Chapter 2

Antimicrobial Resistance of Common Zoonotic Bacteria in the Food Chain: An Emerging Threat

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Additional information is available at the end of the chapter

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Abstract

Antimicrobial resistance in the food chain is currently a subject of a major interest. The excessive use or rather misuse of antimicrobials coupled with a poor hygiene in the food production chain has led to a rise of resistant zoonotic bacteria, commonly transmitted by food. They pose a serious threat to human health. Campylobacteriosis is the leading bacterial food-borne illness and most commonly reported zoonosis in humans in the European Union for more than a decade. Salmonellosis is most frequently diagnosed in food-borne outbreaks. Fluoroquinolones are considered as critically important for treatment of severe cases of both zoonoses in humans. Due to an extremely prevalent resistant isolates, especially from broilers and meat, also the treatment of human *Campylobacter* infections with fluoroquinolones has become compromised. *Salmonella* isolates from poultry and poultry meat tend to be highly resistant to fluoroquinolones as well. Beside the resistance to this group of antibiotics, the threat of multiple drug resistant (MDR) *Campylobacter* and *Salmonella* strains is discussed in the light of most recent reports of animal, food and human clinical surveillance systems.

Keywords: *Campylobacter*, *Salmonella*, antimicrobial resistance, food safety, food production chain, multiple drug resistance

1. Introduction

Antimicrobials are indispensable in human medicine for treating and preventing infectious diseases. In addition, the same classes of antimicrobials are extensively used in livestock not only for the treatment and prevention of infections but also for the growth promotion [1]. The latter has, however, been banned in the European Union (EU) since 2006 [2].

The amounts of antimicrobials utilised in livestock are vast and often exceed those in humans. Data suggest that in the EU approximately 70% of antimicrobials were sold for use in livestock in 2014 [3]. The consumption of antimicrobials in humans and animals has indeed been associated with the occurrence of antimicrobial resistance (AMR) in zoonotic bacteria [3], which are the causative agents of zoonoses and can be transmitted directly between animals and humans or *via* the food chain. AMR in zoonotic bacteria is a subject of major concern.

Even though AMR is an ancient and naturally occurring phenomenon in some bacteria [4], the excessive use of antimicrobials in humans and livestock, as well as poor hygiene conditions and practices in the food production chain, accelerates the emergence of resistance in zoonotic bacteria [5]. The alarming consequence of AMR coupled with the paucity of novel antimicrobials is the rise in the frequency of multidrug resistant (MDR) zoonotic bacteria that may lead to an impaired response to antimicrobial therapy or ultimately even treatment failure [6].

The most current data regarding AMR in zoonotic bacteria are published annually by European Food Safety Authority (EFSA) and European Centre for Disease Control and Prevention (ECDC), but usually with a two-year delay in publishing. According to the recent report on AMR in zoonotic bacteria in 2016 [5], resistance in *Salmonella* and *Campylobacter* is considered of the highest concern. The scope of this review is, therefore, to discuss and emphasise the current trends of AMR in *Salmonella* and *Campylobacter* in the light of the recent EFSA/ECDC report, and the role of whole genome sequencing (WGS) in the surveillance of AMR in *Salmonella* and *Campylobacter* along the food production chain.

2. Common zoonoses in Europe

For more than a decade, campylobacteriosis has been the most common zoonosis in Europe. Salmonellosis is the second most commonly reported enteric infection, and the leading cause of food-borne outbreaks. *Campylobacter* and *Salmonella* combined accounted for almost 95% of the reported and confirmed zoonoses cases in 2016 (**Figure 1**).

Salmonellosis is a food-borne gastrointestinal infection caused by zoonotic bacteria *Salmonella* spp. Several thousand serovars of *Salmonella* spp. *enterica* exist, yet only some are causing disease symptoms. Nontyphoidal serovars are transmitted *via* the food chain, whereas typhoidal serovars, the causative agents of typhoid fever, are restricted to humans [8]. Whilst the majority of nontyphoidal *Salmonella* infections are self-limiting and do not require any antibiotic treatment, some cases result in life-threatening systemic infections that must be treated with antimicrobials, primarily fluoroquinolones (FQ) or third-generation cephalosporins [5]. Resistance to these drugs may jeopardise the efficiency of the antimicrobial therapy.

Campylobacter spp. are common gut commensals of several animal species, especially birds [9], and the leading cause of gastroenteritis in humans, yet the infections often go unreported. The majority of campylobacterioses are caused by two species, namely *Campylobacter jejuni* and *Campylobacter coli*. Symptoms of campylobacteriosis are also usually mild and self-limiting, although some patients with acute infections that can trigger autoimmune inflammatory conditions need to be treated with antimicrobials, primarily macrolides and FQ [5]. Emergence of resistance in *Campylobacter* is common and thus of concern.

