Characterization of quinolone-resistant Enterobacteriaceae strains isolated from poultry in Western Algeria: First report of qnrS in an Enterobacter cloacae

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Received: 21-12-2017, Accepted: 06-03-2018, Published online: 12-04-2018

doi: 10.14202/vetworld.2018.469-473 How to cite this article: Benameur Q, Tali-Maamar H, Assaous F, Guettou B, Boutaiba Benklaouz M, Rahal K, Ben-Mahdi MH (2018) Characterization of quinolone-resistant Enterobacteriaceae strains isolated from poultry in Western Algeria: First report of qnrS in an Enterobacter cloacae, Veterinary World, 11(4): 469-473.

Abstract

Aim: Multidrug-resistant (MDR) Enterobacteriaceae have frequently been reported, in both human and veterinary medicine, from different parts of the world as a consequence of antibiotic usage. However, there is a lack of published data regarding antimicrobial resistance in non-Escherichia coli (E. coli) Enterobacteriaceae from animals in Algeria. This study aimed to evaluate the frequency of resistance to antibiotics with a focus on quinolones and to investigate the presence of qnr genes in Enterobacteriaceae of poultry origin.

Materials and Methods: A total of 310 samples of poultry origin were collected from 2010 to 2014 from broiler and layer farms and hatcheries located in different geographic areas of Western Algeria (including Mostaganem, Oran, Mascara, Relizane, Chlef, Tiaret, and Tissemsilt). Antimicrobial susceptibility testing was performed using disc diffusion assay. Polymerase chain reaction and sequencing accomplished the characterization of qnr genes (qnrA, qnrB, and qnrS).

Results: A total of 253 Enterobacteriaceae strains were isolated in this study. These isolates exhibited high levels of resistance to quinolones and other families of antibiotics. All the strains isolated in this study were resistant to at least one antibiotic. Among them, 233 (92.09%) were considered MDR. Among the 18 randomly selected nalidixic acid (NA)-resistant Enterobacteriaceae isolates, one E. coli and one Enterobacter cloacae were carrying qnrSI. By contrast, qnrA and qnrB were not detected in this study.

Conclusion: This is the first report on the identification of the qnrS gene in E. cloacae isolated from animal source in Algeria. Further studies have to be conducted to determine the real prevalence of qnr genes.

Keywords: Algeria, antimicrobial resistance, Enterobacteriaceae, qnrSI.

Introduction

Quinolones and fluoroquinolones are broad-spectrum antimicrobial agents, extensively used in poultry disease treatment. This widespread use has been associated worldwide with an increased level of resistance, especially in Gram-negative bacteria species in the last decade [1,2]. Multidrug-resistant (MDR) Enterobacteriaceae have frequently been reported, in both human and veterinary medicine, from different parts of the world as a consequence of antibiotic usage [3,4]. In Algeria, the frequency of antimicrobial resistance in Escherichia coli (E. coli) from animals has already been reported by several authors [5-8]. However, there is a lack of published data regarding antimicrobial resistance in non-E. coli Enterobacteriaceae from animals in Algeria. It is admitted that resistance to quinolones results from both chromosomal and plasmid-mediated quinolone resistance (PMQR) mechanisms. Qnr genes represent one of the most important PMQR mechanisms. These genes encode pentapeptide repeat proteins that block the action of ciprofloxacin (CIP) on bacterial DNA gyrase and topoisomerase IV [9,10]. Three major groups of Qnr determinants have been described (QnrA, QnrB, and QnrS), which share between 40% and 60% similarity [11]. These determinants have been identified worldwide in various Enterobacteriaceae, and they have frequently been associated with extended-spectrum β-lactamases (ESBLs) and plasmidic cephalosporinases [12,13]. In Algeria, these determinants have been reported in various human Enterobacteriaceae [14-16]. However, the occurrence of these resistance determinants in isolates of animal origin in Algeria is rarely documented.
This study aimed to evaluate the frequency of resistance to quinolones and other groups of antibiotics in Enterobacteriaceae isolated from poultry in Western Algeria and to investigate the presence of qnr genes in a collection of nalidixic acid (NA)-resistant Enterobacteriaceae isolates.

Materials and Methods

Ethical approval

Ethical approval is not needed to pursue this type of study. However, no chickens were harmed during the collection of samples.

