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Emergence of quinolone resistance among extended-spectrum beta-lactamase-producing Enterobacteriaceae in the Central African Republic: genetic characterization

Thierry Frank*, Jean R Mbecko, Pembe Misatou and Didier Monchy

Abstract

Background: Cross-resistance to quinolones and beta-lactams is frequent in Enterobacteriaceae, due to the wide use of these antibiotics clinically and in the food industry. Prescription of one of these categories of antibiotic may consequently select for bacteria resistant to both categories. Genetic mechanisms of resistance may be secondary to a chromosomal mutation located in quinolone resistance determining region of DNA gyrase or topoisomerase IV or to a plasmid acquisition. The insertion sequence ISCR1 is often associated with qnr and may favour its dissemination in Gram-negative bacteria. The aim of this study was to determine the genetic mechanism of quinolone resistance among extended-spectrum beta-lactamase-producing Enterobacteriaceae strains in the Central African Republic.

Findings: Among seventeen ESBL-producing Enterobacteriaceae isolated from urine, pus or stool between January 2003 and October 2005 in the Central African Republic, nine were resistant to ciprofloxacin (seven from community patients and two from hospitalized patients). The ESBL were previously characterized as CTX-M-15 and SHV-12. Susceptibility to nalidixic acid, norfloxacin and ciprofloxacin, and the minimal inhibitory concentrations of these drugs were determined by disc diffusion and agar dilution methods, respectively. The presence of plasmid-borne ISCR1-qnrA region was determined by PCR and amplicons, if any, were sent for sequencing. Quinolone resistance determining region of DNA gyrase gyrA gene was amplified by PCR and then sequenced for mutation characterization. We found that all CTX-M-producing strains were resistant to the tested quinolones. All the isolates had the same nucleotide mutation at codon 83 of gyrA. Two Escherichia coli strains with the highest MICs were shown to harbour an ISCR1-qnrA1 sequence. This genetic association might favour dissemination of resistance to quinolone and perhaps other antibiotics among Enterobacteriaceae.

Conclusions: This study shows that at least two mechanisms might explain the emerging resistance of Enterobacteriaceae to quinolones in the CAR. Beside the classical topoisomerase mutation, the cause may be acquisition of a plasmid-borne qnrA1. Clinicians and bacteriologists should be made aware of possible dissemination of ISCR1-qnrA1 among Enterobacteriaceae.

Keywords: Quinolones, Betalactams, Antibiotic resistance, Enterobacteriaceae

Background

Quinolones and beta-lactams are widely used antibiotics for the treatment of bacterial infections and are also extensively used in the food-processing industry. Their wide use has triggered increased bacterial resistance worldwide [1]. Quinolone resistance and extended-spectrum beta-lactamase (ESBL) production are often associated in Enterobacteriaceae [1]. The prescription of quinolones may consequently select bacteria that are not only quinolone-resistant but also beta-lactam-resistant and vice versa. The biological mechanisms of resistance to quinolones include impermeability, active efflux, target modification and antibiotic neutralization.
Genetic determination of quinolone resistance is generally linked to chromosomal mutations affecting the quinolone resistance-determining regions (QRDR) of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE) [1]. Mutations in gyrA, the gene encoding for the A subunit of DNA gyrase are the most common mechanism involved in quinolone resistance among Gram negative bacteria [2].

In 1994, the plasmid-borne quinolone resistance genes qnr were identified [1]. The insertion sequence ISCR1 has often been characterized in the genetic environment of qnr and may be involved in dissemination of the gene in Gram-negative bacteria [3].

Quinolone resistance was first identified in the CAR in 2003, among clinical ESBL-producing Enterobacteriaceae. The aim of this study was to determine the genetic mechanism of quinolone resistance of these strains isolated in the Central African Republic (CAR).

**Methods**

Of the seventeen ESBL-producing Enterobacteriaceae isolated from urine, pus or stool samples between January 2003 and October 2005 at the Institut Pasteur de Bangui and previously characterized as CTX-M-15 and SHV-12, nine were resistant to ciprofloxacin (eight Escherichia coli and one Klebsiella pneumoniae) [4]. Seven E.coli strains were isolated from community patients and the two others were isolated from hospitalized patients.

