8

Mutational Polymorphism in the Bacterial Topoisomerase Genes Driven by Treatment with Quinolones

Maja Velhner and Dragica Stojanović
Scientific Veterinary Institute Novi Sad, Serbia

1. Introduction

Overuse of antimicrobial agents in human and veterinary medicine is a serious problem worldwide. Bacteria have developed resistance mechanisms decades ago, even before wide application of antibiotics had started. Naturally they had learned to cope with an unfavourable environment well before man changed it. Bacteria can develop resistance utilizing different mechanisms. Through mobile genetic elements bacteria transfer resistance to progeny, raising concerns about not being able to control the infection (Velhner et al., 2011). An understanding of resistance mechanisms is essential for developing new therapeutics and overcoming current problems in therapy.

Single point mutations in target genes is sufficient to develop resistance (Giraud et al., 1999).

Quinolones are directed to the complex of DNA and the enzyme Gyrase A and/or topoisomerase IV. They disturb replication of bacteria by changing the topology of DNA. Mutations in genes coding these important enzymes enable bacteria to develop resistance, creating a favourable environment for their survival. Gyrase A consists of two subunits encoded by genes *gyrA* and *gyrB*. In the *gyrA* gene, there is a quinolone resistance-determining region (QRDR) where mutations occur, if bacteria are exposed to antimicrobials (rev. by Velhner et al., 2010). The QRDR extends from codon 67 to 106, but upon quinolone treatment, mutations mostly occur on codons 83 and 87 (Yoshida et al., 1990). In the *gyrB* gene, the QRDR region extends from codons 426 to 447 in *E. coli* and also in *Salmonella* (Yoshida et al., 1991). Gyrase A is a target enzyme in gram negative bacteria. Topoisomerase IV is a target enzyme for gram positive and a secondary target in gram negative bacteria. Topoisomerase IV is encoded by two genes termed *parC* and *parE*. In environments where antimicrobials are persistently in use, multiple resistances develop and genes for both enzymes can be mutated. To monitor the level of resistance it is important to estimate minimal inhibitory concentration (MIC) for certain antibiotics since this would tell us what the risk of antimicrobial use is and whether to expect mutations on target genes.

The focus of the present review will be on resistance development in bacteria that cause food-borne illness and are most often found in livestock industry. Three genera have been selected because of their significance in both human and veterinary medicine: *Salmonella*

spp., *Escherichia coli* and *Campylobacter* spp. These bacteria are transferred to humans through the food chain and tend to persist in farms, alimentary and medical settings. Salmonellae are very invasive and spread very quickly from animal to animal. The rate of *Salmonella* Enteritidis isolation in groups of chickens infected orally with 10^2 cfu/0.1 ml (group A), the sentinels (group C) and group of chickens infected orally with 10^4 cfu/0.1ml and sentinels (group D) is presented in Fig 1. The low infective doses did not prevent horizontal spread of SE to contact chickens during 14 days post infection. *Salmonella* Enteritidis could be isolated in highest percent (group C and D) from birds exposed by contact (Velhner et al., 2005). If chickens are infected during the growth *Salmonella* will be transmitted in slaughterhouses, contaminating food. E*scherichia coli* are widely distributed among farm animals. Pathogenic isolates but also commensals, tend to harbour different resistance mechanisms. Genetic polymorphism in target genes is noted in quinolone resistant strains. However, just recently identical extended-spectrum β-lactamase genes were found among *E. coli* isolates from humans and retail chicken meat in The Netherlands. This implicates spread of *E. coli* to people through the food chain, raising concern about prudent use of antimicrobial agents in farm animals. To minimize health problems in humans caused by infection with food borne bacteria, it is also important to develop strict measures to prevent contamination during food processing and handling (Overdevest et al., 2011). *Campylobacter* species gain resistance to quinolones easily and as such present a risk for consumer's safety. Usually they are not pathogenic for animals, but if humans are infected through food this can cause gastrointestinal diseases. Resistance to quinolones is of special concern because proper medical treatment could be difficult if patients are infected with multiple resistant microorganism. Children, elderly people and immunocompromised patients are at highest risk in such situation. Monitoring of antimicrobial resistance is therefore necessary and in most countries is introduced as a part of the national programs in human and veterinary medicine.

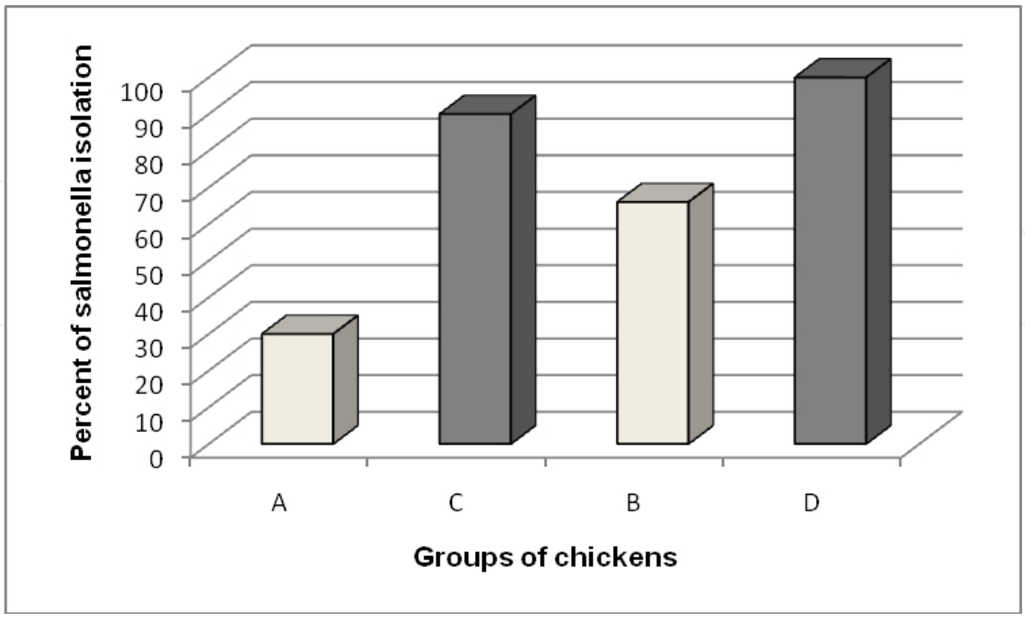


Fig. 1. Incidence of salmonella isolation from cloacal swabs during the experiment. Groups C and D are sentinel chickens.

2. Point mutations in topoisomerase genes of *Salmonella*

There are over 2000 serotypes of the genus *Salmonella* and most of them are pathogens for humans. They are usually transmitted by food of animal origin and animal products. *Salmonellae* are invasive, tend to spread clonally and also gain multiple resistance phenotypes. There is a number of papers dealing with the resistance mechanisms to quinolones in *Salmonella*. Every decade is marked with the predominance of certain serotypes and distinctive resistance patterns. Most frequently quinolones will induce the apparition of point mutations in the *gyrA* gene but there are reports on mutations in other topoisomerase genes. If bacteria are highly resistant to fluoroquinolones (FQ) and MIC exceeds the clinical breakpoint, a double mutant can be expected. Nevertheless in quinolone resistant *Salmonella*, the most frequent mutations are on the *gyrA* gene, while mutations in multiple loci are reported occasionally. We will summarize recent reports concerning mutations in topoisomerase genes and present our current opinion about resistance mechanisms.

Giraud et al. (1999) proposed that in veterinary isolates the most common amino acid substitution in *gyrA* gene is Ser83→Phe, because of wide application in animal husbandry of enrofloxacin, a fluorinated quinolone derivate. A similar result was obtained by spontaneous mutant selection with nalidixic acid (NAL), ciprofloxacin (CIP) and enrofloxacin (ENR). Enrofloxacin selects mutants with Ser83→Phe substitution on *gyrA*, while NAL and CIP favour substitutions on codon 87 (Levy et al., 2004). Therefore mutations on topoisomerase genes depend on antimicrobial agents to which *Salmonella* are exposed (Giraud et al., 2006). In a collection of isolates from farm animals in England and Wales, mutation at codon Ser83 induced higher MIC to ciprofloxacin compared to mutations at Asp87 (Liebana et al., 2002). Mutational polymorphism is prominent in topoisomerase genes in Salmonella and sometimes depends on serotype, country of origin or continent (Escribano et al., 2004; Hopkins et al., 2005; Lindstedt et al., 2004; Piddock et al., 1998; San Martin et al., 2005). The most frequent are mutations on *gyrA*, Ser83→Tyr, Phe or Ala and on codon Asp87→Asn, Gly, Tyr or Lys, (Giraud et al., 2006; Hopkins et al., 2005; Lee et al., 2004). In one clinical isolate of *Salmonella* Enteritidis described in the research work of Lindstedt et al. (2004), a novel mutation was found on *gyrA* at Gly81→His. This mutation coupled with Ser83→Tyr exchange, increased the MIC to CIP to 8 μg/ml (doubled in comparison to single mutant). Mutations on Gly81→Ser do not cause resistance to quinolone and FQ in *Salmonella* Typhimurium when spontaneous mutants are induced *in vitro*. The substitution to serine is too weak to induce resistance to FQ (Reyna et al., 1995). In another experiment Gly81→Cys *in vitro* mutants induced significant increase in MIC to NAL and CIP compared to the parent strain (Giraud et al., 2003). Novel mutants were found in clinical *Salmonella* isolates from patients that were hospitalized because of enteric infection in Tehran, Iran. These novel mutations on *gyrA* are: Ala118→Thr, Ser111→Thr, Arg47→Ser, Leu41→Pro, Asp147→Gly and Gly133→Glu coupled with mutations either on 83 or 87. The significance of these novel substitutions is not known (Hamidian et al., 2011). *Sallmonella* gain resistance to FQ during the therapy. In two immunocompromised patients, *Salmonella* Enteritidis and *Salmonella* Typhimuirum susceptible to FQ were isolated. After the therapy with norfloxacin (patient 1) and cefotaxime (patient 2), the resistant mutants (Ser83→Tyr, Ser83→Phe) were found when the treatment was completed. *In vivo* mutant and pre-therapy

isolates were of the same clone, supporting the opinion that quinolone selection can occur *in vivo* during antibiotic treatment (Ouabdesselam et al., 1996). The role of mutation of *gyrB* to yield quinolone resistance was studied by Gensberg et al. (1995). The authors found a novel mutation on *gyrB* at codon Ser463→Tyr, located outside the QRDR. Complementation experiment with the *E. coli* carrying plasmid pBP548, having the wild type GyraseB, have shown that one isolate, termed L18, regains a susceptible phenotype to quinolones. The mutated region in L18 did not change the hydrophobicity of the enzyme but possibly introduced a conformational change in the molecule. Heisig (1993) and Heisig et al. (1995) reported that in the early 1990's, a *Salmonella* Thyphimuirum var. Copenhagen multiple resistant clonally spread from cattle to humans. These isolates had two point mutations on *gyrA* Ser83→Ala and Asp87→Asn or Tyr. Complementation experiments have provided the evidence that target gene *gyrA*, if mutated, plays a role in resistance to fluoroquinolones and that also *gyrB* is implicated in resistance development. Guerra et al. (2003) found three point mutations in two *Salmonella* Typhimurium var. Copenhagen from the early 1990 that were sent to National Salmonella Reference Laboratory in Germany. The mutations were found on codon Ser83 →Ala and on codon Asp87→Asn in *gyrA*. A novel mutation was found on *gyrB* at codon Ser464→Phe and one additional mutation was found on QRDR of *parC* (Ser80→Ile). Casin et al. (2003) reported that high level resistance to ciprofloxacin was found in two patients (74 year old man, isolate STmA and 3 years old boy, isolate STmB) and that 5 strains had the same point mutations on *gyrA* (Ser83→Phe and Asp87→Asn), while on *gyrB* mutation was found at Ser464→Phe. In STmA, a mutation was found on *parC* (Glu84→Lys) while in an isolate from a 3 year old boy, the mutation was Ser80→Arg. The two isolates were not clonally related. The isolate STmA could acquire resistance during treatment with ciprofloxacin, although it is still hard to explain why the isolate STmB from the boy was floroquinolone resistant and yet the patient had never been exposed to ciprofloxacin. It is possible that both Salmonellas appeared independently from the environment, suggesting the presence of multiple resistant ST in communities in France. A comprehensive research on mutational polymorphism in *Salmonella enterica* in the United Kingdom is provided by Eaves et al., 2004. In this strain collection among 182 isolates, five had a novel substitution on *gyrB*. In *Salmonella enterica* serovar Seftenberg Tyr420 is mutated to Cys and in serovar Newport Arg at 437 was mutated to Leu. These mutations were also found in *Salmonella* Enteritidis and serovar Mbandaka. The authors stated that the mutations occur in close proximity to the quinolone binding pocket, most likely contributing to decreased binding of quinolones. The first report on *parC* mutations in Salmonella was provided by Ling et al. (2003). They discovered that a single point mutation in *parC* confers resistance to quinolones and the highest resistance level was obtained if double mutation occurs in Ser83 and Asp87 on *gyrA* gene. Double mutants were common in clinical isolates reported in the research by Ling et al., 2003. Another interesting observation about *parC* mutations comes from Eaves et al. (2004) who found out that the *parC* mutations, single or coupled with *gyrA* mutation, in *Salmonella* induce lower geometric mean (GMM) and MIC_{50} comparing to single *gyrA* mutant, suggesting compensatory mechanism of *parC* point mutation at 57. This observation was not found by other authors or in *E. coli*, but there is evidence that Thr57→Ser substitution in *parC* (C→G transversion) does not induce quinolone resistance *per se* (Baucheron et al., 2005; Kim et al., 2011).

