ARTICLE IN PRESS

Diagnostic Microbiology and Infectious Disease xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Prevalence of quinolone resistance determinants in non-typhoidal *Salmonella* isolates from human origin in Extremadura, Spain

Maria Jorge Campos [a,e,*], Gonzalo Palomo [a], Lorena Hormeño [a], Silvia Herrera-León [c], Lucas Domínguez [d], Santiago Vadillo [a], Segundo Píriz [a], Alberto Quesada [b]

[a] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Extremadura, Cáceres, Spain
[b] Departamento de Bioquímica, Facultad de Veterinaria, Universidad de Extremadura, Cáceres, Spain
[c] Servicio de Bacteriología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain
[d] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain
[e] Grupo de Investigação em Recursos Marinhos (GIRM), Instituto Politécnico de Leiria (IPL), Peniche, Portugal

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form 15 October 2013
Accepted 15 January 2014
Available online xxxx

Keywords:
Salmonella enterica
Antimicrobial resistance
Quinolone resistance
Mobilisable plasmid
PMQR
Ciprofloxacin
Nalidixic acid
qnrS
gyrA
gyrB
parC
parE

ABSTRACT

Resistance to the quinolones nalidixic acid (NAL) and ciprofloxacin (CIP) and the occurrence of quinolone resistance determinants have been investigated in 300 non-typhoidal *Salmonella* from human origin, isolated in the years between 2004 and 2008, in 6 hospitals within Extremadura (Spain). *Salmonella* Enteritidis was the major serotype found among quinolone-resistant isolates, most of which were clustered by clonal analysis to a single clone, which presented D87 or S83 substitutions in GyrA. Eleven isolates presented the non-classical quinolone resistance phenotype (resistance to CIP and susceptibility to NAL), lacking mutations in the quinolone resistance determinant region of topoisomerase genes. Among them, one *Salmonella* Typhimurium isolate carried a *qnrS1* gene in a low-molecular-weight plasmid, pQnrS1-HLR25, identical to plasmids previously found in the UK, Taiwan, and USA. The occurrence of this genetic element could represent a risk for the horizontal transmission of quinolone resistance among Enterobacteriaceae in the Iberian Peninsula.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

The most common syndrome associated with salmonellosis is a self-limited gastroenteritis that does not require chemotherapy. Sometimes, especially in the case of children, the elderly, and immunocompromised patients or when invasive infection occurs, antibiotics could be required. Fluoroquinolones represent one of the first choices among antimicrobial treatments for severe salmonellosis (Hopkins et al., 2005). However, there is great concern regarding the extensive use of quinolones that positively select resistant isolates and can favor the emergence of resistant isolates.

Quinolones interact with complexes formed between DNA and topoisomerase II or topoisomerase IV, leading to inhibition of cell growth and destruction of bacterial cells (Andriole, 2005). The main mechanism of quinolone resistance is point mutations affecting the quinolone resistance determinant region (QRDR) of the genes encoding topoisomerase II, *gyrA* and *gyrB*, and/or topoisomerase IV, *parC* and *parE*, which might have an additive effect on the level of resistance shown by the microorganism(Fàbrega et al., 2009). Other chromosomal mutation could reduce susceptibility to quinolones by producing the overexpression of efflux pumps AcrAB/TolC or AcrEF/TolC (Fàbrega et al., 2008; Giraud et al., 2006). Plasmid-mediated quinolone resistance (PMQR) mechanisms in Enterobacteriaceae are mediated by *qnr*, *qep*, *aac-(6′)-lb-cr* genes or *oqxAB*, which are rarely found in *Salmonella* (Strahilevitz et al., 2009).

Quinolone resistance among non-typhoidal *Salmonella* has been documented since the early 90s in Spain and other countries (Herrera-León et al., 2011). This study aims to contribute to the field by analyzing the quinolone resistance among strains of *Salmonella enterica* isolated from humans in different locations of Extremadura, the Middle West region of Spain, in the 4 years between 2004 and 2008.

2. Materials and methods

2.1. Bacterial isolates and antimicrobial susceptibility

A total of 300 *Salmonella* isolates were systematically collected from faeces, with the exception of a few strains that were collected

* Corresponding author. Tel.: +351-262783607; fax: +351-262783088.
E-mail address: mcampos@ipleiria.pt (M.J. Campos).

