, FACEPE, and PFA/UEP.

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