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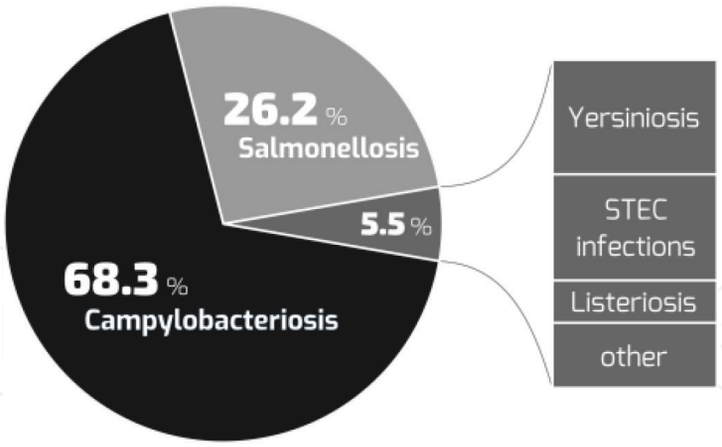

Figure 1. Common zoonoses in Europe in 2016. STEC: Shiga toxin-producing *Escherichia coli* [7].

In the Member States (MS) of the EU monitoring and reporting data on zoonoses and AMR for *Salmonella* and *Campylobacter* from animals, food, feed and humans are mandatory [10]. Comparing AMR data from different countries and assessing trends has long been challenging due to inadequate harmonisation of the methodology and reporting among the MS [11]. Recently, great progress has been made in terms of harmonisation of AMR surveillance programs, especially for food animals and foods with the new legislation [12]. In addition, a protocol for harmonised monitoring of AMR in humans has been developed [13], but it is not a legal document that would obligate the MS to its implementation.

3. *Salmonella*

3.1. Prevalence of nontyphoidal *Salmonella* in the food chain

According to the recent report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016 published by EFSA/ECDC [7], the declining trend of salmonellosis in Europe has ended. In 2016, there were 94,530 confirmed cases of salmonellosis with the highest notification rate per 100,000 population in Eastern Europe (in average 46.9 per 100,000), mostly on the account of Czech Republic (110) and Slovakia (97.7), followed by Northern (20.6), Western (18.8) and Southern Europe (13.0). Additionally, *Salmonella* was most regularly detected in food-borne outbreaks (22.3%), which have resulted in the highest burden of hospitalisations (45.6% of the total number of hospitalised cases) and deaths (50% of the total number of deaths among outbreak cases) [7]. Outbreaks were linked to several sources, e.g., Polish eggs [14], infant formula [15] and sesame seeds [16].

Salmonella was the most prevalent in meat from turkeys (7.74% of the samples tested positive) and from broilers (6.39%), as well as dried seeds (8.0%) [7], which are an important source of infections, especially due to a long shelf life and low moisture [17]. Chicken, turkey and other avian species are commonly inhabited with *Salmonella* without noticeable symptoms [18], which is in addition to the practices in the food production chain [19], considered the highest risk for contamination of meat products. Even though *Salmonella* was significantly less frequently detected in eggs and their products, they remain the most important source

of outbreaks [14, 20], most probably due to a large worldwide consumption coupled with low concentrations of *Salmonella* that cannot be detected [7]. Rapid methods with improved sensitivity are thus needed to address this shortfall, e.g., real-time recombinase polymerase amplification [21] or sequence-based methods.

3.2. Antimicrobial resistance in nontyphoidal *Salmonella* spp.

The most recent data (from 2016) on AMR in *Salmonella* from poultry, meat thereof, and humans are provided by EFSA/ECDC [5], whereas data on other livestock are presented in the last-year report [22]. Generally, as shown in **Figure 2**, the isolates of *Salmonella* spp. along the food production chain tend to be highly resistant to tetracyclines and ciprofloxacin (in average in the EU up to 65%), sulfonamides (56%) and ampicillin (45%) with the highest observed frequency in poultry, meat thereof and pigs, respectively (**Figure 2**). Resistance rates seemed to be higher in Southern or Eastern Europe than in Northern or Western Europe [5, 22]. Such extreme rates of resistance that could indeed reflect an extensive use of these three antimicrobials in livestock [23] are of concern and could be facilitating further dissemination of AMR.

Sulfonamides and ampicillin were the former first-line drugs against salmonellosis [24]. Nevertheless, ampicillin, a critically important antimicrobial [25], is used for treating community acquired pneumonia, complicated severe acute malnutrition and sepsis in neonates and children [26]. In contrast, sulfonamides and tetracyclines are classified as highly important antimicrobials [25]. Sulfonamides are the first-line drugs against urinary tract infections and tetracyclines against *Chlamydia trachomatis* and cholera [26].