High fluoroquinolone resistance in *Salmonella* is not very common and sometimes depends on serovar and the rate of exposition to antimicrobial agents. Heisig et al. (1995) stated that

clinical resistance to fluoroquinolones is rare due to the high antibacterial activity against *Salmonella*, because of the lack of enzymatic inactivation of the drug and because it is hard to transfer such resistance. Fluoroquinolone resistant *Salmonella* Typhimurium mutants selected *in vitro* and *in vivo* have shown a low fitness cost indicated by an impaired growth rate comparing to susceptible strains. However, if a compensatory mutation occurs, bacteria have more chance to survive in the presence of antibiotics. In survivors, the role of overexpressed multidrug pumps is also evident (Giraud et al., 2003). Indeed, most often high resistance to fluoroquinolone is coupled with multiple resistance phenotypes. If bacteria harbor resistance to ampicillin, chloramphenicol, sulfa drugs and fluoroquinolones the therapy of ill individuals that require treatment could be difficult. There is an opinion that in such cases different resistance mechanisms are involved in selecting mutants that are capable of surviving in the environment. Three major mechanisms are: mutation in topoisomerase II and IV, overexpression of efflux pump and decreased outer membrane permeability (Cloeckaert & Chaslus-Dancla, 2001; Giraud et al., 2006; Hopkins et al., 2005).

In Spain, the rate of NAL resistant *Salmonella* isolated from humans increased during the year 1994 and peaked at 2001 to 46.2%. In 2003 NAL resistance was found in 38.5% isolates. Out of 164 non-typhoid *Salmonella* from food collected from 1999 to 2003, 124 isolates were NAL resistant. Point mutations were found dominantly at codon 87 and also codon 83. The first *Salmonella* isolate from humans resistant to CIP was reported in March 2003. Subsequently in the same year, 2 more CIP resistant isolates were found. Three *Salmonella* were isolated from children and two from patients aged 49 and 47. These strains possessed three point mutation in QRDR of the *gyrA* gene (aa substitution were at Ser83→Phe and Asp87→Asn) and additional mutation was found on *parC* at Ser80→Arg (Marimon et al., 2004). Similar increase in number of NAL resistant clinical isolates of *Salmonella enterica* was recorded in Korea. In 1996 NAL resistance was found in 1.8% isolates and by 2000-2002 it increased to 21.8%. Single mutation were at codon 87 (39 strains) and at codon 83 in 4 strains (Choi et al., 2005). An increase from 0.4% in 1996 to 2.3% in 2003 was also recorded for NAL resistant *Salmonella enterica* in the USA. In 14 CIP resistant isolates, double mutations were found at *gyrA* at 83 (Ser83→Tyr or Ser83→Phe) and on 87 (Asp87→Gly or Asp87→Asn). Mutations on *gyrB*, *parC* and *parE* were not detected (Stevenson et al., 2007). *Salmonella* Seftenberg isolated during a nosocomial outbreak in Florida were included in the study of Whichard et al. (2007). In these isolates point mutations were found on *gyrA* at Ser83→Tyr and Asp87→Gly and on *parC* (Ser80→Ile and/or Thr57→Ser) while MIC to CIP was >4μg/ml. Most of the *Salmonella* Seftenberg isolates from their collection also contained mechanism of extended-spectrum cephalosporin resistance. Such co-resistance in *Salmonella* Seftenberg maybe plays a role in the epidemiology of *Salmonella*.

If a high resistance to fluoroquinolone occurs, several point mutations are usually detected in target genes. In *Salmonella* collected in Hong Kong in the period from 1990 to 2001, highly resistant strains to fluoroquinolone were recorded. Two isolates harbour mutations at *gyrA* (Ser83→Phe, Asp87→Asn) and on *parC* at Ser80→Arg. MIC to ciprofloxacine was 8-16 μg/ml for these isolates. In 4 additional isolates, point mutations were found on *gyrA* (Ser83→Phe, Asp87→Gly), in *parC* (Ser80→Arg) and in *parE*, with a new substitution at Ser458→Pro. In these *Salmonella* MIC for ciprofloxacin was even higher (16-32 μg/ml). For the first time a mutation, Thr57→Ser in *parC*, alone or coupled with *gyrA* mutation, was reported. Single *parC* mutant were less susceptible to CIP (MIC < 0.12 μg/ml) suggesting

that such mutation is a marker of low level resistance to FQ (Ling et al., 2003). A first report of infection with fluoroquinolon resistant *Salmonella* in the USA was published by Olsen et al. in 2001. The infection occurred in nursing homes, where *Salmonella enterica* serotype Schwarzenground spread among residents in two distant facilities. The patient, who returned from the Philippines and was hospitalized in New York and afterwards resided in a nursery home, was most likely a vehicle for the transmission. In fact, after comprehensive epidemiological survey *Salmonella* Schwarzenground was found in two environmental samples taken from the mattress and door handle in the room of the ill resident. Twenty nine months *Salmonella* Schwarzenground was present in the environment and this infection lead to the death of 3 people in the nursing home. PFGE pattern was similar for all the strains and the same *gyrA* mutations were found during survey (Ser83→Phe and Asp87→Gly). MIC to ciprofloxacin was 4μg/ml. The first report on clinical *Salmonella* Typhimurium DT12 highly resistant to fluoroquinolones in Japan comes from Nakaya et al. (2003). The STDT12 was isolated from a stool of an infant with diarrhoea. The isolate was resistant to ampicillin, streptomycin, gentamycin, tetracycline, chloramphenicol, sulfamethoxazole/trimetoprim, nalidixic acid and fluoroquinolones. It exhibited resistance also to levofloxacin (MIC 8 μg/ml), ciprofloxacin (MIC 8 μg/ml) and norfloxacin (MIC 16 μg/ml). Three point mutations were distributed on *gyrA* Ser83→Phe and Asp87→Asn and on *parC*, Ser80→Arg. Therapy was successful with fosfomycin, a drug prescribed for babies and children. *Salmonella* isolated from cattle in Japan did not posses such resistance phenotype and could not be linked to this outbreak at the time being. However, in 2005, the spread of multiple resistant *Salmonella enterica* serovar Typhimurium DT104 was found in Japan (Izumiya et al., 2005). The isolates harboured mutations on *gyrA* gene Ser83→Phe and Asp87→Asn and on *parC* Ser80→Arg was found. Besides the same point mutations, similar resistotypes were detected in human and nonhuman isolates in their study. Genetically related ciprofloxacin resistant *Salmonella* Typhimurium and *Salmonella* Choleraesuis were found in Taiwan in people and pigs. In human isolates MIC for ciprofloxacin was 8–64 μg/ml while in pig isolates MIC range from ≥ 0.125 to 64 μg/ml. In *Salmonella* Typhimurium from patients, mutations were found in *gyrA* (Ser83→Phe and Asp87→Gly) and additional mutations were found on *parC* at Ser80→Arg or Glu84→Lys. In *Salmonella* Choleraesuis from patients, *gyrA* mutations were found on codons Ser83→Phe and Asp87→Asn while mutation on *parC* was at Ser80→Ile. The same mutations were found in *Salmonella* Typhimuirum from pigs. In serovar Choleraesuis from pigs the mutations were at Ser83→Phe, Asp87→Asn, or Asp87→Gly coupled with *parC* mutations on Ser80→Ile. Interestingly in both human and pig *Salmonella* Choleraesuis isolates resistant to CIP, the same point mutation was also found on *acrR* (efflux repressor gene) at Gln78→Stp. The molecular typing survey has shown that *Salmonella* Typhimurium and *Salmonella* Choleraesuis spread from animals to humans in Taiwan (Chiu et al., 2004; Hsueh et al., 2004). Other research supports the finding that double and triple mutant of *Salmonella enterica* serovar Choleraesusis circulates in several pig farms and that multiple mutations on topoisomerase genes confer resistance to fluoroqionolones in Taiwan (Huang et al., 2004). Clinical report on ciprofloxacin/ceftriaxone resistant *Salmonella* Choleraesuis infection in three patients admitted to Medical Centre in Southern Taiwan, reveals the same PFGE profile and point mutations on *gyrA* Ser83→Phe, Asp87→Asn and on parC Ser80→Ile. All five isolates from patients also possess the bla_{CMY-2} gene. It was postulated that NAL resistant

Salmonella tend to cause higher death rate comparing to susceptible strains (Ko et al., 2005). Recently, one *Salmonella* Typhi isolate from a patient who returned from India was reported to have high MIC to ciprofloxacin (32μg/ml) and subsequently two point mutations were discovered in *gyrA* Ser83→Phe and Asp87→Gly (Hassing et al., 2011). Recurrent infection with *Salmonella enterica* serovar Typhimurium DT104 occurred in a patient admitted to the hospital in Denmark. Initially the bacteria were susceptible to fluoroquinolones. The patient received low doses of ciprofloxacin and during the next year STDT104 emerged as quinolone resistant (MIC to CIP 0.190 μg/ml), due to the single point mutation, on *gyrA* Ser83→Phe (Kristiansen et al., 2003). In *Salmonella* one single point mutation on *gyrA* gene is sufficient to induce high level resistance to quinolones. Double mutations in *gyrA* or *gyrA* and other target genes for quinolones are not so common as in *E. coli*. However, if double mutations occur it usually leads to elevated or high resistance to FQ. High resistance to quinolones due to double and triple mutations is sometimes reported in patients who returned from Asia and/or Mediterranean countries. It is evident that Salmonella differs from continent to continent and depends on the medical treatment prescribed and overuse of antimicrobials in both human and veterinary medicine. In China it is also possible that stool specimens were sometimes collected after the onset of the disease and after the therapy had already been applied. This is due to easy access to the medication that is not under appropriate control of a physician (Cui et al., 2008). *Salmonella* is also the most frequent cause of traveller's diarrhoea. Clinical isolates obtained in a five year study from the patients who returned from different countries (India, Mexico, Egypt, Peru, Kenya, Ivory Coast, Gambia, Senegal, Mali and Bolivia) were tested for antimicrobial resistance. In quinolone resistant strains a single amino acid substitution was found on Asp87→Tyr and in one isolate a Ser83→Tyr substitution was found. Amino acid substitution was not found on *parC* in this strain collection (Cabrera et al., 2004). *Salmonella enterica* serovar Typhi was found in the year 2006 and 2007, in two patients from Kuwait who returned from Bangladesh. In these isolates four point mutations were detected on QRDR, namely: **Leu55→Trp**, Ser83→Phe, **Asp87→Ala**, **Gln106→Arg** in gyrA gene. On *parC* gene mutations were found on Glu84→Lys, Trp106→Gly and **Tyr128→Asp** (in bold are mutations reported for the first time in *gyrA* and *parC* genes) (Dimitrov et al., 2009). Reduced susceptibility in *Salmonella enterica* isolates from Finland was most frequent in patients who acquired infection abroad. Following mutations were reported on *gyrA* gene: Ser83→Phe, Ser83→Tyr, Asp87→Asn, Gly or Tyr. Mutations on *parC* were not recorded in this survey (Hakanen et al., 1999; Hakanen et al., 2001).

