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Review

Quinolone resistance in the food chain

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Abstract

Antimicrobials are used in pet animals and in animal husbandry for prophylactic and therapeutic reasons and also as growth promoters, causing selective pressure on bacteria of animal origin. The impact of quinolones or quinolone-resistant bacteria on the management of human infections may be associated with three different scenarios. (i) Quinolone-resistant zoonotic bacterial pathogens are selected and food is contaminated during slaughter and/or preparation. (ii) Quinolone-resistant bacteria non-pathogenic to humans are selected in the animal. When the contaminated food is ingested, the bacteria may transfer resistance determinants to other bacteria in the human gut (commensal and potential pathogens). And (iii) quinolones remain in residues of food products, which may allow the selection of antibiotic-resistant bacteria after the food is consumed. In this review, we analyse the abovementioned aspects, emphasising the molecular basis of quinolone resistance in *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp.

Keywords: Resistance; Quinolone; *Salmonella* spp.; *Campylobacter* spp.; *Escherichia coli*; Food chain

1. Introduction

An inevitable side effect of the use of antibiotics is the emergence and dissemination of resistant bacteria and resistance genes. Antimicrobials are used in pet animals and animal husbandry for prophylactic and therapeutic reasons and also as growth promoters, and both provide selective pressure on certain bacteria of animal origin.

The World Health Organization recommended in 1997 and 1999 the discontinuation of antimicrobial growth promoters. A similar recommendation was made by the Institute of Medicine (USA) in 2003 [1]. However, many growth promoters used today outside the European Union are analogues of, and show cross-resistance with, therapeutic antibiotics [2].

Campylobacter and *Salmonella* spp. are not the only concern when considering antimicrobial resistance in major bacteria with food animal reservoirs. When exposed to antimicrobial agents, commensal bacteria may develop resistance and thereafter constitute a reservoir of resistance genes that could be transferred horizontally to pathogenic bacteria. Nowadays, the prevalence of antimicrobial resistance in the commensal bacteria of humans and animals is used as an indicator of the selective pressure of antimicrobial agent use [3,4]. Finally, antibiotics can remain in residues of food products, which allow the selection of antibiotic-resistant bacteria after the food is consumed, or they are released into the environment by animal and human effluents.

Resistant bacteria from animals can infect the human population not only by direct contact but also via food products of animal origin. These resistant bacteria can colonise humans or transfer their resistance genes to other bacteria belonging to the endogenous human flora. As early as 1976 Levy reported the transfer of tetracycline resistance genes between chicken *Escherichia coli* strains, from chicken to chicken and from chickens to humans [5].

In this review, we will discuss the molecular basis of either chromosomal or plasmid-mediated quinolone resistance in *E. coli, Salmonella* spp. and *Campylobacter* spp. as well as the potential impact of the presence of residues of quinolones in food.

2. Quinolone resistance in zoonotic bacterial pathogens

Resistance to quinolones can be achieved in different ways. Acquisition of point mutations in the target genes

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for these antimicrobial agents was the first mechanism of resistance characterised. The protein targets for quinolones are type II topoisomerases, a group of enzymes that catalyse the interconversion of different topological forms of DNA and whose inhibition by interaction with quinolone molecules leads mainly to the inhibition of replication, transcription and decatenation [6]. Type II topoisomerases (DNA gyrase and topoisomerase IV) are heterotetramers of two A subunits (encoded by the *gyrA* gene in DNA gyrase or the *parC* gene in topoisomerase IV) and two B subunits (encoded by the *gyrB* gene in DNA gyrase or the *parE* gene in topoisomerase IV) [7]. Chromosomal mutations generally cluster within the quinolone resistance-determining region (QRDR). These regions have been characterised in each of the four genes, with the QRDR of the A subunits (where the active site of the enzyme is localised) being the region where the most frequent mutations appear [6,8,9].

Some studies have suggested that the minimum inhibitory concentration (MIC) of fluoroquinolones is determined by activity against the primary target. In the case of *E. coli*, the most susceptible enzyme to norfloxacin is the DNA gyrase (18-fold in comparison with topoisomerase IV), whereas in *Staphylococcus aureus* the most susceptible enzyme to norfloxacin is topoisomerase IV (2.5-fold in comparison with gyrase). It has been concluded that the main target in Gram-negative bacteria is DNA gyrase but that it is DNA topoisomerase IV in Gram-positive bacteria. However, recent data suggest that this effect depends on the fluoroquinolone studied [7,10–12].