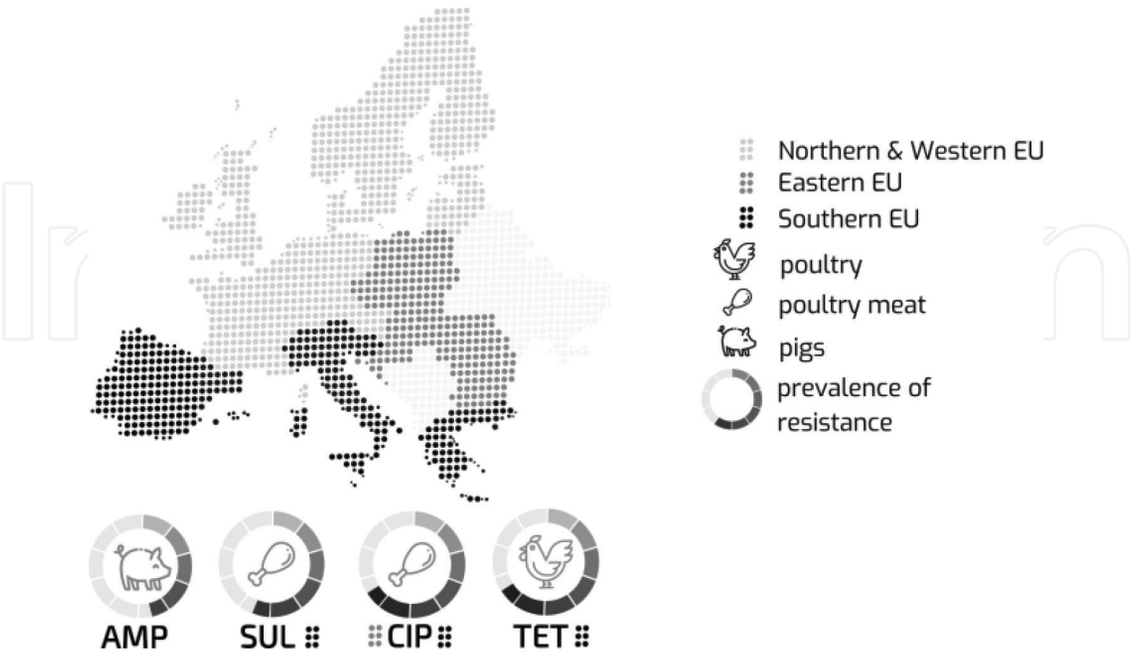

Figure 2. Prevalence of resistance in *Salmonella* spp. AMP: ampicillin, SUL: sulfonamides, CIP: ciprofloxacin, TET: tetracyclines [5, 22].

FQ resistance to ciprofloxacin or nalidixic acid that is reflecting similar genetic mechanisms [11] was remarkably high in isolates from poultry meat (**Figure 2**), followed by poultry [5] and has steeply increased since 2004 [27]. That is of concern, because FQ are in addition to third-generation cephalosporins clinically important for the treatment of salmonellosis and several other infections, yet both may be used in livestock.

3.3. FQ resistance and third-generation cephalosporins resistance in *Salmonella*

Broiler meat (64.7%), broilers (53.8%), turkey (50.5%) and turkey meat (43.7%) were the main sources of FQ resistance. In contrast, isolates from pigs, humans and laying hens had better susceptibility (**Figure 3**) [5, 22]. The ranges of FQ resistance, however, varied extremely among the countries and even regions. In Spain, for example, isolates from pigs in Catalonia (50%) [28] and humans in Extremadura (35%) [29] exhibited much higher levels of FQ resistance than reported by EFSA/ECDC for Spain (7 and 14.8%, respectively).

FQ resistance in isolates from broilers and meat thereof was in average most prevalent in Southern (62.2, 70.1%, respectively) and Eastern Europe (65.5, 71.5%, respectively) and exceeded 90% in Cyprus, Slovenia and Croatia (**Figure 4**). Of note, only a minority of the MS from Northern Europe reported these data [5]. A rapid increase in FQ resistance is evident in Europe, for instance, in isolates from broilers in Spain resistance increased from 0 [30] to 55.6% in just a few years.

Figure 3. Rates of FQ resistance in isolates of *Salmonella* spp. from various sources: broiler meat, broilers, turkeys, turkey meat, laying hens, humans and pigs, respectively [5, 22].