A research work from veterinary field is shortly presented here and clonal spread of *Salmonella* is described. Treatment of pigs infected with quinolone resistant *Salmonella* Typhimuirum DT104 with enrofloxacin cause higher shedding of resistant microorganism comparing to untreated control. This means that antibiotic treatment of pigs before slaughter will induce increased shedding and impose higher risk concerning food safety (Delsol et al., 2004). In chicken experiments it was shown that enrofloxacin treatment cause elevated MIC to ciprofloxacin (MIC 0.12-0.5 μg/ml) if birds are infected with a susceptible strain of *Salmonella enterica* serovar Typhimurium DT104. In the same experiment a multidrug-resistant (MDR) derivate of the same strain appeared in the presence of enrofloxacin, exhibiting higher MIC to CIP (0.25-1μg/ml). This isolate was cyclohexane tolerant, implicating the role of efflux pump in MDR strains (Randall et al., 2005).

```
                              83          87
1. gyrA/ST nctc atccccacggcgattccgcagtgtatgacaccatcgttcgtatggcgcagccattctcgc
2. gyrA/EC ATCC atccccacggcgattccgcagtgtatgacaccatcgttcgtatggcgcagccattctcgc
3. gyrA/9568    ........................aac.............................
4. gyrA/501     ........................aac.............................
5. gyrA/75      ........................aac.............................
6. gyrA/7938    ............ttc.....................................
7. gyrA/74      ........................aac.............................
8. gyrA/33641   ........................aac.............................
9. gyrA/317526  ....................................................
10. gyrA/1898   ........................ggc.............................
11. gyrA/228020 ........................aac.............................


1. gyrA/ST nctc tgcgttacatgctggtggatggtcagggtaacttcggttctattgacggcgactccgcgg
2. gyrA/EC ATCC tgcgttacatgctggtggatggtcagggtaacttcggttctattgacggcgactccgcgg
3. gyrA/9568    ....................................................
4. gyrA/501     ....................................................
5. gyrA/75      ....................................................
6. gyrA/7938    ....................................................
7. gyrA/74      ....................................................
8. gyrA/33641   ....................................................
9. gyrA/317526  ....................................................
10. gyrA/1898   ....................................................
11. gyrA/228020 ....................................................
```

Fig. 2. Multiple alignment of quinolone resistant *Salmonella Enteritidis*, isolated from stool, food and poultry in Serbia. Point mutations are presented in bold. This region encompasses codons 83 and 87. The reference strain is number 2 (*Escherichia coli*, ATCC 25922), in strain 317526 the mutation was not found, implying another mechanism of resistance to quinolones

Intercontinental spread of *Salmonella* Typhimurium definitive phage type 104 (STDT104) is well documented. Clonal spread of *Salmonella* Enteritidis PT1, resistant to quinolones (MIC for nalidixic acid >128 μg/ml) also harbouring one unique point mutation on *gyrA* (Asp87→Tyr) is reported in Ireland by Kilmartin et al. (2005). *Salmonella* Virchow has for many years been a common serotype in Israel. It is resistant to nalidixic acid and possesses the point mutation Asp87→Tyr on *gyrA* gene (Solnik-Isaac, 2007). Since the year 2000, in the Netherlands and Germany *Salmonella* Java clonally spreads and is more prevalent compared to other serovars. This strain is resistant to chloramphenicol, sulphonamide, tetracycline, trimetoprim and often to kanamycin, neomycin and nalidixic acid. It is postulated that S. Java has emerged in the poultry industry due to the frequent use of antibiotics and because vaccination was implemented to eradicate *Salmonella* Enteritidis (van Pelt et al., 2003). Clonal spread of multiple resistant *Salmonella* Infantis (nalidixic acid, streptomycin, sulphonamide and tetracycline) is reported in Hungary where poultry isolates were linked to human's (Nógrády et al., 2007). *Salmonella enterica* serovar Haardt resistant to quinolones with elevated MIC to ciprofloxacin (MIC 0.25-2 μg/ml) was found on chicken meat collected in various stores. Point mutation was detected on *gyrA* (Ser83→Tyr). PFGE analysis implies clonal spread of NAL resistant S. Haardt in Korea (Lee et al., 2008). In Serbia S. Enteritidis isolated from humans, food and poultry was typed by Random Amplified Polymorphic DNA analysis (RAPD). In a collection of 60 strains, 9 were resistant to nalidixic acid. In these

strains 3 different single point mutations were found on *gyrA*, namely Asp87→Asn in 6 strains, Asp87→Gly in 1 strain and Ser83→Phe in one strain (Fig 2). For these isolates, MIC for NAL was 128-512 μg/ml and MIC for CIP was 0.256-0,512 μg/ml. In this strain collection multiple resistances was found in three isolates. *Salmonella* Enteritidis from one day old chicken was resistant to ampicillin (AMP), cephalothin (CFT), nalidixic acid (NAL) and tetracycline (TET). One isolate from stool was resistant to AMP, TET, trimetoprim-sulfamethoxasole (SXT) and another isolate from stool exhibited resistance to AMP, TET, SXT and neomycin (NEO) (Kozoderović et al., 2011). There was an increase of *Salmonella* Enteritidis isolates in Serbia in 2005 and clonal spread was suspected. However, from 2005 to 2008, the percentage of quinolone resistant *Salmonella* Enteritidis appeared to decrease from 9% to 1% in the Northern part of the country (Kozoderović et al., 2009).

3. Point mutations in topoisomerase genes of *E. coli*

Resistance to fluoroquinolones in *E. coli* has been extensively studied. Mutational polymorphism on topoisomerase genes in *E. coli* is frequent in clinical settings but also in commensal. First step mutations occur on *gyrA* gene, most frequently at codon 83, where substitution of Leucine for Serine is found (Chen et al., 2001; Conrad et al., 1996; Everett et al., 1996; Gales et al., 2000; Heisig and Tschorny, 1994; del Mar Tavio et al., 1999). The first report of double mutation in *gyrA* was provided by Heisig et al. (1993). The mutations in *E. coli* isolate 205096, highly resistant to FQ, were located on Ser83→Leu and on Asp87→Gly. Complete sequencing of the gene revealed additional mutation in less conserved region of the *gyrA*, at Asp678→Glu. This mutation is not implicated in FQ resistance. Throughout the coding region, 52 silent mutations were also found. The authors stated that mutations at 83 and 87 encompass part of the enzyme responsible for cleavage and sealing of the DNA. This is necessary to obtain negative supercoiling prior to strand separation. The quinolones prevent contact between DNA and enzyme thus aborting bacterial replication (Drlica & Zhao, 1997). In clinical *E. coli* isolated from urine samples of outpatients who did not receive FQ therapy, a novel mutation was found: Asp87→Tyr. Concomitant mutation of Ser83→Leu and Asp87→Asn or Tyr, induced clinical resistance to CIP (MIC ≥ 8 μg/ml). The significance of the double mutant for high fluoroquinolone resistance is warranted (Vila et al., 1994). In clinical isolates of *E. coli* (MIC to CIP > 1 μg/ml) mutations were found on *gyrA* and *parC* gene. Mutational polymorphism in this strain collection is briefly presented here. Mutant with MIC to CIP of < 1 μg/ml have only one mutation at Ser83→Leu. If MIC to CIP is from 8-128 μg/ml, 3-4 mutations were found on gyrA (Ser83→Leu or Asp87→Tyr or Asn) and parC genes (Ser80→Arg or Ile) and /or Glu84→Lys or Val). In two isolates (1319 and 1383) four mutations were found. The MIC to ciprofloxacin was 64 μg/ml and 128 μg/ml and mutations were arranged as follows: Ser83→Leu, Asp87→Asn, Ser80→Ile and Glu84→Val in isolate marked 1319, while a mutation's on Ser83→Leu, Asp87→Tyr, Ser80→Ile and Glu84→Lys were found in isolate marked 1318. This was the first report of *parC* mutation in *E. coli* (Vila et al., 1996). The role of *parC* mutations in highly quinolone resistant *E. coli* was studied by Kumagai et al., 1996. In the QRDR of the *parC* gene three missense mutations on codons Gly78, Ser80 and Glu84 were described corresponding to the *gyrA* mutations on codons Ser83, Gly81 and Ap87. In this research the importance of mutations in topoisomerase IV, in building resistance to FQ in *E. coli* was emphasized. The

role of mutations on FQ resistance was also studied by Heisig (1996). In *parC* mutant a reduction in MIC to CIP was recorded after introduction of the wild type gene to mutants. If parC$^{1\text{-}80}$ allele (a single mutant) is introduced into isolates that have two point mutations on *parC*, an increase of susceptibility is noted, while introduction of resistant *parC* allele to *gyrA* mutants will induce an increase of MIC to CIP. Mutation of *parC* in *E. coli* having two point mutations on *gyrA* is a prerequisite for high FQ resistance phenotype. The mutations induced by FQ occur in stepwise manner and the primary target for CIP is Gyrase (Bagel et al., 1999). The level of quinolone/fluoroquinolone resistance correlates well with the type and number of mutations on topoisomerase genes. The CLSI breakpoint for FQ is obtained if double mutations on *gyrA* are combined with single or double *parC* mutant (Morgan-Linnell and Zechiedrich 2007; Sáenz et al., 2001). If a mutation on *parC* occurs in a single *gyrA* mutant, second mutation on *gyrA* is selected (Morgan-Linnell and Zechiedrich 2007). Therefore *gyrA* mutation is indispensable for clinical resistance to quinolones and it mostly occurs before *parC* mutations, especially in clinical isolates. Single *parC* mutation does not induce resistance to FQ (Bagel et al., 1999). If *E. coli* is exposed to quinolones, a stepwise mutagenesis may lead to development of a new feature that is beneficial for these bacteria. Usually selection pressure is imposed by application of various quinolones or fluroquinolones in agriculture. In such scenario a single step mutant can become predominate in the population. This will contribute to developing multiple mutations and high resistance to fluoroquinolones in clinical isolates after therapy has been introduced (Piddock et al., 1996). Infection with Fluoroquinolone resistant *E. coli* acquired in hospitals is of special concern. The resistant strains usually evolve independently and the therapy may be difficult to introduce. In the mid 1990, the percent of fluoroquinolone resistant *E. coli* increased in many countries. Lehn et al. (1996) described resistotype and mutations on *gyrA* in a collection of 19 *E. coli* resistant to CIP in hospital settings in Munich, Germany. The majority of isolates were multiple resistant to the following antibiotics: nalidixic acid, ciprofloxacin, norfloxacin, aminoglycosides, co-trimoxazole, ampicillin, ampicillin and sulbactam and piperacillin. Most isolates obtained for the study were from Urology Department, from Gynecology, Surgery and Internal Medicine. PFGE typing implicates diversity of the strains but resistotype was quite similar. Also, the mutations on topisomerase were documented on Ser83→Leu, Asp87→Asn in 16 strains. In two strains Asp was mutated to Gly. Resistance of *E. coli* to FQ is equally reported in developed and developing countries. At highest risk are patients that are hospitalized and treated with antibiotic. Several studies have dealt with various aspects of infection with *E. coli* in hospitals and outpatient clinics. In a study from Taiwan, a total of 1203 isolates of *E. coli* from 44 hospitals were tested on antimicrobial susceptibility and it was found that 11.3% isolates were resistant to FQ. Decreased susceptibility to CIP was found in 21.7% isolates. A single point mutation was found on *gyrA* among FQ resistant and isolates with reduced susceptibility. The authors postulated that such single mutation is a prerequisite for resistance development. In FQ highly resistant strains (MIC >32μg/ml) three or four point mutations were found in QRDR of the *gyrA*, and *parC*. These included substitution at Ser83→Leu, Asp87→Asn, coupled also with Ser80→Ile (9 isolates) or Ser80→Arg (one isolate), Ala81→Pro (one isolate) and Glu84→Gly or Lys (one isolate). The survey in Taiwan reveals high risk on FQ resistance in clinical *E. coli* isolates. It was noted that patients diagnosed with cancer were predisposed to infection with resistant *E. coli*. Nalidixic acid and pipemidic acid are still in medical practice and these practices pose a risk for higher FQ resistance in Taiwan (McDonald et al., 2001). Two or more mutation on topisomerase II and