0732-8893/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.010

Please cite this article as: Campos MJ, et al, Prevalence of quinolone resistance determinants in non-typhoidal *Salmonella* isolates from human origin in Extremadura, Spain..., Diagn Microbiol Infect Dis (2014), http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.010

ARTICLE IN PRESS

from blood (n = 5), urine (1), and from a wound (1), in 6 hospitals located in the Extremadura region, namely, Hospital San Pedro de Alcantara (n = 68), Cáceres; Hospital Ciudad de Cória (26), Cória; Hospital de Llerena (49), Llerena; Hospital Campo Arañuelo (57), Navalmoral de la Mata; Hospital de Mérida (71), Mérida; and Hospital Virgen del Prado (29), Plasencia. Sample collections were made from June 2007 to June 2008, with the exceptions of the Navalmoral and Plasencia hospitals, where sampling was performed from 2004 to 2008 and during 2007 (January-December), respectively. Only one isolate per clinical episode or outbreak was considered. The MICs of the quinolones nalidixic acid (NAL) and ciprofloxacin (CIP) were determined by using the 2-fold broth microdilution reference method (ISO 20776–1:2006; DIN, 2007). Epidemiological cut-off value (ECOFF) used in this work was defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) as being 0.064 μg/mL for CIP and 16 μg/mL for NAL. Resistant isolates were considered when their MIC was higher than the ECOFFs.

2.2. Serotyping, phage typing, and pulse typing

Antigenic formula was determined by slide agglutination with commercial antisera in the Spanish National Reference Laboratory of *Salmonella* and *Shigella* (LNRSSE), Majadahonda, Madrid. Phage typing was performed according to previously published protocols with phages and interpreting criteria provided by the Health Protection Agency (formerly Public Health Laboratory Service, Colindale), London, England (Anderson et al., 1977; Ward et al., 1987). DNA for pulsed-field gel electrophoresis (PFGE) was prepared according to a standard protocol (Ribot et al., 2006). Agarose plugs were digested with 50 U of *XbaI* (Thermo Fisher Scientific, Waltham, MA, USA), and fragments were separated in 1% agarose gel (Conda-Pronadisa, Madrid, Spain) using a Chef-DR III System (BioRad, Hercules, CA, USA) during 20 h with a constant voltage of 6 V, a linear ramp of 2.2–54.2 s *Salmonella braenderup* H9812 (PulseNet) was used as a size marker, and the gels were stained with SYBR Safe DNA gel stain (Thermo Fisher Scientific). Images of PFGE were obtained in an image system Fluor-S MultImager (BioRad) and were analysed with Quantity One 4.4.0 (BioRad). A Dice coefficient of 1% was used to construct the dendogram with Bionumerics 5.2 (Applied Maths, Sint-Martens-Latem, Belgium).

2.3. PCR amplification and sequencing of quinolone resistance determinants

Total DNA was extracted by using a simplified boiling method and directly utilized for PCR reactions. Primers and PCR conditions previously described were used to amplify the QRDR of topoisomerase genes *gyrA*, *gyrB*, *parC*, and *parE* (Giraud et al., 1999; Randall et al., 2005), and the PMQR determinants *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA*, and *aac(6′)-Ib-cr* (Cattoir et al., 2007; Cavaco et al., 2009; Kim et al., 2009; Park et al., 2006; Wang et al., 2009). All isolates presenting resistance to any of the tested quinolones were screened for these determinants, as well as 23 randomly selected sensitive *Salmonella* isolated from different hospitals in different years. Positive controls for PCR were kindly provided by G. A. Jacoby from Lahey Clinic, Boston, USA (*qnrA1*, *qnrS1*, *qnrB1*, and *aac(6′)-Ib-cr*); M. Wang from Fudan University, Shanghai, China (*qnrC*); L. Cavaco from National Food Institute, Copenhagen, Denmark (*qnrD*), and K. Yamane from the National Institute of Infectious Diseases, Tokyo, Japan (*qepA*). The PCR mix contained 0.2 mmol/L dNTPs (Biotools, Madrid, Spain), 0.5 μmol/L of each primer (StabVida, Caparica, Portugal), 0.025 U/μL Taq polymerase (Biotools), 1× PCR buffer containing 1.5 mmol/L $MgCl_2$ (Biotools), and 1 μL DNA template for a total volume of 50 μL. Amplicons were purified (Speed PCR Clean-Up Kit; Biotools) and sequenced by StabVida (Portugal). Plasmids were extracted by using the High Pure Plasmid Isolation Kit (Roche, Basel, Switzerland).

3. Results

3.1. Antimicrobial susceptibility and topoisomerase-QRDR genotypes

Almost one third of the non-typhoidal *S. enterica* isolates presented resistance to NAL or CIP (Table 1). All the isolates sharing quinolone resistance (n = 115) were screened for mutations in the QRDR of topoisomerase genes, which revealed that most of them (81% of quinolone resistant isolates) presented mutations in *gyrA* and/or *parC* (Table 2), but none in *gyrB* or *parE*. By contrast, the same screening performed on 23 quinolone-sensitive isolates, randomly selected from the collection, detected 11 isolates (47.8%) carrying the substitution T57S in ParC and no substitution in GyrA. It has been shown previously that this substitution in ParC is not a true marker for quinolone resistance (Baucheron et al., 2004; Gunell et al., 2009; Lunn et al., 2010; Seminati et al., 2005; Weill et al., 2006), but instead, it is a serotype-specific polymorphism. Indeed, linked mutations at the QRDRs of *gyrA* and *parC* (substitutions D87 and T57, respectively) did not seem to increase resistance more than the single mutation of *gyrA*. The MIC_{90} values for CIP seemed higher among isolates carrying GyrA substitution S83 than D87, although the number of isolates with the first genotype was very low, especially for S83Y substitutions, which were those presenting the lowest susceptibility (Table 2).

The two major serotypes among *Salmonella* isolates, Typhimurium and Enteritidis, were represented unequally among quinolone-resistant phenotypes and genotypes, despite having identical level of quinolone resistance. Indeed, *Salmonella* Enteritidis dominated among quinolone-resistant isolates with GyrA substitutions D87Y or D87G (Table 2), while both serotypes were similarly well represented in the group of isolates lacking any substitution. The serotypes with a frequency smaller than five (Blockley, Brandenburg, Bredeney, Hadar, Indiana, Montevideo, and Newport) lead the set of isolates carrying ParC or GyrA plus ParC substitutions.

3.2. Clonal analysis of quinolone-resistant isolates

Macrorestriction and PFGE analysis of isolates belonging to the 2 most frequent serotypes, Typhimurium and Enteritidis, originated 56 and 22 pulse types, respectively. Among quinolone-resistant isolates (resistant to NAL or CIP), 24 *Salmonella* Typhimurium and 82 *Salmonella* Enteritidis represented 20 and 16 different pulse types, respectively (Table 3). Thus, genetic diversity was much higher among quinolone-resistant isolates of *Salmonella* Typhimurium. However, 58 isolates with serotype Enteritidis presented the same pulse type, EN02, which corresponded to a widely distributed clone carrying quinolone resistance (Palomo et al., 2013). The remaining group of quinolone-resistant *Salmonella* Enteritidis, excluding EN02, comprised 24 isolates corresponding to 15 pulse types, a clonal variety similar to that of *Salmonella* Typhimurium isolates (Table 3).

Table 1
Quinolone-resistance of *S. enterica* serotypes.

Serotypes	n[a]	Resistance (%)			
		NAL	CIP	NAL and CIP	NAL or CIP
S. Enteritidis	115	67.8	64.3	60.9	71.3
S. Typhimurium	125	16.0	17.6	14.4	19.2
S. Muenchen	10	0.0	0.0	0	0
S. Brandenburg	5	20.0	20.0	0	40.0
Other serotypes[b]	45	11.1	17.8	11.1	17.8
Total	300	34.7	35.0	31.0	7.7

[a] Total number of isolates.
[b] Includes 27 least frequent serotypes (n < 5).

Please cite this article as: Campos MJ, et al, Prevalence of quinolone resistance determinants in non-typhoidal *Salmonella* isolates from human origin in Extremadura, Spain..., Diagn Microbiol Infect Dis (2014), http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.010

Download English Version:

https://daneshyari.com/en/article/6115739

Download Persian Version:

https://daneshyari.com/article/6115739

Daneshyari.com