The second mechanism of resistance is a decrease in intracellular accumulation of the antibiotic. This can occur by decreasing uptake or by increasing efflux of the drug [6]. Entry of fluoroquinolones, which are hydrophilic molecules, into the bacterial cell is through specific outer membrane proteins (porins). It is thought that all bacteria have efflux pumps, many of which are multidrug pumps, meaning that a number of different antibacterial agents can be recognised as potential substrates [7,13]. There are five transport protein superfamilies: the major facilitator superfamily (MFS); the ATP-binding cassette (ABC) family; the resistance/nodulation/division (RND) family; the small multidrug resistance (SMR) family; and the multidrug and toxic compound extrusion (MATE) family. These antibiotic efflux pumps utilise the energy of the proton-motive force to export antibiotics from the cell, with the exception of the ABC family that utilises the energy generated from the hydrolysis of ATP [14,15].

Bacterial strains that express efflux-mediated quinolone resistance show cross-resistance to a number of structurally unrelated antimicrobial agents (such as tetracyclines, chloramphenicol, β-lactams, trimethoprim, aminoglycosides and toxic compounds) owing to the broad substrate specificity of these efflux systems, which are capable of accommodating a variety of clinically relevant antimicrobial agents in addition to fluoroquinolones [13,14,16–18].

In Enterobacteriaceae, the main fluoroquinolone efflux system is encoded by *acrAB*/*tolC* genes, an efflux pump that belongs to the RND family (which is widely distributed in Gram-negative bacteria). It is formed by three proteins: AcrA, a periplasmic protein; AcrB, an energy-dependent transport system in the inner membrane; and TolC, an outer membrane protein. A related multidrug efflux system, encoded by *acrEF*, accommodates the same antibiotics but in this case there is no expression under wild-type conditions, whereas *acrAB* is constitutively expressed in wild-type cells and plays a significant role in intrinsic resistance. AcrAB expression is under the control of four known regulators: (i) AcrR, which acts as a repressor; (ii) MarA, a positive regulator that confers a multiple antibiotic resistance (MAR) phenotype and is repressed by MarR; (iii) SoxS, a positive regulator that mediates cell response to oxidative stress and is turned on by SoxR; and (iv) RobA, a small protein that binds to the *E. coli* replication origin and some stress gene promoters and contributes to a MAR phenotype. Some strains can become resistant because, in addition of QRDR mutations, they can acquire new amino acid changes in AcrR and MarR that inactivate these repressors and lead to overexpression of the efflux pump. Otherwise, mutations can also appear in *soxR*, conferring a permanently activated state that increases the SoxS level and, consequently, efflux pump overexpression [9,14,16,18–20].

For a long time it was thought that quinolone resistance was only spread vertically. However, in 1998 the horizontal transmission of plasmid-mediated quinolone resistance genes was reported (discussed below).

3. Mechanisms of resistance to quinolones in *Escherichia coli*

The most important mutations leading to a quinolone-resistant phenotype in *E. coli* are in the *gyrA* gene, mainly amino acids Ser83 → Leu and Asp87 → Asn (this position can be changed to several other less frequent amino acids such as Val, Tyr and Gly), and in the *parC* gene (Ser80 → Arg (Ile can also be found) and Glu84 → Val (Gly can also be found)) [10,21–26]. Nakamura et al. [23] found that mutations in the *gyrB* gene also contribute to low-level quinolone resistance. Yoshida et al. [26] evaluated mutations in *gyrB* and found two possible mutations: Asp426 → Asn (associated with a higher level of quinolone and fluoroquinolone resistance) and Lys447 → Glu (associated with hypersusceptibility to fluoroquinolones but nalidixic acid resistance). However, in *E. coli* clinical isolates this does not appear to be a common phenomenon [24]. Although mutations in the *parE* gene do not appear to play a major role in the acquisition of quinolone resistance in *E. coli* clinical isolates [27], Sorlozano et al. [25] have recently described the acquisition of a new mutation within the QRDR of the *parE* gene (not previously detected) at position 458 (Ser → Ala). Statistical analysis has impli-