IV were found in a hospital strain collection in South Korea. In fact, the number of acquired mutations was proportional to MIC for ciprofloxacin. The CIP resistant isolates were divided into 4 groups depending of the number of mutations in topoisomerase genes. It was concluded that second step mutant will induce double mutation on *parC* and that double *parC* mutant are highly resistant to fluoroquinolones. All of the *parE* mutations were outside the QRDR. Three novel *parE* mutations were reported on Leu445→Ile, Ser458→Pro and Ser458→Trp. These single mutations on *parE* significantly increased MIC to CIP, norfloxacin and gatifloxacin. In this strain collection three to four mutations on topisomerase II and IV were common. Mutations on *gyrB* were not found. Combination of double mutations on *par*C or single *par*C and *parE* mutation increases MIC of ciprofloxacin (Chan Moon et al., 2010). Mutational polymorphism was found in clinical isolates of *E. coli* in New Delhi, India, revealing that a number of isolates possess several mutation on topisomerase genes and that mutation on *parE* was prominent since 44.4% of isolates are mutated on *parE*, outside of QRDR. The substitutions found on *parE* were Ser458→Ala and Glu460→Asp. Mutations on both genes (*parC* and *parE*) being the secondary target for FQ contributed to higher MIC >0.25 μg/ml for ciprofloxacin (Bansal and Tandon, 2011). Asian Network for Surveillance of Resistant Pathogens provided 68 *E. coli* isolates resistant to FQ, to carry out a research on geographic distribution of mutational polymorphism. Clonal spread was not evident. Continentally distributed isolates possessed a variety of mutation in topoisomerase genes but those with three or more mutations were increasingly resistant to CIP. This high increase was attributed also to the mutation of the *parE* gene (Uchida et al., 2010). *E. coli* isolates from healthy patients in Teaching Hospital and Microbiology Department at the University in Ghana were studied with respect to FQ resistance. About 50 to 90% of faecal isolates from healthy individuals were multiple resistant to the following antimicrobial agents: ampicillin, streptomycin, tetracycline, sulphonamides and trimethoprim. The obtained results revealed that in the year 2008, 18.2% of isolates were resistant to NAL and 9.9% were resistant to FQ. In resistant isolates the most frequent substitution was Ser80→Leu often combined with Asp87→Asn in *gyrA* and on *parC* the most frequent substitution was at Ser80→Ile. The mutation in topoisomerase that was found was attributed to old and recently introduced drugs in clinical practice in Ghana. Presence of multiple resistances in commensal microflora in patients is noted and the prudent use of fluoroquinolones is warranted (Namboodiri et al., 2011). Bacteria change phenotype, depending on the selection pressure. This has been shown in research on resistance mechanisms in laboratory mutants and clinical isolates. We have learned that careful use of antibiotics is essential and must be under control or it will be impossible to cope with the infections acquired in hospitals and the environments from which subsequent transfer of resistant bacteria is common. Topoisomerase mutators can have prohibitive fitness cost or might have selection advantage.

E. coli with single point mutation on *gyrA* was found in veterinary isolates and it was suggested that the digestive tract of animals can be a reservoir of low level resistance to quinolones. If such bacteria find their way to humans through the food chain, clinical resistance will occur in patients that needs therapy (Everett et al., 1996). A heterogenic population of quinolone resistant *E. coli* collected from poultry, poultry farmers and poultry slaughterers was described by van den Bogaard et al. (2001). Only in few isolates a link between poultry and poultry farmers was noted, implying that *E. coli* from animals could also infect people. Mutations on topoisomerase genes in *E. coli* isolated from faeces of

healthy chickens in six broiler and 4 breeder farms in Korea was described by Lee et al. (2005). All isolates resistant to ciprofloxacin and enrofloxacin exhibited mutations on Ser83→Leu. Many isolates also harbour second mutation on Asp87→Asn, Ala, Gly, His or Tyr. In this strain collection, mutations were also found on *parC*, the most prevalent being Ser80→Arg, Ile, Phe and Gly and in some isolates, mutation were found on Glu84→Lys, Ile and Tyr. The MIC breakpoint to CIP and ENR was >3 μg/ml and 2 μg/ml respectfully. The isolates with two mutations distributed on *gyrA* and *parC* had elevated MIC to ciprofloxacin (0.5-3 μg/ml) while MIC to ENR was 1 to 32 μg/ml. Isolates presented by greater MIC than clinical breakpoint had two mutations on *gyrA* (at codons 83 and 87) with or without substitution on *parC* . Khan et al. (2005) found out that the poultry litter collected from poultry and turkey farms in the Arkansas, USA becomes an important source of quinolone resistant *E. coli*. In isolates resistant to CIP (> 2 μg/ml) two point mutations were found at Ser83→Leu and Asp87→Asn. Single point mutation in MIC < 2 μg/ml was found on Ser83→Leu. Beef, pork and poultry are considered as the main reservoir of quinolone resistant *E. coli*, *Salmonella* and *Campylobacter* (Mayrhofer et al., 2004). Nine *E. col*i isolates from wild birds that died from septicemia were tested on quinolone resistance. It was found that all the strains had one mutation on *gyrA* gene at Ser80→Leu. Mutations on other target genes (*gyrB*, *parC* and *parE*) were not found (Jiménez Gómez et al., 2004). The high incidence of quinolone resistant *E .coli* from nosocomial and community acquired infections is linked to a high incidence of FQ resistant *E. coli* in poultry and pigs. The clear connection between human and poultry isolates was not, however, found in Spain but it was postulated that the infection of humans occurs via contaminated food of animal origin Garau et al. (1999). Indeed, it is difficult to explicitly claim that the link exists between poultry related food and humans but the similarities are present in resistant isolates, compared to susceptible strains. The general statement is that the misuse of quinolones largely contributes to the spread of resistant bacteria through the food chain (Johnson et al., 2006).

It is difficult to explain how FQ resistance evolves in *E. coli* and how it spreads in clinical settings. It seems that *E. coli* mutates to a higher extent compared to *Salmonellae* and is capable of accumulating several point mutations at the same time or in a stepwise manner. Since the FQ resistant phenotype arises exclusively after *de novo* mutations (Mooij et al., 2005) it seems that microorganisms harbouring one single amino acid substitution are prone to mutate and more easily survive in the environment. High mutation rates could also lead to the development of deleterious mutations. Strong mutators therefore do not always have an evolutionary advantage. If the high mutation rate of *E. coli* will not favour its survival, then the only way to explain the presence of resistant population in a certain zone is exposure of bacteria to antimicrobials. *E. coli* tends not to spread clonally in hospital settings (Lautenbach et al., 2006) and also in the environment (Khan et al., 2005). It is not clear whether isolates with multiple mutations on target genes have some evolutionary advantage so it appears that multiple mutations have low fitness cost. If such mutations are accumulated, compensatory mutations will support growth. On the contrary there are examples where multiple mutations have actually increased bacterial fitness. This also takes place in the absence of antibiotics. Mutations on regulatory genes have the highest influence on bacteria fitness, since those genes regulate transcription and also efflux mechanisms (Marcusson et al., 2009).

4. Point mutations in topoisomerase genes in *Campylobacter* spp

Infections caused by *Campylobacter* usually have a very silent course in animals (Stojanov et al., 2004). However, Campylobacteriosis is the most frequent reason for gastroenteritis in humans. If food producing animals (pigs and poultry) are treated with enrofloxacin (a fluoroquinolone antibiotic) *Campylobacter* develops resistance that lasts well beyond withdrawal of the therapy (Delsol et al., 2004; Griggs et al., 2005; Luo et al., 2005). Subsequently food can be contaminated during processing, increasing the possibility of transmission to humans. Ciprofloxacin resistant Campylobacter emerged on the European continent in the early 1990 and this coincides with the agricultural practice to treat animals with enrofloxacin. At that time in the United Kingdom human *Campylobacter* infections were reported most frequently in travellers returned from abroad, while ciprofloxacin resistant *Campylobacter* was found in poultry carcasses imported from Europe (Gaunt and Piddock, 1996). In Northern Ireland, ciprofloxacin resistance of *Campylobacter* rose in 1998. This was attributed to dietary habits of the consumers in the UK. Chicken and pork meat consumption increased because of Bovine spongioform encephalopathy. Subsequently the import of poultry meat from the European countries might have contributed to the spread of fluoroquinolone resistance (Moore et al., 2001). The National Antimicrobial Resistance Monitoring Program (NARMS) was conducted from 1997 to 2001 to identify susceptibility of *Campylobacter* for the following antimicrobials: chloramphenicol, ciprofloxacin, clindamycin, erythromycin, nalidixic acid, tetracycline, azithromycin and gentamycin. Isolates were collected based on a questionnaire that included: history of recent illness, exposure to animals, food consumption or travelling. Retail chicken meat products from domestic brand were also included in the study. An increase of ciprofloxacin resistant *Campylobacter* from 13% in 1997 to 21% in 2001 was found. Foreign travel was identified as a risk of FQ resistant *Campylobacter* infection in humans but also consumption of chicken meat. The increase of CIP resistant *Campylobacter* coincides with the increasing use of fluoroquinolones in human medicine and livestock industry in the USA (Gupta et al., 2004). Infection with CIP resistant *Campylobacter* in a Minnesota community was related to travels abroad and seasonal peaks were identified. Overall increase in *Campylobacter* isolates resistant to CIP from the year 1996 to 1998 was attributed also to domestically acquired infections. Poultry meat and products were identified as a source of infection and a genetic correlation between human and poultry isolates was determined (Smith et al., 1999). However, *Campylobacter* is genetically quite diverse and clear links between food of animal origin and humans is not easily confirmed. In fact, diverse *Campylobacter* isolates were noted on a single swine farms and slaughter plant in the USA (Thakur and Gebreyes, 2005). Similar results were obtained in Senegal in a two-year period, applying multilocus sequence approach in *Campylobacter* isolated on chicken carcasses. Allelic profiles in *Campylobacter* jejuni (Kinana et al., 2006) and *Campylobacter* coli (Kinana et al., 2007a) imply genetic differences in this strain collection. The link between genotype and quinolone resistance was not found with certainty in their research. Even for the isolates of the same sequence type, different silent mutations were found on *gyrA* gene. In the research conducted in France an increase in FQ resistant *Campylobacter* was recorded for the period 1986 to 2004. The FQ resistance pattern was similar between human, pig and poultry isolates. In *C. coli* the overall resistance rate was higher comparing to *C.* jejuni. In pigs, *C.* coli predominated over *C.* jejuni and it was more frequently resistant to CIP. The higher rate of *C.* coli isolation in pigs and chickens is not

explained. The FQ resistance pattern was similar in *C.* jejuni isolates found in humans and broilers. Resistance decreased to lesser extend in human C. jejuni comparing to chicken. This is explained in part by restrictive use of antimicrobials as food additives in livestock industry and may also be related to various routes of infection in humans and therapeutic practice in human medicine (Gallay et al., 2007). Overall, it has been shown that people are infected from food of animal origin and that intensive use of quinolones and fluoroquinolones in livestock industry undoubtedly contributes to the development of resistance. Molecular typing of the *Campylobacter* species revealed quite diverse genetic backgrounds and apparent clonal distribution was not identified. The prudent use of FQ antibiotics is necessary to build up safe environment and safe food production.