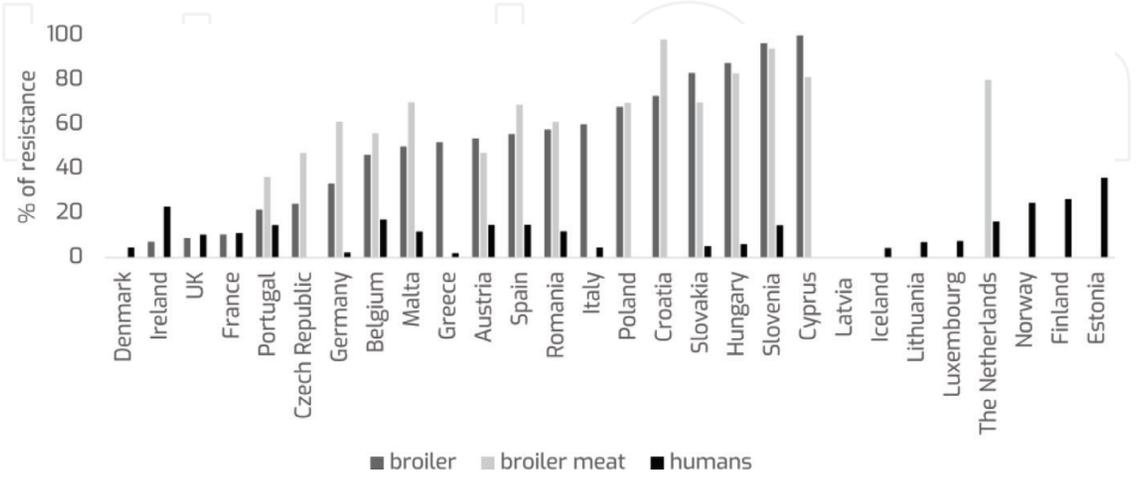

Figure 4. Prevalence of FQ-resistant isolates of *Salmonella* spp. from broilers, meat thereof and humans in Europe. UK: United Kingdom [5].

In contrast, the proportions of FQ resistance in humans were significantly lower (11%) [5] and has remained at a relatively stable level since 2009 [31]. The biggest share of FQ resistance was detected in Northern Europe, mostly on the account of Estonia (36.0%), Finland (26.3%), Norway (24.7%) and Ireland (22.9%) (**Figure 4**). Human-associated serovars commonly detected in Europe [32–35] that frequently exhibited FQ resistance were *S.* Infantis (23.4%) and *S.* Kentucky (85.8%) [5].

FQ resistance is a result of a complex mechanism, but it is still not fully understood [36]. Many point mutations in the genes encoding for gyrase and topoisomerase, the two enzymes that are inhibited by FQ, were identified as the causative agents [37]. In addition, plasmids may harbour genes for efflux pumps, target protection proteins or drug-modifying enzymes [38].

Resistance to third-generation cephalosporins was rare in humans as well as in livestock [5], yet when combined with FQ resistance, it poses a serious risk to human health, in terms of reducing the efficiency of these drugs against salmonellosis and thus leaving only the reserve antimicrobials as a feasible therapy option [24]. Resistance to cephalosporins is conferred by genes encoding for AmpC β-lactamase as well as for various extended spectrum β-lactamase (ESBL) that can be located on plasmids. Such isolates were observed in Germany [39] and are assumed to have clonally spread from livestock to humans. Worryingly, in Portugal, more than a third of the isolates from broiler meat (39.4%) exhibited resistance to cefotaxime and ceftazidime and in Italy 12% from broilers [5]. In addition, combined resistance to FQ and cephalosporins was detected in poultry and humans in Spain, Belgium and France [40, 41].

Salmonella in the food production chain presents an important reservoir of genetic resistance determinants, which could be mobilised and transferred *via* the food chain to either other human pathogens or commensal bacteria [42]. Notably, importation of meat products [43] and travelling in endemic areas [44], where the rates of resistance to critically important antimicrobials are alarmingly high [45], were linked to the global spread of MDR strains.

3.4. MDR and combined resistance to fluoroquinolones and third-generation cephalosporins

In general, 26.5% of the human isolates of *Salmonella* and 50.3% of broiler meat displayed MDR phenotype (defined as resistant to at least three antimicrobials of the nine antimicrobial classes tested). The highest prevalence of MDR isolates from humans was observed in Portugal (51%) and from broiler meat in Slovenia (100%) [5]. MDR strains isolated from pigs in Germany were associated with integrons, which might have an important role in dissemination of resistance [46].

The majority of MDR isolates belonged to serovars *S.* Infantis and *S.* Kentucky. Among human isolates of *S.* Kentucky, which is the seventh most common serovar, MDR was recorded at extremely high levels (76.3%) [5]. *S.* Kentucky ST198 clone that is displaying high-level resistance to ciprofloxacin and frequently also to amoxicillin, streptomycin, spectinomycin, gentamicin, sulfamethoxazole and tetracycline has been imported from North Africa and has been widely spread across Europe in humans and food production chain [32]. In addition, acquisition of extended-spectrum β-lactamase, plasmid-encoded cephalosporinase or carbapenemase in this clone was detected in Mediterranean area [47] and in Poland [33]. Combined resistance was also detected in *S.* Kentucky from humans and livestock in Belgium, Luxembourg, Malta, the Netherlands and Germany [5].

S. Infantis was the most prevalent serovar in broilers and the fourth among human infections. Multi-drug resistance to FQ, sulfonamides and tetracyclines was observed frequently in the isolates from broilers (75.3%), broiler meat (72.6%), as well as in isolates from humans in two MS (Austria and Slovenia) that together with Hungary and Croatia accounted for a majority of the *S.* Infantis isolates from broilers. This indicates the presence of a specific MDR clone prevalent in this geographical region [5]. In addition, resistance to cephalosporins was recorded in the isolates from either humans, food or poultry in Great Britain [48], Switzerland [34], Italy [35], as well as in the USA [49], in some cases located on a plasmid and thus conferring a risk of transfer. Such strains can be transmitted from broilers and broiler meat to humans and may lead to human infections [35].