Bacterial strains

From December 2010 to January 2014, 253 non-duplicate Enterobacteriaceae strains were isolated from 310 samples received in the Regional Veterinary Laboratory of Mostaganem, Algeria, for routinely control of Salmonella. All samples were collected by veterinarians controlling from farms and hatcheries located in different geographic areas of Western Algeria (including Mostaganem, Oran, Mascara, Relizane, Chlef, Tiaret, and Tissemsilt). The samples nature received were healthy and diseased broiler and layer breeders, 1-day-old broiler and layer chicks, broilers, laying hens, and farm swabs. The isolates were recovered from internal organs (liver, spleen, or pericardium), fecal samples, or farm swabs. For the primary isolation, one mL of sample was inoculated with nine mL of buffered peptone water vortexed and incubated at 37°C overnight. Then, a drop of broth was streaked on MacConkey agar and incubated at 37°C overnight. The Enterobacteriaceae isolates were identified biochemically by classical biochemical tests and the API 20E system (bioMérieux, Marcy l’Étoile, France).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of all isolated Enterobacteriaceae strains was tested following Clinical and Laboratory Standards Institute (CLSI) guidelines [17]. The isolates were tested against a panel of 12 antimicrobials: Nalidixic acid (NA, 30 µg), flumequin (UB, 30 µg), ciprofloxacin (CIP, 5 µg), ampicillin (AM, 10 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), cefotaxime (XNL, 30 µg), tetracycline (TE, 30 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), neomycin (N, 30 µg), gentamicin (CN, 15 µg), chloramphenicol (C, 30 µg), and colistin (CT, 50 µg) (Bio-Rad, Marnes la Coquette, France). Results were obtained after incubating samples for 16–18 h at 37°C and were interpreted according to CLSI previously cited guidelines. E. coli ATCC 25922 was used as a quality control strain.

Polymerase chain reaction (PCR) and DNA sequencing

A total of 18 MDR isolates randomly selected among NA-resistant Enterobacteriaceae isolates were screened by multiplex PCR amplification of qnrA, qnrB, and qnrS as previously described [18-20], after extraction of total DNA by the boiling method. Primers used were as follows: for qnrA, 5'-TTCTCACGCGCAGATTTTGAG and 5'-TGCCAGCACAGTCTTGAC, to give a 571 pb product; for qnrB, 5'-TGCCGAAAAATT(GA)ACAGAA and 5'-GACGACACAG(TC)GCCTGGTAG, to give a 594 pb product; and for qnrS, 5'-GAGCGTGCAACTTGCGGTAG and 5'-GACGTGCTAACTTGGGTAG, to give a 388 pb product. All the six primers were added to the template DNA and PCR mix (Invitrogen, Carlsbad, CA). The following cycle conditions were used: Initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 45 s and amplification at 72°C for 1 min, and a final extension at 72°C for 10 min. Negative controls (without DNA template) were included in each run. Amplification products were provisionally identified from their sizes in agarose gels. Amplification products were separated by electrophoresis, on 1.5% ethidium bromide-stained agarose gels in 1 × TBE buffer at 150 V for 1 h, and then visualized under ultraviolet light. PCR amplicons were confirmed by sequencing and the DNA sequences obtained were compared with those in the GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