Antibiotic susceptibility was determined by the diffusion disc method (Comité de l’Antibiogramme de la Société Française de Microbiologie). The minimal inhibitory concentrations (MICs) of nalidixic acid, norfloxacin and ciprofloxacin were measured by the agar dilution method.

PCR was carried out on genomic DNA extracted with the commercial Qiagen DNA Mini Kit (Qiagen, Courtaboeuf, France) at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 55 °C for 40 s and extension at 72 °C for 60 s, followed by 72 °C for 7 min.

The ciprofloxacin-resistant isolates were screened for the qnrA gene by PCR with specific primers targeting ISCR1, 5’-TGAAACAGAAAAACGCCAAGG-3’ (ISC R1qnr upper), and qnrA, 5’-GCAGCActTTACTC-CAAGG-3’ (ISC R1qnr lower). The primers targeting ISCR1-qnrA1 were designed with Oligo-4 software. The sequences encoding QRDR of DNA gyrase were amplified with gyrA6 5’-CGA CCT TGC GAG AGA AAT-3’ and gyrA631R 5’-GTT CCA TCA TCA GCC CTT CAA-3’ [5].

All amplicons were sequenced at MWG Biotech France (http://www.mwg-biotech.com) and analysed with program ChromasPro 2.0 and blastN software (http://gb-admin@ncbi.nlm.nih.gov) (GenBank accession numbers for the sequenced ISCR1-qnrA1 region are JF728153 and JF728154).

All patients have given their agreement to participate in this study prior the samples collection. This study was reviewed and approved by the scientific committee board of University of Bangui in charge of the validation of scientific protocol studies in the CAR.

**Results and discussion**

Susceptibility testing showed that all CTX-M-producing strains were resistant to nalidixic acid, norfloxacin and ciprofloxacin. The MIC of nalidixic acid and norfloxacin was 64 μg/ml and that of ciprofloxacin was 32 μg/ml for six E. coli and the K. pneumoniae strains. For two E. coli isolates, the MICs were higher, and PCR targeting ISCR1-qnrA amplified a 1050-bp fragment from these two isolates which was shown by DNA sequence analysis to be ISCR1-qnrA1. We could not determine the MICs values of the two qnrA-positive E. coli by the agar dilution method because they exceeded the highest concentrations of antibiotics tested in this study (32 μg/ml for ciprofloxacin and 64 μg/ml for the nalidixic acid).

Other publications have shown that qnrA increased the MICs of quinolones by 10-fold in Enterobacteriaceae [6]. The combination of an insertion sequence ISCR1 with qnrA1 could ensure dissemination of quinolone resistance to both ESBL-producing and ESBL-negative Enterobacteriaceae. The ISCR1 element is also capable of mobilizing qnrA1 in multiple drug resistance regions, generally located on plasmids that mediate resistance to multiple antibiotics.

Sequence analysis of the gene portion encoding the QRDR of GyrA showed the same amino acid change in GyrA (Ser83-Leu) in all our isolates. Nucleotide mutations at codon 83 are the commonest GyrA substitutions found in quinolone-resistant E. coli and the serine residue is most commonly substituted by a leucine [7].

**Conclusion**

We have shown that the emerging resistance of Enterobacteriaceae to quinolones in the CAR may be due to a topoisomerase mutation or a plasmid-borne qnrA1 acquisition. It would be interesting to sequence the DNA gyrase gyrB and topoisomerase IV parC and parE genes, to screen for the other qnr genes (B and S) and to characterize the environment of ISCR1-qnrA1, which has already been found in several sul1-type complex integrons. The possible dissemination of ISCR1-qnrA1 among Enterobacteriaceae requires vigilance by bacteriology laboratories in the CAR. It will also be important to inform clinicians of the potential risk associated with prescribing quinolones and beta-lactams.
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Authors’ contributions
TF: carried out the molecular genetic studies, validated the results of antibiotic susceptibility testing, participated in nucleotide sequence analysis and drafted the manuscript.
JRM: participated in bacteriological isolation of Enterobacteriaceae strains. PM: participated in bacteriological isolation of Enterobacteriaceae strains. DM: helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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