4. *Campylobacter*

4.1. Prevalence of *Campylobacter* in the food chain

Whilst *Campylobacter,* with 246,307 confirmed infections in 2016 and 6.1% increase relative to 2015, accounted for the majority of zoonoses in Europe, the death toll was low (0.03%). The highest notification rate per 100,000 population was observed in Eastern Europe (71.4), followed by Western (65.7), Southern (56.3) and Northern Europe (55.0). Czech Republic (228.2) and Slovakia (140.5) were the countries with the highest prevalence [7].

Campylobacters were most frequently detected in turkeys (65.3%) and meat thereof (11%) as well as in broilers (27.3%) and meat thereof (36.7%) [7], making the poultry food production chain the main source of contamination. This is in concordance to data from several reports [50, 51]. The prevalence in retail poultry meat was, however, reported even up to almost 90% [50]. *Campylobacter* was also detected in cattle [52], pigs [53] and sheep [54]. Contaminated farm environment or equipment as well as the presence of *Campylobacter* in other animals and wildlife, were significantly associated with the prevalence of *Campylobacter* in poultry [55]. Furthermore, recent data suggest that human clinical *C. jejuni* isolates in Central Europe can be attributed to domesticated poultry, cattle livestock and environmental sources [56].

Outbreaks can be traced back to several sources (e.g., raw milk [57], water [58] and chicken liver pate [59]) and even associated with antimicrobial-resistant strains [57]. However, a limited number of highly contaminated products are most probably responsible for the majority of *Campylobacter* infections. Effective and harmonised surveillance systems, especially in the poultry food production chain, that are oriented towards categorising risks should thus be established [50].

4.2. Antimicrobial resistance in *Campylobacter*

Campylobacter spp. in 2016 displayed extremely high resistance levels to FQ, which is particularly worrisome, as FQ are used as the first-line drugs against campylobacteriosis. Consequently, in some EU countries, FQ therapy of campylobacteriosis is no longer feasible. In average, the highest share of resistant isolates was detected in poultry and meat thereof, especially in the Member States of Southern and Eastern Europe (**Figure 5**) [5]. Data suggest that the use of FQ in livestock, specifically pigs, selects for FQ-resistant strains and accelerates the dissemination of such strains [60].

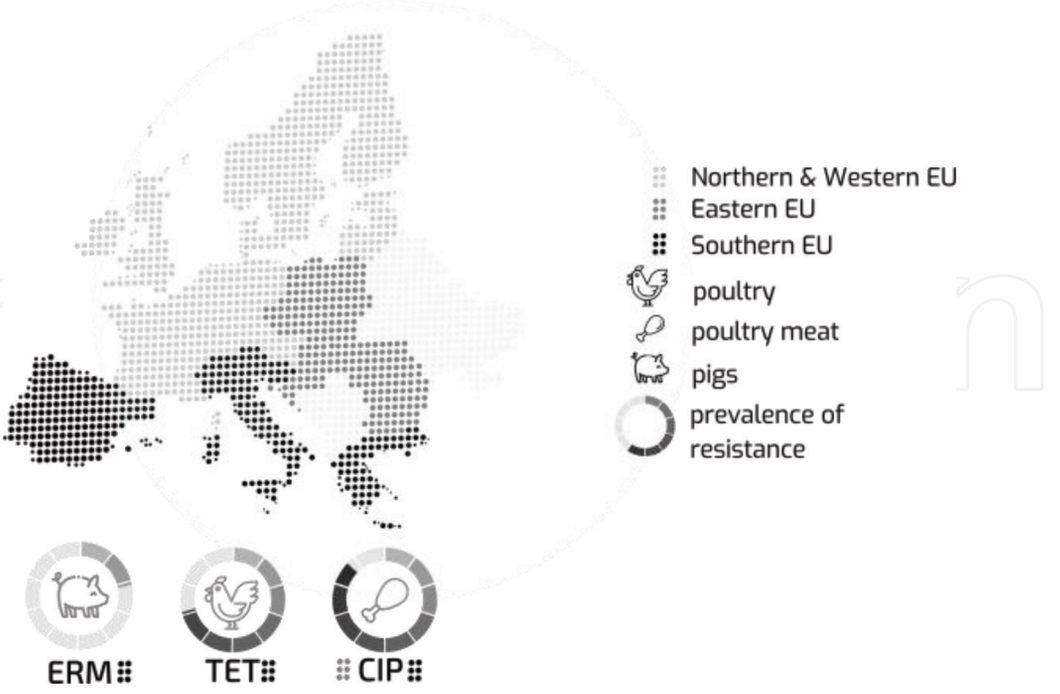

Figure 5. Prevalence of resistance in *Campylobacter* spp. ERM: erythromycin, CIP: ciprofloxacin, TET: tetracyclines [5, 22].