Results and Discussion

Antimicrobial susceptibility of Enterobacteriaceae isolates

Two hundred and fifty three Enterobacteriaceae strains were isolated from 310 poultry samples received in the Regional Veterinary Laboratory of Mostaganem, Northwestern Algeria. The isolates consisted of 134 E. coli, 55 Enterobacter cloacae (E. cloacae), 42 Klebsiella pneumoniae (K. pneumoniae), 10 Proteus mirabilis, 7 Serratia marcescens, and 5 Providencia rettgeri. The percentage of antimicrobial resistance of the predominant Enterobacteriaceae strains isolated in this study is shown in the Figure-1. E. coli isolates showed a high resistance rate to particular antimicrobials, notably TE 94.77% (n=127), NA 94.03% (n=126), AM 94.03% (n=126), UB 93.28% (n=125), CIP 85.10% (n=114), and SXT 76.11% (n=102). Among E. cloacae isolates, the highest proportion of resistance was toward AM 90.90% (n=50), followed by NA 83.63% (n=46), UB 76.36% (n=42), TE 74.54% (n=41), CIP 65.45% (n=36), and SXT 52.72% (n=29). Resistance of K. pneumoniae to AM, NA, UB, CIP, TE, SXT, and AMC was, respectively, observed in 100% (n=42), 92.85% (n=39), 92.85% (n=39), 90.47% (n=38), 85.71% (n=36), 57.14% (n=24), and 57.14% (n=24) of the isolates. All the isolates examined in this study were resistant to at least one antibiotic. Among them, 233 (92.09%) were considered MDR (resistant to three or more different antimicrobial agents belonging to different classes of antibiotics) (Table-1). K. pneumoniae and E. coli are the most common opportunistic Enterobacteriaceae, and their growing cross-resistance...
to quinolones is a critical problem [21,22]. In Algeria, there is a lack of published data regarding antimicrobial resistance in non-*E. coli* and non-*Salmonella* spp. Enterobacteriaceae of animal origin. Resistance of *E. coli* isolates to quinolones was far higher compared to previous studies conducted in Algeria [6,7]. In view of the whole range of antibiotics available in Algeria and the increasing and inappropriate use of quinolones in poultry farms, the globally high incidence of antibiotic resistance observed in this study is not really surprising.

**Qnr occurrence**

The qnr multiplex PCR allowed the detection of two positive isolates (one *E. coli* and one *E. cloacae*), both of them were carrying qnrS1 (Table-2), whereas qnrA and qnrB were not identified in any of the 18 randomly selected isolates. In Algeria, several studies allowed the detection of qnr determinants in human clinical isolates [12-14]. However, few studies reported their presence in isolates of animal origin. qnrA has been recently identified in ESBL-producing *E. coli* isolates from poultry [23], and qnrS1 and qnrB5 in ESBL-producing *E. coli* isolates from companion animals [24]. To the best of our knowledge, this is the first description of qnrS genes in an *E. cloacae* isolate from animal source in Algeria. All of the previously cited Algerian studies detected the presence of qnr determinants in ESBL-producing isolates. However, no study reported their presence in non-ESBL Enterobacteriaceae in our country. The qnrS gene has been previously detected in several *Salmonella* strains isolated from poultry source in Denmark, Germany, and Netherland [25-27] and was also reported in *E. coli* isolates from food-producing animals in China and Nigeria [28,29]. Typically, qnrB was considered to be the most prevalent PMQR gene in Enterobacteriaceae isolates [11]. The other 16 NA-resistant Enterobacteriaceae isolates tested in this study were negative for the three qnr genes investigated. However, they were not tested for other qnr determinants (qnrC, qnrD, and qnrVC). Thus, this preliminary study have to be completed by further investigations of other PMQR determinants, including qnrC, qnrD, qnrVC, aac(6')-Ib-cr, qepA, and oqxAB. qnr genes have been either detected alone or in association with ESBL genes in a range of bacterial species [30]. Recently, qnrA or qnrS determinants were identified in non-ESBL-positive isolates harboring TEM-1 or LAP-1 [31]. As previously reported, our results confirmed that the spread of these genes can be independent and not always associated with *bla* EM genes [2,31].

*E. coli* harboring qnrS1 gene, detected in our study, was resistant to CIP, whereas the qnrS1-positive *E. cloacae* were susceptible to CIP. Quinolone resistance has been described to be transmitted by plasmids carrying qnr genes [32], resulting in low-level quinolone resistance and it can facilitate the selection of quinolone-resistant mutants with higher-level resistance [33,34]. The transferability of CIP-resistant *E. coli* or mobile resistance determinants from chickens to humans has been indicated in several studies [35,36]. Since the zoonotic transfer of fluoroquinolone-resistant bacteria is of concern from a human health perspective, the reverse scenario - the transfer of fluoroquinolone-resistant bacteria from humans to animals - warrants equal consideration, which may be responsible for therapeutic failures in animals.

**Conclusions**

This study revealed high levels of antimicrobial resistance to antibiotics with a focus on quinolones in Enterobacteriaceae isolates. This is the first detection of qnrS in *E. cloacae* isolates from the animal in Algeria. The emergence of PMQR thus may contribute by several means to the rapid and deleterious increase in bacterial resistance to fluoroquinolones. These fluoroquinolone-resistant bacteria may be transferred from animals to humans and vice versa, increasing the risk of treatment failure. Therefore, implementation of more efficient preventive measures at all levels of broiler and layer industries is becoming mandatory.