The ability of *Campylobacter* to acquire resistance to quinolones/fluoroquinolones is rather impressive. The most frequent missense mutation in *gyrA* gene is Thr86→Ile. The codon 86 in *Campylobacter* corresponds to *gyrA* codon 83 in *E. coli* and Salmonella (Beckmann et al., 2004; Griggs et al., 2005; Hakanen et al., 2002; Sonnevend et al., 2006; Wang et al., 1993, Zirnstein et al., 2000). This single point mutation confers resistance to ciprofloxacin and MIC is $\geq$ 32μg/ml. Less frequent mutation in QRDR of the *gyrA* in *Campylobacter* are Asp90→Asn, Thr86→Lys, Thr86→Ala, Thr86→Val and Asp90→Tyr. Double mutant are found on Thr86→Ile-Pro104→Ser and Thr86→Ile-Asp90→Asn (rev by Payot et al., 2006). *Campylobacter* isolates originating from broilers, turkeys and humans resistant and sensitive to NAL were examined for mutations within QRDR. In 135 resistant strains the most frequent mutation was Thr86→Ile and thereafter mutations were found on Thr86→Ala, Asp90→Asn and Pro104→Ser. It is not known whether mutation on Pro104→Ser influence the resistance to FQ. In susceptible isolates as well as in resistant strains, silent mutations were on codons Gly78, His 81, Gly110 and Ser119. Genetic variation is therefore common in *C.* jejuni isolated from chickens, turkeys and humans (Beckman et al., 2004). Mutations on *gyrB* gene are rare in *Campylobacter* resistant to NAL and CIP. Piddock et al. (2003) reported silent mutation on *gyrB* in few isolates of *Campylobacter* species. In clinical isolates of *C.* coli, *C.* jejuni, *C.* lari and *C.* fetus obtained from UK, Germany and the Netherlands, from the period 1990 to 1995, the most frequent mutation on *gyrA* was Thr86→Ile. In one isolate mutation on Asp90→Asn was found and one isolate was double mutant (Thr86→Ile-Pro104→Ser). Silent mutations were found on *gyrA* in *C. jejuni* suggesting genetic and epidemiological differences between the isolates. In Senegal silent mutation of *gyrB* was found in a collection of isolates from chicken carcasses. These silent mutations were identified from codons 371-540. Besides silent mutations the dominant transition on *gyrA* was Thr86→Ile and six isolates mutated on Thr86→Ala. The amino acid substitution Thr86→Ala was found also in two chicken isolates susceptible to CIP and MIC to NAL was 16 μg/ml. In 17 isolates mutations were found at Asn203→Ser, downstream *gyrA* and in 3 isolates mutations were found also at Ala206→Thr. The *parC* gene could not be amplified (Kinana et al., 2007b). In a strain collection of CIP resistant *C. coli*, isolated from humans, food and animals in Italy, a novel mutation at codon 86 was described. This was a double nucleotide substitution at ACT → GTT changing Thr86→Val in three *C.* coli isolates. This type of transition did not induce additional increase of MIC to CIP. In *C.* coli different *gyrA* alleles were found, but the mutations were silent, pointing only to the genetic diversity of the unrelated isolates from Italy. Mutation Thr86→Ile on *gyrA* was associated with high MIC to ciprofloxacin (MIC $\geq$32 μg/ml) (Carattoli et al., 2002). In six clinical isolates from the

research of McIver et al. (2004) mutation was identified on Thr86→Ile, but in laboratory derived mutants three non-synonymous substitution were identified at Asp90→Asn, Asp85→Tyr and Thr86→Ala. This was a first report of double mutation Asp85→Tyr-Thr86→Ile in *Campylobacter* after *in vitro* exposition to sub-inhibitory concentration of ciprofloxacin. Silent mutations apparently do not improve *in vivo* fitness cost of the resistant *Campylobacter* nor its colonization ability, which is preserved even in the absence of antibiotics. Its viability is solely due to single point mutation in *gyrA* gene. This is probably the reason why FQ resistant *Campylobacter* persist in farm animals over a long period and because once contaminated with such bacteria the farm presents a hazard in food production (Luo et al., 2005). Double *gyrA* mutant is reported from Thailand in *C.* coli isolated from pigs. Mutations were found in *gyrA* at Thr86→Ile and Gly119→Ser and in three isolates also amino acid substitution on *gyrB* were Lys382→Gln or Arg. These additional mutations did not increase MIC to CIP or NAL, so its possible role in resistance mechanism needs to be determined in the future (Ekkapobyotin et al., 2008). Double mutations on *gyrA* (Thr86→Ile) and *parC* (Arg139→Gln) were found in clinical isolates of *C.* jejuni in patient that were treated with fluoroquinolones because of profound diarrhea. The mutation on *parC* gene is rare and is coupled with amino acid transition on *gyrA*. The influence of *parC* mutation on MIC for CIP is not clear (Gibreel et al., 1998).

Implications of gene mutations to NAL resistance phenotype was studied by Jesse et al. (2006) and an observation was made that single point mutation on Thr86→Ala confers the resistance to NAL but not to CIP. Most isolates from their strain collection, obtained from chicken carcasses and cattle faeces, also possessed one or more silent mutations in the *gyrA* gene. Silent mutations, double mutant in *gyrA* gene and also mutations outside of the QRDR/gyrA, usually do not change the MIC significantly but contribute in allelic diversity and in that respect can be used as a typing tool or in research on correlation between strains found in humans and animals. Multilocus sequence *fla* typing successfully discriminated *C.* jejuni and *C.* coli in the strain collection from the Institute of Veterinary Bacteriology at the University of Bern, Switzerland. This method can be used in epidemiology research and in studying phylogenetic relation and divergence in large collection of isolates over a longer period of time (Korezak et al., 2009). The *fla* Restriction Fragment Length Polymorphism (RFLP) is a good alternative in research on genetic diversity of *Campylobacter* spp. (Keller & Perreten, 2006, Sonnevend et al., 2006). *Campylobacter fetus* subsp. fetus (wild type isolate and laboratory obtained mutants) is intrinsically resistant to NAL and resistance to CIP is obtained from the single transition of Asp91→Tyr. The MIC to CIP does not exceed 8μg/ml for laboratory mutants. For clinical isolates the obtained MIC was 16 μg/ml (Taylor & Chau, 1997). *Campylobacter fetus* was isolated in two immunodeficient patients. The clinical symptoms of gastrointestinal disorder relapsed after therapy with fluoroquinolones. In re-isolated *Campylobacter* a mutation on *gyrA* gene was found at Asp87→Thr. The authors stated that in immunodeficient patients it is very important to control resistance status before, during and after the treatment to enable successful therapy and prevent a failure (Meier et al., 1998). *Campylobacter hyointestinalis* subsp. hyointestinalis, isolated from reindeer and bovine fecal samples from the northern Finland were tested on antimicrobial resistance and *gyrA* mutations obtained *in vitro*. *C. hyointsetinalis* is intrinsically resistant to NAL and susceptible to CIP. In isolate naturally resistant to NAL an amino acid substitution Thr86→Ile was found. The same transition (Thr86→Ile) was recorded from *in vitro* obtained mutants (grown in a gradually increasing concentration of ciprofloxacin) with MIC of $\geq$64 μg/ml. In the strains that exhibit

MIC to CIP ≤32 μg/ml, *gyrA* was not mutated implicating other mechanism of inherent resistance, was not mutated implicating other mechanism of inherent resistance (Laatu et al., 2005).

Mutational polymorphism in QRDR region of the *gyrA* gene in *Campylobacter* species is not exclusively driven by antimicrobial therapy in livestock industry but also occurs after therapy of patients. To decrease spreading of resistant bacteria in agriculture, since 2005 fluoroquinolones have been banned in poultry and swine industry in the USA. Other antimicrobial agents must be used to treat *E. coli* infections in pigs and poultry in North America. The most important approach to combat resistance in bacteria is therefore good management, food safety and good cooking practice. It is hard to expect that the problems of antibiotic resistance will be entirely resolved since bacteria find ways to survive under selective pressure created by man but also in their own environment and communities. Permanent monitoring of antimicrobial resistance and resistance mechanism is essential and must be carried out in each country under national programs and preferable supported by international projects. Point mutations in a single gene of topoisomerase enzymes are a good example of the smart game that bacteria play to survive and spread in nature.

5. Conclusion

Antibiotic resistance monitoring is compulsory in developed countries but is also conducted at similar level in developing countries. Since resistance development is attributed to the use of antimicrobial agents in clinical therapy and livestock industry, medical and veterinary sector is equally involved in resistance monitoring. We have learned that resistance to "old" antibiotics can lead to bacteria that inherit more than one mechanism of resistance, that could develop MDR phenotype and that the new feature is sometimes fitness cost effective. Mutations on target genes selected *in vitro* do not necessarily resemble the nature of mutational frequency *in vivo*, but with certainty enable scientist to understand how resistance develops and what the risk is of antibiotic use. Epidemiologists have put much effort to explain the spread of resistant bacteria and to find their origin. They have tried to explain their intercontinental appearance. Vehicles for the transmission are numerous and it is usually impossible to find the exact path of their spread in humans and also animals. By utilizing molecular biology methods, clones and links that are attributed to their transfer from one setting to another can be identified. Mutations on topoisomerase genes determine the genetic background of the bacteria and in some instances increase the success of their survival in nature.

6. Acknowledgment

This paper is dedicated to late Dr Slavko Đurišić, who was our mentor, dear colleague and friend. We thank Mrs Lidija Orčić for English editing. This work is supported by a grant from the Ministry of Education and Science, Republic of Serbia, Project number TR 31071

7. References

Bagel S,; Hüllen V, Wiedemann B. & Haisig P. (1999). Imapct of *gyrA* and *parC* mutations on quinolone resistance, doubling time and supercoiling degree of *Escherichia coli*. Antimicrobial Agents and Chemotherapy, Vol.43, No.4, pp. 868-875, ISSN 0066-4804

Bansal S. & Tandon V. (2011). Contribution of mutations in DNA gyrase and topisomerase IV genes to ciprofloxacin resistance *in Escherichia coli* clinical isolates. International Journal of Antimicrobial Agents, Vol.37, pp. 253-255, ISSN 0924-8579

Baucheron S,; Chiu CH. & Butaye P. (2005). High-level resistance to fluoroquinolones linked to mutations in *gyrA* and *parC* and *parE* in *Salmonella enterica* serovar Schwarzengrund isolates from humans in Taiwan. Antimicrobial Agents and Chemotherapy, Vol.49, No.2, pp. 862-863, ISSN 0066-4804

Beckman L.; Müller M, Luber P., Schrader C., Bartelt E. & Klein G. (2004). Analysis of *gyrA* mutations in quinolone-resistant and susceptible *Campylobacter jejuni* isolates from retail poultry and human clinical isolates by non-radioactive single-strand conformation polymorphism analysis and DNA sequencing. Journal of Applied Microbiology, Vol. 96. pp. 1040-1047, ISSN 1364-5072

Cabrera R,; Ruiz J, Marco F, Oliveira I, Arroyo M, Aladueña A, Usera MA, Teresa Jiménez De Anita MT, Gascón J. & Vila J. (2004). Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing traveler's diarrhea. Antimicrobial Agents and Chemotherapy, Vol.48, No.10, pp 3934-3939, ISSN 0066-4804

Carattoli A,; Dionisi AM. & Luzzi I. (2002). Use of a LightCycler *gyrA* mutation assay for identification of ciprofloxacin-resistant *Campylobacter coli*. FEMS Microbiology Letters, Vol.214, pp. 87-93, ISSN 0378-1097

Casin I.; Breuil J., Jean Pierre D., Guelpa C. & Collatz E.(2003). Fluoroquinolone resistance linked to GyrA, GyrB and ParC mutations in *Salmonella enterica* Typhimurium isolates in humans. Emerging Infectious Diseases, Vol.9, No.11, pp. 1455-1457 ISSN 1080-6040

Chan Moon D,; Yong Seol S, Gurung M, Sook Jin J, Hee Choi C, Kim J, Chul Lee Y, Taek Cho D. & Chul Lee JC. (2010). Emergence of a new mutation and its accumulation in the topoisomerase IV gene confers high levels of resistance to fluoroquinolones in *Escherichia coli* isolates. International Journal of Antimicrobial Agents, Vol.35, pp. 76-79, ISSN 0924-8579