In general, resistance to erythromycin, the second clinically important antimicrobial for treating campylobacteriosis, is generally uncommon; however, more resistant isolates were detected in pigs (21.6% in 2015), as seen in **Figure 5** [22], which could reflect a wide use of macrolides for the treatment of common infections in pigs [61]. In contrast to *C. jejuni* from humans (2.1%), markedly higher erythromycin resistance level was observed in *C. coli* (11.0%), with the highest proportion in Estonia (63.2%) and Portugal (50%). Similar trends could be observed in isolates from livestock and food [5]. Similarly, high levels of resistance (62.4%) were recorded in *C. coli* from pigs in Spain [22] and even higher in *Campylobacter* isolates from poultry meat in Italy (72.1%) [62].

Resistance to macrolides in *Campylobacter* most commonly occurs *via* chromosomal mutations in 23S rRNA [63] that reduces the binding affinity of macrolides to the binding site. These mutations were, however, demonstrated to have a fitness cost and to slow growth rates [64]. Recently, transferrable erythromycin resistance, conferred by the rRNA methylase *erm(B)* gene and located on either plasmids or associated with chromosomal multidrug resistance genomic islands, was detected in humans and livestock [65].

Southern Europe in average recorded higher prevalence of resistance to tetracyclines, which may also be used for the treatment of campylobacteriosis in humans, and is, in addition to FQ resistance, a very common feature [5]. Marked variations in tetracycline resistance could be observed between *C. coli* and *C. jejuni,* countries and sources of isolation. Resistance rates varied from very low (<10%) in *C. coli* from pigs in Sweden [66], moderate in *Campylobacter* spp. from cattle in Poland (20.9%) [52], *C. jejuni* from broiler carcasses in Belgium (47%) [67] and *C. jejuni* from chicken meat in France (53.6%) [51] to extremely high in isolates of *C. coli* from pigs in France (93%) [66], as well as *Campylobacter* spp. from quails in Portugal (96.7%) [68]. In general, *C. coli* exhibited higher resistance levels [5].

4.3. FQ resistance and combined/MDR resistance in *Campylobacter*

FQ resistance first emerged in Southeast Asia early in the 1990s with a rapid increase from 0 to 84% over the period of 4 years [69] and has been widely spread to the other parts of the world, which might be due to an enhanced fitness of FQ-resistant isolates [70]. Significant portion of infections with FQ-resistant *Campylobacter* could be acquired through travel [71]. Extreme rates of FQ resistance in endemic areas [72] are therefore of concern.

In Europe, FQ resistance in *Campylobacter* spp. was extremely high, but it varied among the sources of isolation, species and countries. In average, isolates of *C. coli* exhibited markedly higher resistance rates than isolates of *C. jejuni*. As seen in **Figure 6**, turkey (96.8% in *C. coli* and 76.2% in *C. jejuni*) and meat thereof (100% in *C. coli*, 74.5% in *C. jejuni*) presented the main sources of resistance, closely followed by broilers and meat thereof [5].

A rapid increase in FQ resistance in *Campylobacter* is evident in the last 14 years [27]. In Slovenia, for instance, the resistance level to nalidixic acid rapidly increased in isolates from broiler meat from 49.1% in 2001–2003 [73] to 78.6% in 2006 [11]. In 2016, in average, 77.3% of *Campylobacter* spp. from broilers exhibited FQ resistance [5]. Recent data suggest the presence and clonal spread of FQ-resistant *C. jejuni* clonal complex ST-21 in central Europe (Slovenia, Germany, Austria) [74].

FQ resistance in *C. jejuni* (**Figure 7**) from broilers (in average 66.9%) varied from 8.4% in Finland to 97.9% in Latvia [5]. Furthermore, in 2014, Latvia reported on 100% resistant isolates

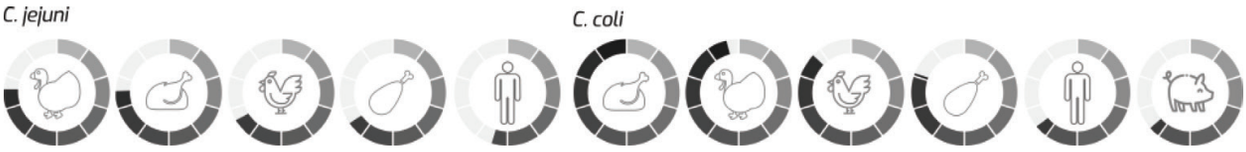

Figure 6. Sources and prevalence of FQ-resistant isolates of *C. jejuni* (turkey, turkey meat, broilers, broiler meat and human, respectively) and *C. coli* (turkey meat, turkeys, broilers, broiler meat, humans and pigs, respectively) [5, 22].