![Figure-1](Available at www.veterinaryworld.org/Vol.11/April-2018/10.pdf)

**Figure-1:** Antimicrobial resistance among Enterobacteriaceae. AM=Ampicillin, AMC=Amoxicillin/clavulanic acid, XNL=Ceftiofur, NA=Nalidixic acid, UB=Flumequin, CIP=Ciprofloxacin, TE=Tetracycline, SXT=Trimethoprim/sulfamethoxazole, N=Neomycin, CN=Gentamicin, C=Chloramphenicol, CT=Colistin.
Table-1: MDR in Enterobacteriaceae isolates.

| Organism            | Total No. | Number of MDR isolates (%) |
|---------------------|-----------|----------------------------|
| E. coli (S1)        | 134       | 125 (93.28)                |
| E. coli (S2)        | 65        | 52 (94.54)                 |
| K. pneumoniae       | 42        | 39 (92.85)                 |
| P. mirabilis        | 10        | 8 (80.00)                  |
| S. marcescens       | 7         | 5 (71.42)                  |
| P. rettgeri         | 5         | 4 (80.00)                  |
| Total               | 253       | 233 (92.09)                |

MDR=Multidrug resistant, E. coli=Escherichia coli, E. cloacae=Enterobacter cloacae, K. pneumoniae=Klebsiella pneumoniae, P. mirabilis=Proteus mirabilis, S. marcescens=Serratia marcescens, P. rettgeri=Providencia rettgeri

Table-2: Enterobacteriaceae isolates tested by PCR for the determination of qnr determinants.

| Strain           | qnr | Antimicrobial resistance pattern          |
|------------------|-----|------------------------------------------|
| E. coli (S1)     | /   | NA, UB, CIP, AM, SXT                     |
| E. coli (S2)     | /   | NAL, UB, CIP, AM, TE                     |
| E. coli (S3)     | /   | NA, UB, CIP, AM, SXT, TE                 |
| E. coli (S4)     | /   | NA, UB, CIP, AM, SXT, TE                 |
| E. coli (S5)     | /   | NAL, UB, CIP, SXT, CN                   |
| E. coli (S6)     | qnrS1 | NAL, UB, CIP, AM, SXT, CN               |
| E. coli (S7)     | /   | NAL, UB, CIP, AM, SXT, N                |
| E. coli (S8)     | /   | NAL, UB, CIP, SXT, AM, MIC              |
| P. rettgeri (S9) | /   | NAL, UB, CIP, AM, SXT, AM               |
| E. coli (S10)    | /   | NAL, UB, SXT, TE                        |
| K. pneumoniae (S11)| /  | NAL, UB, AM, SXT, T/C                   |
| E. cloacae (S12) | qnrS1 | NAL, UB, AM, SXT, T/C                   |
| E. coli (S13)    | /   | NAL, UB, AM, SXT, AM                   |
| E. coli (S14)    | /   | NAL, UB, AM, SXT                        |
| E. coli (S15)    | /   | NAL, UB, AM, SXT                        |
| E. coli (S16)    | /   | NAL, UB, CIP, SXT, AM                   |
| E. coli (S17)    | /   | NAL, UB, CIP, SXT, N                    |
| E. coli (S18)    | /   | NAL, UB, CIP, SXT                      |

NA=Nalidixic acid; UB=flumequine; CIP=Ciprofloxacin; AM=Ampicillin; AMC=Amoxicillin/clavulanic acid; SXT=Trimethoprim/sulfamethoxazole; TE=Tetracycline; N=Neomycin; CN=Gentamicin; C=Chloramphenicol

ACKNOWLEDGMENTS

The authors appreciate the staff of the veterinary practitioners for providing samples. A special thanks to Dr. Ben-Mahdi Tarek, the ex-Director of the Regional Veterinary Laboratory of Mostaganem, Algeria, Dr. Benbernou Sennia, Dr. Sebai Ali, Dr. Bouziri Abduljalal, and Ms. Benkhamkham Naziha, for their skilled assistance in the laboratory work. This study was supported by internal funding.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS’ CONTRIBUTIONS

QB, FA, BG, and MBB carried out the main research works and analyzed the main data in the experiments. HT, KR, and MHB have supervised the laboratory work and approved the final version of the manuscript. All authors read and approved the final manuscript.
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