Chen JY,; Siu LK, Chen YH, Lu PL, HoM. & Peng CF. (2001). Molecular epidemiology and mutations at *gyrA* and *parC* genes of ciprofloxacin resistant *Escherichia coli* isolates from a Taiwan medical centre. Microbial Drug Resistance, Vol.7, No.1, pp. 47-53, ISSN 1076-6294

Chiu CH,; Wu TL, Su LH, Liu JW. & Chu C. (2004). Fluoroquinolone resistance in *Salmonella enterica* serotype Choleraesuis Taiwan, 2000-2003. Emerging Infectious Diseases, Vol.10, No.9, pp. 1674-1676, ISSN 1080-6040

Chiu CH.; Su LH, Chu C, Chia JH, Wu TL, Lin TY, Lee YS. & Ou JT. (2004). Isolation of *Salmoenella eneterica* serotype Choleraesuis resistant to ceftriaxone and ciprofloxacin. Lancet, Vol.363 (April 17), Issue 9417, pp. 1285-1286, ISSN 0140-6736

Choi SH,; Woo JH, Lee JE, Park SJ, Choo EJ, Kwak YG, Kim MN, Choi MS, Lee NY, Lee BK, Kim NJ, Jeong JY, Ryu J. & Kimm YS. (2005). Increasing incidence of quinolone resistance in human non-typhoid *Salmonella enterica* isolates in Korea and mechanisms involved in quinolone resistance. Journal of Antimicrobial Chemotherapy, Vol.56, pp. 1111-1114, ISSN 0305-7453

Cloeckaert A. & Chaslus-Dancla E. (2001). Mechanisms of quinolone resistance in *Salmonella*. Veterinary Record, Vol. 32, pp. 291-300, ISSN 0042-4900

Conrad S, Oethinger M, Kaifel K, Klotz G, Marre R. & Kern WV. (1996). *gyrA* mutations in high-level fluoroquinolone-resistant clinical isolates of *Escherichia coli*. Journal of Antimicrobial Chemotherapy, Vol.38, pp. 443-455, ISSN 0305-7453

Cui S, Li J, Sun Z, Hu C, Jin S, Guo Y, Ran L. & Ma Y. (2008). Ciprofloxacin-resistant Salmonella enterica serotype Typhimuirum, China. Emerging Infectious Diseases, Vol.14, No.3, pp. 493-495, ISSN 1080-6040

del Mar Tavio M,; Vila J, Ruiz J, Martin-Sánchez AM. & Jiménez de Anta MT. (1999). Mechanisms involved in the development of resistance to fluoroquinolones in *Escherichia coli* isolates. Journal of Antimicrobial Chemotherapy, Vol.44, pp. 735-742, ISSN 0305-7453

Delsol A.; Woodward MJ. & Roe JM (2004). Effect of 5 day enrofloxacin treatment on *Salmonella enterica* serotype Typhimurium DT104 in the pig. Journal of Antimicrobial Chemotherapy, Vol.53, pp. 396-398 ISSN 0305-7453

Delsol AA, Sunderland J, Woodward MJ, Pumbwe L, Piddock LJV. & Roe JM. (2004). Emergence of fluoroquinolone resistance in the native *Campylobacter coli* population of pigs exposed to enrofloxacin. Journal of Antimicrobial Chemotherapy, Vol.53, pp. 872-874, ISSN 0305-7453

Dimitrov T, Dashti AA, Albaksami O, Udo EE, Jadaon MM. & Albert MJ. (2009). Ciprofloxacin-resistant *Salmonella enterica* serovar Typhi from Kuwait with novel mutations in *gyA* and *parC* genes. Journal of Clinical Microbiology, Vol.47, No.1, pp 208-211, ISSN 0095-1137

Drlica K. & Zhao X. (1997). DNA gyrase, topoisomerase IV and the 4-Quinolones. Microbiology and Molecular Biology Reviews, Vol.61, No.3, pp. 377-392, ISSN 1092-2172

Eaves DJ.; Randall L., Gray DT, Buckley A., Woodward MJ., White AP. & Piddock LJV. (2004). Prevalence of mutations within the quinolone resistance-determining region of *gyrA*, *gyrB*, *parC* and *parE* and association with antibiotic resistance in quinolone-resistant *Salmonella enterica*. Antimicrobial Agents and Chemotherapy, Vol.48, No.10, pp. 4012-4015, ISSN 0066-4804

Ekkapobyotin C,; Padungtod P. & Chuanchuen R. (2008). Antimicrobial resistance of *Campylobacter coli* isolates from swine. International Journal of Food Microbiology, Vol.128, pp. 325-328, ISSN 0168-1605

Escribano I,; Rodríguez JC. & Royo G. (2004). Mutations in the *gyrA* in *Salmonella enterica* clinical isolates with decreased ciprofloxacin susceptibility. International Journal of Antimicrobial Agents, Vol.24, pp. 102-105, ISSN 0924-8579

Everett MJ, Jin YF, Ricci V. & Piddock LJV. (1996). Contributions of individual mechanisms to fluoroquionolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. Antimicrobial Agents and Chemotherapy, Vol.40, No.10, pp. 2380-2386, ISSN 0066-4804

Gales AC, Gordon KA, Wilke WW, Pfaller MA. & Jones RN. (2000). Occurrence of single point *gyrA* mutations among ciprofloxacin-susceptible *Escherichia coli* isolates causing urinary tract infections in Latin America. Diagnostic Microbiology and Infectious Disease, Vol.36, pp. 61-64, ISSN 0732-8893

Gallay A.; Prouzet-Mauléon V., Kempf I., Lehours P., Labadi L., Camou C., Denis M., de Valk H., Desenclos JC. & Mégraud F. (2007). *Campylobacter* antimicrobial drug

resistance among humans, broiler chickens, and pigs, France. Emerging Infectious Diseases, Vol.13, No.2, pp .259-266, ISSN 1080-6040

Garau J.; Xercavins M., Rodríiguez-Caraballeira M., Ramón Gómez-Vera J., Coll I., Vidal D., Llovet T. & Ruíz-Bremón A.(1999). Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrobial Agents and Chemotherapy, Vol.43, No.11, pp. 736-2741, ISSN 0066-1137

Gaunt PN. & Piddock LJV. (1996). Ciprofloxacin resistant *Campylobacter* spp. in humans: an epidemiological and laboratory study. Journal of Antimicrobial Chemotherapy, Vol.37, pp. 747-757, ISSN 0305-7453

Gensberg K,; Jin YF. & Piddock LJV (1995). A novel *gyrB* mutation in a fluoroquinolone-resistant clinical isolate of *Salmonella* typhimurium. FEMS Microbiology Letters, Vol.132, pp. 57-60, ISSN 0378-1097

Gibreel A,; Sjögren E, Kaijser B, Wretlind B. & Sköld O. (1998). Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated with mutational change in *gyrA* and *parC*. Antimicrobial Agents and Chemotherapy, Vol.42, No.12, pp. 3276-3278, ISSN 0066-4804

Giraud E,; Cloeckaert A, Baucheron S, Mouline C. & Chaslus-Dancla E. (2003). Fitness cost of fluoroquinolone resistance in *Salmonella enterica* serovar Typhimurium. Journal of Medical Microbiology, Vol.52, pp 697-703, ISSN 0022-2615

Giraud E.; Baucheron S. & Cloeckaert A.(2006). Resistance to fluoroquinolones in Salmonella: emerging mechanisms and resistance prevention strategies. Microbes and Infection, Vol.8, pp. 1937-1944, ISSN 1286-4579

Giraud E.; Brisabois A., Martel JL. & Chaslus-Dancla E. (1999). Comparative studies of mutations in animal isolates and experimental in vitro and in vivo-selected mutants of *Salmonella* spp., suggest a counterselection of highly fluoroquinolone-resistant strains in the field. Antimicrobial Agents and Chemotherapy, Vol. 43, No.9, pp. 2131-2137, ISSN 0066-4804

Grrigs DJ; Johnson MM, Frost JA, Humphrey T, Jørgensen F. & Piddock LJV. (2005). Incidence and mechanism of ciprofloxacin resistance in *Campylobacter* spp., isolated from commercial poultry flocks in the United Kingdom before, during and after fluoroquinolone treatment. Antimicrobial Agents and Chemotherapy, Vol.49, No.2, pp. 699-707, ISSN 0066-4804

Guerra B.; Malorny B., Schroeter A. & Helmuth R. (2003). Multiple resistance mechanisms in fluoroquinolone-resistant *Salmonella* isolates from Germany. Antimicrobial Agents and Chemotherapy, Vol.47, No.6, pp. 2059, ISSN 0066-4804

Gupta A,; Nelson JM, Barrett TJ, Tauxe RV, Rossiter SP, Frideman CR, Joyce KW, Smith KE, Jones TF, Hawkins MA, Shiferaw B, Beebe JL, Vugia DJ, Rabatsky-Ehr T, Benson JA, Root TP. & Angulo FJ. (2004). Antimicrobial resistance among *Campylobacter* strains, United States, 1997-2001. Emerging Infectious Diseases, Vol.10, No.6, pp. 1102-1109, ISSN 1080-6040

Hakanen A,; Jalava J, Kotilainen P, Jousimies-Somer H, Siitonen A. & Huovinen P. (2002). *gyrA* polymorphism in *Campylobacter jejuni*: Detection of *gyrA* mutations in 162 C. jejuni isolates by single-strand conformation polymorphism and DNA sequencing. Antimicrobial Agents and Chemotherapy, Vol.46, No.8, pp. 2644-2647, ISSN 0066-4804

Hamidian M,; Tajbakhsh M, Tohidopour A, Rahbar M, Reza Zali M. & Walther-Rasmussen J. (2011). Detection of novel *gyrA* mutation in nalidixic acid-resistance isolates of Salmonella enterica from patients with diarrhea. International Journal of Antimicrobial Agents, vol.37, pp. 360-364, ISSN 0924-8579

Hassing RJ,; Menezes GA, van Pelt W, Petit PL, van Genderen PJ. & Goessens WHF. (2011). Analysis of mechanisms involved in reduced susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi and Paratyphi A isolates from travellers to Southeast Asia. International Journal of Antimicrobial Agents, Vol.37, pp. 240-243, ISSN 0924-8579

Heisig P, Schedletzky H. & Falkenstein-Paul H. (1993). Mutations in the *gyrA* gene of a highly fluoroquinolone-resistant clinical isolate of *Escherichia coli*. Antimicrobial Agents and Chemotherapy, Vol 37, No.4, pp. 696-701, ISSN 0066-4804

Heisig P,; (1996). Genetic evidence for a role of *parC* mutations in development of high-level fluoroquinolone resistance in *Eschericihia coli*. Antimicrobial Agents and Chemotherapy, Vol.40, No.4, pp. 879-885, ISSN 0066-4804

Heisig P. & Tschorny R. (1994). Characterization of fluoroquinolone-resistant mutants of *Escherichia coli* selected in vitro. Antimicrobial Agents and Chemotherapy, Vol.38, No.6, pp. 1284-1291, ISSN 0066-4804

Heisig P.; Kratz B, Halle E, Gräser Y, Altwegg M, Rabsch W. & Faber JP (1995). Identification of DNA Gyrase A mutations in ciprofloxacin-resistant isolates of *Salmonella typhimurium* from men and cattle in Germany. Microbial Drug Resistance, Vol.1, No3, pp. 211-218, ISSN 1076-6254

Hopkins KL.; Davies RH. & Threlfall EJ.(2005). Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: Recent developments. International Journal of Antimicrobial Agents, Vol.25, pp. 358-373, ISSN 0924-8579

Hsueh PR,; Teng LJ, Tseng SP, Chang CF, Wan JH, Yan JJ, Lee CM, Chuang YC, Huang WK, Yang D, Shyr JM, Yu KW, Wang LS, Lu JJ, Ko WC, Wu JJ, Chang FY, Yang YC, Lau YJ, Liu YC, Liu CY, Ho SW. & Luh KT (2004). Ciprofloxacin-resistant *Salmonella enterica* typhimurium and Choleraesuis from pigs to humans, Taiwan. Emerging Infectious Diseases, Vol.10, No.1, pp. 60-68, ISSN 1080-6040 http://www.eurosurveillance.org/ViewArticle.aspx?Articled=398