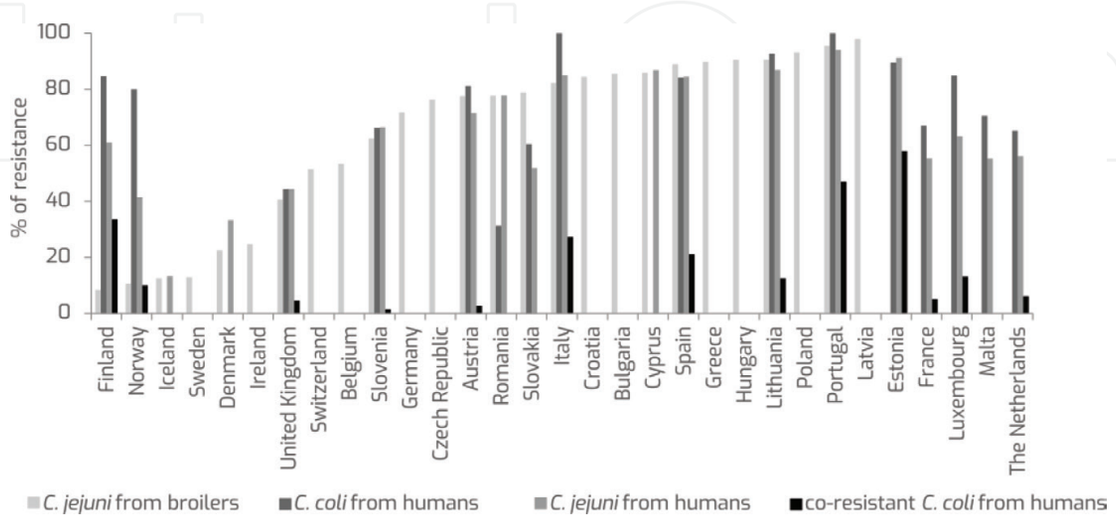

Figure 7. Rates of FQ resistance in *C. jejuni* from broilers and humans, in *C. coli* from humans and combined resistance to FQ and erythromycin in *C. coli* from humans [5].

of *Campylobacter* from chicken [75]. In humans, the highest rates of FQ resistance were reported for *C. coli* from Italy and Portugal (100%) and in for *C. jejuni* from Portugal and Estonia (>90%). Notably, 9 out of 19 EU MS recorded 80–100% resistance rates for *C. coli* (**Figure 7**) [5].

Overall, 9.2% of human *C. coli* exhibited combined resistance to ciprofloxacin, erythromycin and tetracycline with resistance rates ranging from 0 to 57.9% (Estonia), which is shown in **Figure 7** [5]. Erythromycin resistance is often associated with MDR phenotype [63]. In Finland, for example, 94.7% of *Campylobacter* isolates from humans were, in addition to erythromycin, resistant to FQ, and 73.7% to tetracycline [76]. Combined resistance to the first-line drugs may be associated with adverse events such as delayed recovery, invasive illness and prolonged treatment with feasible alternative antimicrobials [77, 78].

FQ resistance in *C. jejuni* and *C. coli* can be mediated through specific point mutations in *gyrA* gene, encoding for DNA gyrase or through chromosomally encoded multidrug efflux pump. The two mechanisms work synergistically [79]. Efflux pumps in *Campylobacter,* primarily CmeABC, are involved in resistance to broad spectrum of antimicrobials, including macrolides and quinolones [80], as well as cross-resistance to other compounds such as bile salts [81]. Therapeutic application of efflux pump inhibitors (e.g., epigallocatechin gallate) that were shown to restore macrolide efficacy could be a feasible treatment option in combination with the macrolide therapy [80, 82–84].

5. The role of whole genome sequencing in the surveillance of antimicrobial resistance of common zoonotic bacteria

The EU harmonised system on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (EC Decision 2013/652/EU; EFSA, EDSA) [12] is based on the phenotypic assessment of AMR of selected bacterial species (*Salmonella, Campylobacter, E. coli*) in selected food-producing animal species (poultry, pigs, cattle) and food products (chicken, pork, beef meat), using dilution methods (ISO standard 20776-1:2006, ISO standard 20776-2:2007) and EUCAST epidemiological cut-off values (ECOFF-values) as interpretative criteria (EC Decision 2013/652/EU) [12]. In accordance with this legislation, 170 isolates are examined for antimicrobial susceptibility to a panel of 15 antimicrobial substances, for each combination of bacterial species and type of sample of animal population or food category each year by each member state. In 2016, ECDC also published EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* isolates, aimed to increase the comparability of AMR data collected at the EU level from different Member States, and to improve the comparison of data among human isolates and isolates from animals and food products [13]. Beside dilution method as a preferred method, disk diffusion and gradient strip diffusion method are also allowed.

On the isolate level, genotyping of human isolates is also recommended, in terms of the assessment of resistance mechanisms and detection of the epidemic spread of resistance, particularly multi-drug resistant *Salmonella,* but it is not required in reporting [13].