Huang TM,; Chang YF. & Chang CF. (2004). Detection of mutations in the *gyrA* gene and class I integron from quinolone-resistant *Salmonella enterica* serovar Choleresuis isolated in Taiwan. Veterinary Microbiology, Vol.100, pp. 247-254, ISSN 0378-1135

Izumiya H,; Mori K, Kurazono T, Yamaguchi M, Higashide M, Konishi N, Kai A, Morita K, Terajima J. & Watanabe H. (2005). Characterization of isolates of *Salmonella enterica* serovar Typhimurium displaying high level fluoroquinolone resistance in Japan. Journal of Clinical Microbiology, Vol.43, No.10, pp. 5074-5079, ISSN 0095-1137

Jesse TW,; Englen MD, Pittenger-Alley LG. & Fedorka-Cray PJ. (2006). Two distinct mutations in *gyrA* to ciprofloxacin and nalidixic acid resistance in *Campylobacter coli* and *Campylobacter jejuni* isolated from chickens and beef cattle. Journal of Applied Microbiology, Vol.100, pp. 682-688, ISSN 1364-5072

Jiménez Gómez PA,; García de los Ríos JE, Mendoza Rojas A, de Pedro Ramonet P, Garcia Albiach R. & Reche Sainz MP. (2004). Molecular basis of quinolone resistance in *Escherichia coli* from wild birds. The Canadian Journal of Veterinary Research, Vol.68, pp. 229-231, ISSN 0830-9000

Johnson JR.; Kuskowski MA., Menard M., Gajewski A., Xercavins M. & Garau J.(2006). Similarity between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. The Journal of Infectious Diseases, Vol.194, pp. 71-78, ISSN 0022-1899

Keller J. & Perreten V. (2006). Genetic diversity in fluoroquinolone and macrolide-resistant *Campylobacter coli* from pigs. Veterinary Microbiology, Vol.113, pp. 103-108, ISSN 0378-1135

Khan AA.; Nawaz M.S., West S, Khan SA. & Lin J. (2005). Isolation and molecular characterization of fluoroquinolone-resistant *Escherichia coli* from poultry litter. Poultry Science , Vol. 84, pp. 61-66, ISSN 0032-5791

Kilmartin D.; Morris D., O'Hare C., Corbett-Feeney G. & Cormican M. (2005). Clonal expansion may account for high levels of quinolone resistance in *Salmonella enterica* serovar Enteritidis. Applied and Environmental Microbiology, Vol.71, No.5, pp. 2587-2591, ISSN 0099-2240

Kim KY,; Park JH, Kwak HS. & Woo GJ. (2011). Characterization of the quinolone resistance mechanism in foodborne *Salmonella* isolates with high nalidixic acid resistance. International Journal of Food Microbiology, Vol.146, pp. 52-56, ISSN 0168-1605

Kinana AD,; Cardinale E, Bahsoun I, Tall F, Sire JM, Breurec S, Garin B, Saad-Bouh Boye C. & Perrier-Gros-Claude JD. (2007a). *Campylobacter coli* isolates derived from chickens in Senegal: Diversity, genetic exchange with *Campylobacter jejuni* and quinolone resistance. Research in Microbiology, Vol.158, pp. 138-142, ISSN 0923-2508

Kinana AD,; Cardinale E, Bahsoun I, Tall F, Sire JM, Garin B, Saad-Bouh Boye C, Dromigny JA. & Perrier-Gros-Claude JD. (2007b). Analysis of topoisomerase mutations in fluoroquinolone-resistant and susceptible *Campylobacter jejuni* strains isolated in Senegal. International Journal of Antimicrobial Agents, Vol.29, pp.397-401, ISSN 0924-8579

Kinana AD,; Cardinale E, Tall F, Bahsoun I, Sire JM, Garin B, Breurec S, Saad-Bouh Boye C. & Perrier-Gros-Claude JD. (2006). Genetic diversity and quinolone resistance in *Campylobacter jejuni* isolates from poultry in Senegal. Applied and Environmental Microbiology, Vol72, No.5, pp. 3309-3313, ISSN 0099-2240

Ko WC,; Yan JJ, Yu WL, Lee HC, Lee NY, Wang LR. & Chuang YC. (2005). A new therapeutic challenge for old pathogens: Community-acquired invasive infections caused by ceftriaxone and ciprofloxacin resistant *Salmonella enterica* serotype Choleraesuis. Clinical Infectious Diseases, Vol.40, pp. 315-8, ISSN 1058-4838

Korezak BM,; Zurfluh M, Emler S, Kuhn-Oertli J. & Kuhnert P. (2009). Multiplex strategy for multilocus sequence typing *fla* typing and genetic determination of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates collected in Switzerland. Journal of Clinical Microbiology, Vol.47, No.7, pp. 1996-2007, ISSN 0095-1137

Kozoderović G., Jelesić Z. & Velhner M.: Resistance to quinolones among *Salmonella* Enteritidis isolates in Vojvodina. Microbiologia Balkanica 2009, Book of abstracts and Programme, 6th Balkan Congress of Microbiology, Ohrid, 28-31. Oktober, Macedonia, Skopje, Macedonian Medical Association, 2009, p. 120, ISSN 0025-1097

Kozoderović G.; Velhner M., Jelesić Z., Stojanov I., Petrović T., Stojanović D. & Golić N. (2011). Molecular typing and antimicrobial resistance of *Salmonella* Enteritidis

isolated from poultry, food and humans in Serbia. Folia Microbiologica, Vol. 56, pp. 66-71, ISSN 0015-5632

Kristiansen MAM,; Sandvang D. & Rasmussen TB. (2003). In vivo development of quinolone resistance in *Salmonella enterica* serotype Typhimuirum DT104. Journal of Clinical Microbiology, Vol.41, No.9, pp. 4462-4464, ISSN 0095-1137

Kumagai Y,; Kato JI, Hoshino K, Akasaka T, Sato K. & Ikeda H. (1996). Quinolone-resistant mutants of *Escherichia coli* DNA topoisomerase IV *parC* gene. Antimicrobial Agents and Chemothreapy, Vol.40, No.3, pp. 710-714, ISSN 0066-1137

Laatu M,; Rautelin H. & Hänninen ML. (2005). Susceptibility of *Campylobacter hyointestinalis* subsp. *hyointestinalis* to antimicrobial agents and characterization of quionolone-resistant strains. Journal of Antimicrobial Chemotherapy, Vol.55, pp. 182-187, ISSN 0305-7453

Lautenbach E,; Fishman NO, Metlay JP, Mao X., Bilker WB, Tolomeo P. & Nachamkin I. (2006). Phenotypic and genotypic characterization of fecal *Escherichia coli* isolates with decreased susceptibility to fluoroquinolones: Results from a large hospital-based surveillance initiative. The Journal of Infectious Diseases, Vol.194, pp. 79-85, ISSN 0022-1899

Lee K,; Lee M, Lim J, Jung J, Park Y. & Lee Y. (2008). Contamination of chicken meat with *Salmonella enterica* serovar Haardt with nalidixic acid resistance and reduced fluoroquinolone susceptibility. Journal of Microbiology Biotechnology, Vol.18, No.11, pp. 1853.-1857, ISSN 1017-7825

Lee YJ,; Kim KS, Kim JH. & Tak RB. (2004). *Salmonella gallinarum gyrA* mutations associated with fluoroquinolone resistance. Avian Pathology, Vol.33, No.2, pp 251-257, ISSN 0307-9457

Lee YJ.; Cho JK., Kim KS., Tak RB., Kim AR., Kim JW., Im SK. & Kim BH.(2005). Fluoroquinolone resistance and *gyrA* and *parC* mutations of *Escherichia coli* isolated from chickens. The Journal of Microbiology, Vol.43, No.5, pp. 391-397, ISSN 1225-8873

Lehn N,; Stöwer-Hoffmann J, Kott T, Strassner C, Wagner H, Krönke M. & Schneider-Brachert W. (1996). Characterization of clinical isolates of *Escherichia coli* showing high levels of fluoroquinolone resistance. Journal of Clinical Microbiology, Vol.34, No.3, pp. 597-602, ISSN 0095-1137

Levy DD.; Sharma B. & Cebula TA.(2004). Single-nucleotide polymorphism mutation spectra and resistance to quinolones in Salmonella enterica Serovar Enteritidis with a mutator phenotype. Antimicrobial Agents and Chemotherapy, Vol.48, No.7, pp. 2355-2363, ISSN 0066-4804

Liebana E.; Clouting C, Cassar CA, Randall LP, Walker RA, Threlfall EJ, Clifton-Hadley FA, Ridley AM. & Davies RH. (2002). Comparison of *gyrA* mutations, cyclohexane resistance, and the presence of Class I integrons in *Salmonella enterica* from farm animals in England and Wales. Journal of Clinical Microbiology, Vol.40, No.4, pp. 1481-1486, ISSN 0095-1137

Lindstedt BA,; Aas L. & Kapperud G. (2004). Geographically dependent distribution of *gyrA* gene mutations at codons 83 and 87 in *Salmonella* Hadar, and a novel codon 81 Gly to His mutation in *Salmonella* Enteritidis. APMIS, Vol.112, No. 3, pp. 165-71, ISSN 0963-4641

Ling JM.; Chan EW., Lam AW. & Cheng AF. (2003). Mutations in topoisomerase genes of fluoroquinolone-resistant Salmonellae in Hong Kong. Antimicrobial Agents and Chemotherapy, Vol.47, No.11, pp. 3567-3573, ISSN 0066-4804

Luo N,; Pereira S, Sahin O, Lin J, Huang S, Michel L. & Zhang Q. (2005). Enhanced *in vivo* fitness of floroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. PNAS, Vol.102, No.3, pp. 541-546, ISSN 0027-8424

Luo N,; Sahin O, Lin J, Michel LO. & Zhang Q. (2003). In vivo selection of *Campylobacter* isolates with high levels of fluoroquinolne resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. Antimicrobial Agents and Chemotherapy, Vol47, No.1, pp. 390-394, ISSN 0066-4804

Marcusson LL,; Frimodt-Moller N. & Hughes D. (2009). Interplay in the selection of fluoroquinolone resistance and bacterial fitness. PLOSPathogens, Vol.5, No.8, pp. 1-7, ISSN 1553-7366

Marimon JM, Gomariz M, Zigorraga, Cilla G. & Pérez-Trallero E. (2004). Increasing prevalence of quinolone resistance in human nontyphoid *Salmonella enterica* isolates obtained in Spain from 1981 to 2003. Antimicrobial Agents and Chemotherapy, Vol.48, No.10, pp. 3789-3793, ISSN 0066-4804

Mayrhofer S.; Paulsen P., Smulders FJM. & Hilbert F. (2004). Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. International Journal of Food Microbiology, Vol.97, pp. 23-29, ISSN 0168-1605

Mc Donald CL,; Chen FJ, Lo HJ, Yin HC, Lu PL, Huang CH, Chen P, Lauderdale TL & Ho M. (2001). Emergence of reduced susceptibility and resistance to fluoroquinolones in *Escherichia coli* in Taiwan and contributions of distinct selective pressures. Antimicrobial Agents and Chemotherapy, Vol.45, No.11, pp. 3084-3091, ISSN 0066-4804

McIver CJ,; Hogan TR, White PA. & Tapsall JW. (2004). Patterns of quinolone susceptibility in *Campylobacter jejuni* associated with different *gyrA* mutations. Pathology, Vol.36, No.2, pp. 166-169, ISSN 0031-3025

Meier PA,; Dooley DP, Jorgensen JH, Sanders CC, Huang WM. & Patterson JE. (1998). Development of quinolone-resistant *Campylobacter fetus* bacteriemia in human immunodeficiency virus/infected patients. Tha Journal of Infectious Diseases, Vol.177, pp. 951-954, ISSN 0022-1899