In recent years, the development of high-throughput technologies and platforms for massive DNA sequencing, and genomics tools has opened new possibilities also in the surveillance of AMR in common zoonotic bacteria. WGS, together with appropriate databases, general (NCBI, ENA) or specialised for AMR (ARG-ANNOT, ResFinder, CARD, RED-DB, Bacmet), bioinformatic tools (BLAST) and platforms enable detection of antibiotic resistance genetic loci in the genomes of bacterial isolates or microbiomes and reveal the mechanisms leading to AMR. While WGS offers very rapid and efficient tool for detection of the antibiotic resistance genes (ARG) in genomes of individual bacterial isolates, the main issue remains how to predict from these data the actual antimicrobial susceptibility, and epidemiological or clinical cut-off values [85]. However, differentiation among isolates with acquired or intrinsic resistance on the basis of phenotypic MIC determinations only is also not totally accurate. Furthermore, it should be considered that also the strains that contain the genes associated with antimicrobial resistance but do not exhibit phenotypic resistance present certain risk for the horizontal spread when consumed.

The usefulness of WGS for antimicrobial resistance surveillance was confirmed in several studies. Examination of 640 nontyphoidal *Salmonella* isolates from retail meat and human clinical samples identified known resistance genes and phenotypic resistance to 14 antimicrobials, where the correlation between resistance genotypes and phenotypes was close to 100% for most classes of antibiotics, and lower for aminoglycosides and beta-lactams [86]**.** In addition to known ARG, several unique resistance genes were found, more in the human isolates (n = 59) than in the retail meat isolates (n = 36). The authors concluded that the use of more appropriate MIC breakpoints and inclusion of new AGSs in the databases will further improve the correlations between phenotypic and genotypic observations. For *Salmonella typhimurium* isolates (n = 50) from Danish pigs, high concordance (99.74%) between phenotypic and predicted antimicrobial susceptibility was observed as well [87]. Phenotypic resistance to quinolones and fluoroquinolones due to chromosomal mutations, however, could not be detected by ResFinder platform.

Genomic approach is increasingly used also in the developing of control methods and identification of antimicrobial resistance markers for evidence-based decisions in epidemiology and surveillance of foodborne diseases. OMICS datasets have been found as a powerful tool to complement current studies that are starting to be used also in some risk assessment areas. In a current comprehensive study "Syst-OMICS," 4500 *Salmonella* genomes will be sequenced and analysis pipeline built in order to study *Salmonella* genome evolution, antibiotic resistance and virulence genes [88]. The data of the first 3377 genomes already sequenced are stored in the newly established *Salmonella* Foodborne Syst-OMICS database (SalFoS, https://salfos.ibis.ulaval.ca/). Their analysis identified 1003 unique resistomes, composed of combinations of 195 different genes. Surprisingly, the two most frequently observed resistomes accounted for 23% of the *Salmonella* strains examined.

Comparative genomics of the WGS was successfully used also in the examination of 589 *Campylobacter* isolates from retail chicken meat exhibiting phenotypic resistance to 9 antimicrobials [89]. For most antimicrobial agents (ciprofloxacin, nalidixic acid, gentamicin, azithromycin, erythromycin and clindamycin), the observed phenotypic resistance, determined on the basis

of the comparison of measured MICs with established ECOFF cut-off values, was in accordance with the presence of the known resistance genes or mutations. In the case of telithromycin, however, the observed point mutations in the 23S rRNA, which is a well-known mechanism of resistance to these classes of antimicrobials, did not regularly cause phenotypic resistance. Another recent study on C. *jejuni* isolates from the poultry (n = 502) demonstrated successful use of genomics in the study of fluoroquinolone resistance [90]. The isolates were clustered according to the presence/absence of the *gyrA* mutations causing fluoroquinolone resistance. Beside the WGS of isolates from the mentioned study, previously published (ENA) *Campylobacter* genomes were included in the comparative analyses of the genomes. Although no significant associations were found between trade patterns, antimicrobial use in livestock and population of *C. jejuni*, this approach proved to be successful, especially when big datasets are available.

In conclusion, comparative genomics of WGS is increasingly used in the prediction of phenotypic antimicrobial resistance and surveillance of antimicrobial resistance of common zoonotic bacteria. However, as it is based on the detection of already known ARG, the success is highly dependent on the quality of databases, which need to be regularly updated with newly discovered resistance mechanisms and well-curated.

6. Conclusion

Antimicrobial resistant zoonotic bacteria pose a serious risk to human health. High rates of FQ resistance in both *Salmonella* and *Campylobacter* are of concern due to their wide use for the treatment of human infections. In some regions of the EU, the level of FQ resistance is so high that *Campylobacter* infections cannot be treated with FQ anymore. In addition, the emergence of multi-drug resistant *Salmonella* and *Campylobacter* further limits the therapy options and is possibly associated with adverse treatment effects. Greater efforts are needed to limit the wide spread of AMR in zoonotic bacteria—implementation of antimicrobial stewardship, especially in the developing countries, development of novel antimicrobials, improvement of practices in the food production chain, reduction of the amounts of antimicrobials sold for use in livestock, improvement of AMR surveillance programs, in terms of greater harmonisation, and application of rapid sequence-based methods in the routine surveillance of antimicrobial resistance.

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Conflict of interest

No competing financial interests exist.

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