Mooij MJ, Schouten I, Vos G, van Belkum A, Vandenbroucke-Grauls CMJE, Savelkoul PHM. & Schultsz C. (2005). Class 1 integrons in ciprofloxacin-resistant *Escherichia coli* strains from two Dutch hospitals. Clinical Microbiology and Infection, Vol.11, pp. 898-902, ISSN 1198-743X

Moore JE,; Crowe M, Heaney N. & Crothers E. (2001). Antibiotic resistance in *Campylobacter* spp. isolated from human faeces (1980-2000) and foods (1887-2000) in Northern Ireland: an update. Journal of Antimicrobial Chemotherapy, Vol.48, pp. 455-457, ISSN 0305-7453

Morgan-Linnell SK. & Zechiedrich L. (2007). Contributions of the combined effect of topoisomerase mutations toward fluoroquinolone resistance in *Escherichia coli*. Antimicrobial Agents and Chemotherapy, Vol.51, No.11, pp. 4205-4208, ISSN 0066-4804

Nakaya H.; Yasuhara A, Yoshimura K, Oshihoi Y, Izumiya H. & Watanabe H (2003). Life threatening infantile diarrhea from fluoroquinolone-resistant *Salmonella enterica*

Typhimurium with mutations in both *gyrA* and *parC*. Emerging Infectious Diseases, Vol.9, No.2, pp. 255-257, ISSN 1080-6040

Namboodiri SS,; Opintan JA, Lijek RS, Newman MJ. & Okeke IN. (2011). Quinolone resistance in *Escherichia coli* from Accra, Ghana. BMC Microbiology, Vol.11:44, ISSN 1471-2180

Nógrády N.; Tóth Á, Kostyák Á, Pászti J. & Nagy B. (2007). Emergence of multidrug-resistant clones of *Salmonella* infantis in broiler chickens and humans in Hungary. Journal of Antimicrobial Chemotherapy, Vol.60, pp. 645-648, ISSN 0305-7453

Olsen SJ.; De Bess EE, McGivern TE, Marano N, Eby T, Mauvais S., Balan VK, Zirnstein G., Cieslak PR. & Angulo FJ (2001). A nosocomial outbreak of fluoroquinolone-resistant salmonella infection. New England Journal of Medicine, Vol.344, No.21, (May 24), pp. 1572-1578, ISSN 0028-4793

Ouabdesselam S,; Tankovic J. & Soussy CJ, (1996). Quinolone resistance mutations in the *gyrA* gene in clinical isolates of Salmonella. Microbial Drug Resistance, Vol. 2, No.3, pp. 299-302, ISSN 1076-6254

Overdevest I,; Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X. & Kluytmans J. (2011). Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerging Infectious Diseases, Vol. 17, No. 7 pp. 1216-1222, ISSN 1080-6040

Payot S.; Bolla JM., Corcoran D., Fanning S., Mégraud F. & Zhang Q.(2006). Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* spp. Microbes and Infection, Vol.8, pp. 1967-1971, ISSN 1286-4579

Piddock LJV (1996). Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy. Journal of Antimicrobial Chemotherapy, Vol. 38, pp. 1-3, ISSN 0924-8579

Piddock LJV, Ricci V, Pumbwe L, Everett MJ. & Griggs DJ. (2003). Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. Journal of Antimicrobial Chemotherapy, Vol.51, pp. 19-26, ISSN 0305-7453

Piddock LJV.; Ricci V., McLaren I. & Griggs DJ. (1998). Role of mutation in the *gyrA* and *parC* genes of nalidixic-acid-resistant salmonella serotypes isolated from animals in the United Kingdom. Journal of Antimicrobial Chemotherapy, Vol.41, pp. 635-641, ISSN 0305-7453

Randall LP.; Eaves DJ, Cooles SW, Ricci V, Buckley A, Woodward MJ. & Piddock LJV (2005). Fluoroquinolone treatment of experimental *Salmonella enterica* serovar Typhimuirum DT104 infections in chickens select for both *gyrA* mutations and changes in efflux pump gene expression. Journal of Antimicrobial Chemotherapy, Vol.56, pp. 297-306, ISSN 0305-7453

Reyna F,; Huesca M, González V. & Fuchs LY. (1995). *Salmonella typhimurium gyrA* mutations associated with fluoroquinolone resistance. Antimicrobial Agents and Chemotherapy, Vol.39, No.7, pp. 1621-1623, ISSN 0066-4804

Sáenz Y,; Zarazaga Z, Briñas L, Ruiz-Larrea F. & Torres C. (2001). Mutations in *gyrA* and *parC* genes in nalidixic acid-resistant *Escherichia coli* strains from food products,

humans and animals. Journal of Antimicrobial Chemotherapy, Vol51, pp. 1001-1005, ISSN 0305-7453

San Martin B,.; Lapierre L., Toro C., Bravo V., Cornejo J., Hormazabal JC. & Borie C. (2005). Isolation and molecular characterization of quinolone resistant *Salmonella* spp from poultry farms. Veterinary Microbiology, Vol.110, pp. 239-244, ISSN 0378-1135

Smith KE,; Besser JM, Hedberg CW, Leano FT, Bender JF, Wicklund JH, Johnson BP, Moore KA. & Osterholm MT.(1999). Quinolone-resistant Campylobacter jejuni infections in Minnesota, 1992-1998. New England Journal of Medicine, Vol.340,m (May 20), pp.1525-1532, ISSN 0028-4793

Solnik-Isaac H.; Weinberger M, Tabak M, Ben-David A, Shachar D. & Yaron S. (2007). Quinolone resistance of *Salmonella enterica* serovar Virchow isolates from human and poultry in Israel: Evidence for clonal expansion. Journal of Clinical Microbiology, Vol. 45, No.8, pp. 2575-2579, ISSN 0095-1137

Sonnevend A,; Rotimi VO, Kolodziejek J, Usmani A, Nowotny N. & Pál T. (2006). High level of ciprofloxacin resistance and its molecular background among *Campylobacter jejuni* strains isolated in the United Arab Emirates. Journal of Medical Microbiology, Vol.55, pp. 1533-1538, ISSN 0022-2615

Stevenson JE, Gay K, Barrett TJ, Medalla F., Chiller TM. & Angulo FJ. (2007). Increase in nalidixic acid resistance among non-typhi *Salmonella enterica* isolates in the United States from 1996 to 2003. Antimicrobial Agents and Chemotherapy, Vol.51, No.1, pp. 195-197, ISSN 0066-4809

Stojanov IM,; Velhner M. & Orlić DB. (2004). Presence of Campylobacter spp. in nature. Zbornik Matice Srpske, No.107, pp. 75-83, ISSN 0352-4906

Taylor DE. & Chau ASS. (1997). Cloning and nucleotide sequence of the *gyrA* gene from *Campylobacter fetus* subsp fetus ATCC 27374 and characterization of ciprofloxacin-resistant laboratory and clinical isolates. Antimicrobial Agents and Chemotherapy, Vol.41, No.3, pp. 665-671, ISSN 0066-4804

Thakur S. & Gebreyes WA. (2005). *Campylobacter coli* in swine production: Antimicrobial resistance mechanisms and molecular epidemiology. Journal of Clinical Microbiology, Vol.43, No.11, pp. 5705-5714, ISSN 0095-1137

Uchida Y,; Mochimaru T, Morokuma Y, Kiyosuke M, Fujise M, Eto F, Harada Y, Kadowaki M, Shimono N. & Kang D. (2010). Geographic distribution of fluoroquinolone-resistant *Escherichia coli*. International Journal of Antimicrobial Agents, Vol. 35, pp. 387-391, ISSN 0924-8579

van den Bogaard AE,; London N, Driessen C. & Stobberingh EE. (2001). Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy, Vol.47, pp. 763-771 ISSN 0305-7453

van Pelt W.; van der Zee H, Wannet WJB, van de Giessen AW, Mevius DJ, Bolder NM, Komijn RE. & van Duynhoven YTHP (2003). Explosive increase of Salmonella Java in poultry in the Netherlands: Consequences for public health. Eurosurveillance, Vol.8, No.2, pii=398. Available online:

Velhner M.; Petrović J, Stojanov I., Ratajac R. & Stojanović D. (2011). Mehanizmi prenošenja rezistencije kod bakterija, Arhiv Veterinarske Medicine, Vol.3, pp. -92, ISSN 1820-9955.

Velhner M.; Stojanov I, Potkonjak D, Kapetanov M., Orlić D. & Rašić Z. (2005). Salmonella Enteritidis isolation from broiler chickens infected with low doses. Acta Veterinaria, Vol. 55, pp. 183-191, ISSN 0567-8315.

Velhner Maja,; Kozoderović Gordana, Jelesić Zora, Stojanov I, Ratajac R. & Stojanović Dragica. (2010). Rezistencija bakterija na hinolone i njen uticaj na zdravlje ljudi i životinja. Veterinarski glasnik, Vol.64, No.3-4, pp. 277-285, ISSN 0350-2457

Vila J, Ruiz J, Goñi P. & Jimeneze de Anta MT.(1996). Detection of mutations in *parC* in quinolone-resistant clinical isolates of *Escherichia coli*. Antimicrobial Agents and Chemotherapy, Vol 40, No.2, pp. 491-493, ISSN 0066-4804

Vila J, Ruiz J, Marco F, Barcelo A, Goñi P, Giralt E. & Jimeneze de Anta T. (1994). Association between double mutation in *gyrA* gene of ciprofloxacin-resistant clinical isolates of *Eschericihia coli* and MICs. Antimicrobial Agents and Chemotherapy, Vol. 38, No10, pp. 2477-2479, ISSN

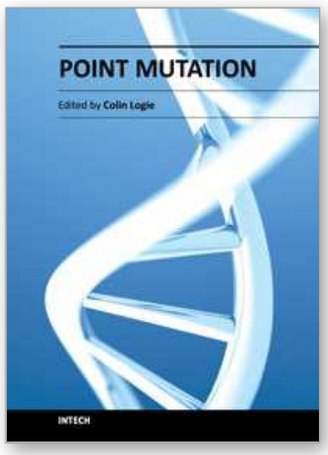
Wang Y,; Huang WM. & Taylor DE. (1993). Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrobial Agents and Chemotherapy, Vol.37, No.3, pp. 457-463, ISSN 00664804

Whichard JM, Gay K, Stevenson JE, Joyce KJ, Cooper KL, Omondi M, Medalla F, Jacoby GA. & Barrett TJ. (2007). Human *Salmonella* and concurrent decreased susceptibility to quinolones and extended-spectrum cephalosporins. Emerging Infectious Diseases, Vol.13, No.11, pp. 1681-1687, ISSN 1080-6040

Yoshida H,; Nakamura M, Bogaki M, Ito H, Kojima T, Hattori H. & Nakamura S. (1993). Mechanism of action of quinolones against *Escherichia coli* DNA gyrase. Antimicrobial Agents and Chemotherapy, Vol.37, No.4, pp. 839-845, ISSN 0066-4804

Yoshida H.; Bogaki M., Nakamura M. & Nakamura S. (1990). Quinolone resistance-determining region in the DNA Gyrase *gyrA* gene of *Escherichia coli*. Antimicrobial Agents and Chemotherapy, Vol. 34, No.6, pp. 1271-1272, ISSN 0066-4804

Zirnstein G, Helsel L, Li Y, Swaminathan B. & Besser J. (2000). Characterization of *gyrA* mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. FEMS Microbiology Letters, Vol. 190, pp. 1-7, ISSN 0378-1097



Point Mutation
Edited by Dr Colin Logie

ISBN 978-953-51-0331-8
Hard cover, 352 pages
Publisher InTech
Published online 21, March, 2012
Published in print edition March, 2012

This book concerns the signatures left behind in chromosomes by the forces that drive DNA code evolution in the form of DNA nucleotide substitutions. Since the genetic code predetermines the molecular basis of life, it could have been about any aspect of biology. As it happens, it is largely about recent adaptation of pathogens and their human host. Nine chapters are medically oriented, two are bioinformatics-oriented and one is technological, describing the state of the art in synthetic point mutagenesis. What stands out in this book is the increasing rate at which DNA data has been amassed in the course of the past decade and how knowledge in this vibrant research field is currently being translated in the medical world.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen