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Monitoring AMR in *Campylobacter jejuni* from Italy in the last 10 years (2011–2021): Microbiological and WGS data risk assessment

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Abstract

Campylobacter jejuni is considered as the main pathogen in human food-borne outbreaks worldwide. Over the past years, several studies have reported antimicrobial resistance (AMR) in *C. jejuni* strains. In Europe, the official monitoring of AMR comprises the testing of *Campylobacter* spp. from food-producing animals because this microorganism is responsible for human infections and usually predominant in poultry. Food-producing animals are considered to be a major source of campylobacteriosis through contamination of food products. Concerns are growing due to the current classification of *C. jejuni* by the WHO as a 'high priority pathogen' due to the emergence of resistance to multiple drugs such as those belonging to the fluoroquinolones, macrolides and other classes, which limits the treatment alternatives. Knowledge about the contributions of different food sources to gastrointestinal disease is fundamental to prioritise food safety interventions and to establish proper control strategies. Assessing the genetic diversity among *Campylobacter* species is essential to the understanding of their epidemiology and population structure. Using a population genetic approach and grouping the isolates into sequence types within different clonal complexes, it is possible to investigate the source of the human cases. The work programme was aimed for the fellow to assess the AMR of *C. jejuni* isolated from humans, poultry and birds from wild and urban Italian habitats. Given the public health concern represented by resistant pathogens in food-producing animals and the paucity of data about this topic in Italy, the aim was to identify correlations between phenotypic and genotypic AMR and comparing the origin of the isolates. The work programme allowed the fellow to acquire knowledge, skills and competencies on the web-based tools used by IZSAM to process the NGS data and perform bioinformatics analyses for the identification of epidemiological clusters, the study of AMR patterns in *C. jejuni* isolates, and the assessment of the human exposure to such AMR pathogens. Furthermore, the fellow became able to transfer the acquired knowledge through innovative web-based didactical tools applied to WGS and clustering of specific food-borne pathogens, with particular reference to *C. jejuni*. To achieve this objective, 2,734 *C. jejuni* strains isolated from domestic and wild animals and humans, during the period 2011–2021 were analysed. The resistance phenotypes of the isolates were determined using the microdilution method with EUCAST breakpoints, for the following antibiotics: nalidixic acid, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, streptomycin, tetracycline. The data were complemented by WGS data for each strain, uploaded in the Italian information system for the collection and analysis of complete genome sequence of pathogens isolated from animal, food and environment (GENPAT) developed and maintained at IZSAM; information like clonal complex and sequence type to understand the phylogenetical distance between strains according to their origins were also considered. This work underlines that a better knowledge of the resistance levels of *C. jejuni* is necessary, and mandatory monitoring of *Campylobacter* species in the different animal productions is strongly suggested.

Keywords: Campylobacter, antimicrobial resistance (AMR), multidrug resistance (MDR), multilocus sequence typing, resistance genes, MLST

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1. Introduction

Antibiotic-resistant bacteria can spread through many routes. When antimicrobial resistance (AMR) occurs in zoonotic bacteria present in animals and food it can also compromise the effective treatment of infectious diseases in humans.

In the field of food safety, policymakers need to protect consumers from risks related to the food chain and to establish the best control options to reduce such risks. Scientists and risk assessors are examining the factors which may lead to the presence of antimicrobial-resistant bacteria in food and animals to provide appropriate scientific advice to decision-makers (Allard et al., 2012).

1.1. Description of the pathogen

Campylobacter is a Gram-negative, aerobic, microaerophilic, mobile bacterium. It presents pleomorphic forms, being observed as curved, spiral, comma and coccoid bacilli, the last one is observed especially in old cultures (Baker, 2009). They can grow at two temperatures: 37°C and 42°C (Best et al., 2005). The taxonomy of this genus has changed dramatically since its discovery, in 1963, by Sebald and Véron (Buettner et al., 2010); (Cecilia et al., 2013). Currently, it comprises 25 species, 2 provisional species and 8 subspecies, many of which are of clinical and economic importance (Cevenini et al., 1985). *Campylobacter jejuni* is one of the most important species, which comprises two subspecies: *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*. The *jejuni* subspecies, referred to as *C. jejuni*, have been recognised as the most isolated bacterium from humans with gastroenteritis since 1970. In addition, it is involved in other diseases (Diaz-Sanchez et al., 2013), such as proctitis, septicaemia, meningitis, abortion and autoimmune diseases (Reiter's syndrome and Guillain-Barré syndrome) (Baker, 2009). In chickens and other species of birds, as well as in dogs, pigs, sheep and livestock, *C. jejuni* is considered a commensal organism, making it one of the most important pathogens present and transmitted by foods of animal origin (Dingle et al., 2001). Most cases of *Campylobacter enteritis* do not require treatment, as they are generally short-lived, self-limited events. However, when symptoms are prolonged or very severe, antimicrobial therapy is necessary. For these occasions, erythromycin is the antibiotic of choice. Some *Campylobacter* species are resistant to penicillin, ampicillin and cephalosporins. The increase in resistance to fluoroquinolones coincides with their administration in poultry and veterinary medicine in general. Most strains of *C. jejuni* are still susceptible to erythromycin, azithromycin, gentamicin, tetracycline and chloramphenicol. Erythromycin and azithromycin shorten the duration of illness when given early in gastrointestinal infection (EFSA and ECDC, 2014; EFSA Antimicrobial Resistance, 2021).

1.2. Situation

Campylobacter is the most common cause of bacterial gastroenteritis in Europe. The incidence of human campylobacteriosis is increasing worldwide, as well as the number of isolates resistant to fluoroquinolones which are one of the primary classes of antimicrobials used to treat *Campylobacter* infection in human therapy and thus considered of high public concern (Kittl et al., 2013). In 2020, in the European Union, *Campylobacter* is still the most commonly reported cause of bacterial food-borne illness, as it has been since 2005. It represented more than 60% of all the reported cases in 2020 (EFSA and ECDC, 2021). Poultry is a natural reservoir of *Campylobacter* species, constituting the most important source of human infection. The consumption of undercooked poultry meat or the mishandling of raw poultry products is considered to be the main risk factor associated with human campylobacteriosis (Lucarelli et al., 2016; Manfreda et al., 2016). In Italy, the notification rate was 1.410 reported human cases per 100.000 population (Di Giannatale et al., 2016; EFSA and ECDC, 2021). Human clinical cases are not regularly reported; Therefore, the real incidence of the disease in man has not been established. However, a pilot surveillance programme for *Campylobacter* infection in humans has been recently implemented. The preliminary results confirmed previous observations (Parkhill et al., 2000; Mughini Gras et al., 2012), namely that the pathogen is widespread, and that human cases frequently occur, particularly in children (Pendleton et al., 2013). With regard to animals and animal products, studies carried out in Italy have demonstrated the presence of *Campylobacter* in poultry, poultry products and pork meat (Piantieri et al., 1985; Pezzotti et al., 2003a,b; Piccirillo et al., 2014; EFSA and ECDC, 2021). However, the presence of the pathogen at a national level needs to be better investigated.

1.3. EFSA's Role in AMR

EFSA provides independent scientific support and advice to risk managers on the risks to human and animal health related to the possible emergence, spread, and transfer of AMR in the food chain and animals. EFSA takes an integrated approach to its work on antimicrobial resistance involving a number of its Scientific Panels and Units as it is a concern for the entire food chain. On this topic, EFSA cooperates closely with other relevant EU agencies such as the European Centre for Disease Prevention and Control (ECDC) and the European Medicines Agency (EMA) (Allard et al., 2012).

1.4. NGS in Campylobacteriosis

The investigation of the food-borne disease outbreaks related to the origin of the pathogen strains could be time-consuming in most cases. The available molecular tools, including the 'gold standard' pulsed-field gel electrophoresis (PFGE) method, do not always distinguish the outbreak-related strains from other genetically similar strains unassociated with the same outbreak (Sánchez de la Barquera and Herrera, 2002; Lévesque et al., 2008; Man, 2011). Thus, next-generation sequencing (NGS) tools and whole genome sequencing (WGS) in particular, provide a powerful approach for epidemiological trace-back efforts (On, 2005). The tracking and tracing of living animals and animal-derived food within the food chain is part of the monitoring process (Harlizius et al., 2004; Sarhangi et al., 2021). In addition to epidemiological tracing, NGS provides additional data to investigators that can include identification of specific markers for detection efforts and assessment of unique virulence factors that may be strain specific.

The lack of a method for distinguishing *Campylobacter* strains makes it more difficult to trace back sources in outbreaks. Multiple typing methods have been developed including antibiotic resistance, phage typing, serotyping and several emerging genetically based methods. Advanced research in the field of genome-based methods (multilocus sequence typing and microarrays) has allowed the identification of different clonal groupings of *C. jejuni* (Troso et al., 1997; Manning et al., 2003; Kittl et al., 2013a). Also, the development of real-time PCR Taqman allelic discrimination assays permitted the rapid detection of *C. jejuni* isolates and preliminary strain identification (Véron and Chatelain, 1973). However, the high plasticity of *Campylobacter* relegates the use of some of the genotyping technology in advance of more advantageous methods like WGS (Sebald and Veron, 1963; Troso et al., 1997; Young et al., 2007 Stahl and Stintzi, 2011). The development of WGS of *C. jejuni* offers the opportunity to detect genes and proteins involved in the pathogenesis of *Campylobacter* and also genes of unknown function (Zanetti et al., 1996; Zhang et al., 2009). The understanding of the function of many of these proteins and genes could lead to the increasing of knowledge of the role of *Campylobacter* in the colonisation of chicken gut, the immune response and finally could cause the improvement of current practices and potential intervention strategies for the control of campylobacteriosis (Zhang et al., 2009).

1.5. Data collection

The development of integrated information systems is a guarantee for farmers and consumers because these systems allow to turn data into actions. The Italian Ministry of Health appointed the IZSAM as National Reference Centre for WGS of microbial pathogens: database and bioinformatics analysis. For this reason, the IZSAM has realised a platform (GENPAT) (Genpat Platform, 2017) for the collection and storage of genomic sequences of pathogenic microorganisms, to perform bioinformatic analyses, to archive and to share the results. IZSAM provides the technical infrastructure, developers team, and service desk needed for the management of data, ensuring the interoperability with other national information systems in the veterinary field through an online platform. The GENPAT system provides IT tools and data that are quickly available, usable and helpful in outbreak situations allowing to link molecular typing and bioinformatics analyses result with time and geographical position of sampling as well as the others epidemiological information available. In this way, it is possible to compare the classification results obtained by WGS for two or more distinct isolates and to measure their relatedness in order to support an epidemiological investigation as experienced in 2015 during a severe listeriosis outbreak in the Marche region (Duranti et al., 2018).

Access to information allows knowing the health risk to which the livestock and food processing chain is exposed and how the risks can affect public health, performing real-time assessments to adjust technical and organisational responses, plan actions and check their effectiveness afterward. The national platform for the collection and analysis of complete genome sequence together with the veterinary information systems adopted in Italy during the years fully meets these needs (Cito et al., 2018).

2. Description of the work programme

2.1. Aims

The work programme allowed the fellow to acquire knowledge, skills and competencies on the application of NGS methods and bioinformatics analyses for the identification of epidemiological clusters, the study of AMR patterns in *C. jejuni* isolates and the assessment of the human exposure to such AMR pathogens. Furthermore, the fellow became able to transfer the acquired knowledge through innovative web-based didactical tools applied to WGS and clustering of specific food-borne pathogens, with particular reference to *C. jejuni*.

2.2. Activities/Methods

WP1. Acquisition of knowledge and skills to use didactic web-based tools (February–April 2021)

The fellow had the opportunity to acquire new knowledge and skills through theoretical and practical training on didactic web-based tools. The training was carried out at distance in cooperation with subject matter experts and trainers working at the ISO 9001:2015 certified International Centre for Veterinary Training and Information (CIFIV). This learning-by-doing approach allowed the fellow to apply his knowledge by contributing to the development of technical and scientific content for an e-learning course devoted to professionals engaged with genomics applied to food safety risk assessment (e.g. microbiologists, epidemiologists, bioinformaticians, statisticians and veterinarians). This WP allowed maximising the impact of the fellowship by enhancing cascade training and scalability of the acquired competencies to other scientific domains.

WP2. NGS technology and production of WGS data (March–May)

NGS has been used for WGS of viral and bacterial pathogens, and it is also applied for metagenomics and transcriptomic studies of microorganisms. At the genomic laboratory, the fellow acquired knowledge and practical skills on different NGS technologies, starting from the sample (the bacterial isolate or different samples for metagenomics analysis).

Different aspects of the WGS workflow were taken into consideration:

- General requirements for a genomic laboratory, use of the laboratory management system, and specific worksheets.
- DNA extraction for short and long-read sequencing, evaluation of quantity and quality of DNA.
- Sequencing on Illumina and Nanopore NGS platforms, quality metrics of the NGS run.
- Best practices for quality management of the WGS workflow, use of positive and negative control, genomic library Quality Control.
- Principles of validation of WGS workflow.
- Participation in proficiency tests for microbial WGS.

WP3. Collection, normalisation and organisation of data (June–August)

IZSAM collects and registers a well-defined set of data for each sample tested in its laboratories. In addition, several samples were collected in the framework of national control plans (all related data are registered into the National Veterinary Information System) and European and national projects managed by IZSAM. All these factors allowed IZSAM to retrieve relevant epidemiological data for all tested samples.

The WP3 activity was focused on data related to a selection of *Campylobacter* isolates obtained from different sources, at various stages of the chicken meat production chain (farm, slaughterhouse and retail). A detailed data analysis plan was prepared, including the description of the dataset retrieved, the type of data quality checks to be performed and the format of the resulting validated databases, using data from 2,734 *C. jejuni* strains isolated from domestic and wild animals and humans, during the period 2011–2021.

WP4. Bioinformatics analysis (July–October)

The fellow was involved in the development of a detailed data analysis plan, including the description of the bioinformatics pipelines used to analyse WGS data and metadata obtained from the *Campylobacter* isolates arriving at IZSAM. The data were analysed to identify 'epi-clusters', considering the outcomes of the molecular typing methods using WGS data, such as *in silico* MLST, and gene-by-gene or single nucleotide polymorphisms (SNPs)-based analysis. Moreover, different bioinformatic tools such as Resfinder, ARIBA, Plasmidfinder and databases like CARD were applied to WGS data aiming to identify the AMR genes of interest and associated mobile genetic elements within the genome of *Campylobacter* isolates.

WP5 Exposure assessment (November–December)

The results of the analysis performed in WP4 allowed to development of AMR risk assessment models, considering different exposure pathways. For instance, data concerning the dissemination and persistence of *C. jejuni* in the farm's environment and the production chain were taken into account together with the presence of AMR determinants and genetic mobile elements in the strain's genome.

2.3. Secondary activities

The fellow has assisted and completed complementary activities to reinforce the experience (description in Appendix A).

3. Results and conclusions

3.1. AMR

The proportion of completely susceptible strains was very similar in isolates from humans and domestic animals (67,63% and 61,55%, respectively), while strains from the wild animal population found a significantly higher prevalence (95,49%). Most of these strains were collected from domestic animals (95,01%), largely poultry samples (81,21%), that showed a high level of resistance to nalidixic acid, ciprofloxacin and tetracycline (67,39%, 67,27% and 55,63%, respectively). The human isolates reproduced the same patterns reinforcing the direct association between the increase in the resistance profiles over time with veterinary practices in the control of pathogens in poultry (Moore et al., 2006; Ruiz-Palacios, 2007). Fluoroquinolones had very high rates of resistant profiles since 2011 with a stable trend over the last decade. Conversely, erythromycin showed a slight increase in resistance levels mostly for poultry and swine, while wild animals and human strains had stable trends. In conclusion, antibiotics released in animal production environments can interfere with the development of resistance profiles.

3.2. Genetic approach

Within the samples we have identified 11 clonal complexes (CCs) (443-49-179-446-42-206-354-45-353-828-21), the most prevalent being CC-828, CC-21 and CC-353, with 30,5%, 19,7% and 16,9% respectively. This CCs were often described in the literature for its global distribution. Despite the high genetic diversity usually detected within *Campylobacter* populations, a remarkable similarity among isolate collections on a national and international scale, even across different continents, can be documented (Di Giannatale et al., 2016; Cito et al., 2018). CC-21 is a multihost lineage shared among different sources (De Haan et al., 2010), one of the largest CCs found to date, comprising 26% of all the isolates submitted to PubMLST, with a total of 152 different STs (https://pubmlst.org/campylobacter). This CC is frequently associated with cases of human disease and is one of the most frequently reported CCs in poultry (Dingle et al., 2001; Kärenlampi et al., 2007; Kittl et al., 2013b; Manfreda et al., 2016; EFSA and ECDC, 2021); however, CC-21 has also been found in cattle (Pezzotti et al., 2003; EFSA and ECDC, 2021), sheep (Lucarelli et al., 2016), turkey (Lo Schiavo et al., 1991) and the environment (Dingle et al., 2001). Several studies were performed in Italy and show how common this CC is not only in chickens but also in strains isolated from turkeys, cattle and bulk tank milk (Lo Schiavo et al., 1991; Bianchini et al., 2014a,b). The most common CC among chicken *C. jejuni* isolates was the CC-21 according to that documented worldwide (Pezzotti et al., 2003b; EFSA and ECDA, 2014; Lucarelli et al., 2016; Cito et al., 2018; Sarhangi et al., 2021). CC-828 was also described as predominant and with similar results to CC-21 and shared between chicken and human campylobacteriosis (Pezzotti et al., 2003a). The predominance of the CC-828 and CC-353 was expected since several studies demonstrated that these CCs are globally spread in both human and chicken *Campylobacter* populations (Sánchez de la Barquera and Herrera, 2002; Pezzotti et al., 2003b; Colles and Maiden, 2012; Lucarelli et al., 2016; Cito et al., 2018).

The most diverse CCs are related to more prevalent sequence types. This proposes that probably their diversity is a mirror of their replication frequency and circulation which affects their gene content and efficiency (On, 2005; Ragimbeau et al., 2008). This data demonstrates the importance of rapid reporting of gastroenteritis cases to local public health authorities to perform a timely epidemiological and microbiological investigation. Mandatory notification for *Campylobacter* would be useful for better estimation of the disease, as well as identification of the source of infection and proper control measures to protect public health (Sheppard et al., 2009a,b). The more prevalent CCs we have found

are concordant to those found worldwide, keeping track of molecular epidemiology provides a universal picture of the movement of dominant *Campylobacter* strains.

3.3. Conclusions from the participation in the EU-FORA programme

It was a valuable opportunity for the fellow to obtain experience in AMR risk assessment of a food-borne pathogen like *C. jejuni*. This was also an excellent opportunity to consolidate his specialised knowledge and skills in food safety, particularly in bacterial microbiology, by working according to European and international guidelines and standards for AMR. The general risk assessment methodology applied for this specific project is expected to be further extended and applied by the fellow in future positions, to expand knowledge. Moreover, the EU-FORA programme provided a great environment to build a strong professional and personal network that will be an open door for future collaborations and references.

References

Allard MW, Luo Y, Strain E, Li C, Keys CE, Son I, Stones R, Musser SM and Brown EW, 2012. High-resolution clustering of *Salmonella enterica* serovar Montevideo strains using a next-generation sequencing approach. BMC Genomics, 13, 1–19.

Baker CD, 2009. Red Book Atlas de enfermedades infecciosas en pediatria/Red Book Atlas of Pediatric Infectious Diseases. Ed. Médica Panamericana.

Best EL, Fox AJ, Frost JA and Bolton FJ, 2005. Real-time single-nucleotide polymorphism profiling using Taqman technology for rapid recognition of *Campylobacter jejuni* clonal complexes. Journal of Medical Microbiology, 54, 919–925.

Bianchini V, Borella L, Benedetti V, Parisi A, Miccolupo A, Santoro E, Luini M, 2014a. Prevalence in bulk tank milk and epidemiology of *Campylobacter jejuni* in dairy herds in Northern Italy. Applied and Environmental Microbiology, 80, 1832–1837.

Bianchini V, Luini M, Borella L, Parisi A, Jonas R, Kittl S and Kuhnert P, 2014b. Genotypes and antibiotic resistances of *Campylobacter jejuni* isolated from cattle and pigeons in dairy farms. International Journal of Environmental Research and Public Health, 11, 7154–7162.

Buettner S, Wieland B, Staerk KDC and Regula G, 2010. Risk attribution of *Campylobacter* infection by age group using exposure modeling. Epidemiology & Infection, 138, 1748–1761.

Cecilia HC, Arreola MGA and Graciela CE, 2013. *Campylobacter jejuni*: ¿ A forgotten bacteria? Its situation in Mexico. Enfermedades Infecciosas Y Microbiología, 33, 77–84.

Cevenini R, Varoli O, Rumpianesi F, Mazzaracchio R, Nanetti A and La Placa M, 1985. A two-year longitudinal study on the etiology of acute diarrhea in young children in Northern Italy. Microbiologica, 8, 51–58.

Cito F, Di Pasquale A, Cammà C and Cito P, 2018. The Italian information system for the collection and analysis of complete genome sequence of pathogens isolated from animal, food, and environment. International Journal of Infectious Diseases, 73, 296–297.

Colles FM and Maiden MCJ, 2012. *Campylobacter* sequence typing databases: applications and prospects. Microbiology, 158, 2695–2709.

De Haan CPA, Kivistö R, Hakkinen M, Rautelin H and Hänninen ML, 2010. Decreasing trend of overlapping multilocus sequence types between human and chicken *Campylobacter jejuni* isolates over a decade in Finland. Applied and Environmental Microbiology, 76, 5228–5236.

Di Giannatale E, Garofolo G, Alessiani A, Di Donato G, Candeloro L, Vencia W, Decastelli L and Marotta F, 2016. Tracing back clinical Campylobacter jejuni in the Northwest of Italy and assessing their potential source. Frontiers in Microbiology, 7, 887.

Diaz-Sanchez S, Hanning I, Pendleton S and D'Souza D, 2013. Next-generation sequencing: the future of molecular genetics in poultry production and food safety. Poultry Science, 92, 562–572.

Dingle KE, Colles FM, Wareing DRA, Ure R, Fox AJ, Bolton FE, Maiden MCJ, 2001. Multilocus sequence typing system for *Campylobacter* jejuni. Journal of Clinical Microbiology, 39, 14–23.

Duranti A, Sabbatucci M, Blasi G, Acciari VA, Ancora M, Bella A, Busani L, Centorame P, Cammà C, Conti F, De Medici D, Di Domenico M, Di Marzio V, Filippini G, Fiore A, Fisichella S, Gattuso A, Gianfranceschi M, Graziani C, Guidi F, Marcacci M, Marfoglia C, Neri D, Orsini M, Ottaviani D, Petruzzelli A, Pezzotti P, Rizzo C, Ruolo A, Scavia G, Scuota S, Tagliavento G, Tibaldi A, Tonucci F, Torresi M, Migliorati G and Pomilio F, 2018. A severe outbreak of listeriosis in central Italy with a rare pulsotype associated with processed pork products. Journal of Medical Microbiology, 67, 1351–1360.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2014. The European Union Summary REPORT on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA Journal 2014;12(2):3547, 55 pp. https://doi.org/10.2903/j.efsa.2014.3547

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021. The European Union one health 2020 zoonoses report. EFSA Journal, 2021;19(12):6971, 324 pp. https://doi.org/10.2903/j.efsa.2021.6971

EFSA Antimicrobial Resistance, 2021. Available online: https://www.efsa.europa.eu/en/topics/topic/antimicrobial-resistance

Genpat Platform, 2017. Ministero della Salute, con Decreto del 30 maggio 2017 (G.U.R.I. n. 196 del 23 agosto 2017), ha attivato il Centro di Referenza Nazionale per Sequenze Genomiche di microrganismi patogeni: banca dati e analisi di bioinformatica presso la sede centrale dell'IZSAM Available online: https://genpat.izs.it/cmdbuild/ui/#login

Harlizius B, van Wijk R and Merks JW, 2004. Genomics for food safety and sustainable animal production. Journal of Biotechnology, 113, 33–42.

Kärenlampi R, Rautelin H, Schönberg-Norio D, Paulin L and Hänninen ML, 2007. Longitudinal study of Finnish *Campylobacter jejuni* and *C. coli* isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. Applied and Environmental Microbiology, 73, 148–155.

Kittl S, Heckel G, Korczak BM and Kuhnert P, 2013a. Source attribution of human *Campylobacter* isolates by MLST and fla-typing and association of genotypes with quinolone resistance. PLoS One, 8, e81796.

Kittl S, Korczak BM, Niederer L, Baumgartner A, Buettner S, Overesch G and Kuhnert P, 2013b. Comparison of genotypes and antibiotic resistances of *Campylobacter jejuni* and *Campylobacter coli* on chicken retail meat and at slaughter. Applied and Environmental Microbiology, 79, 3875–3878.

Lévesque S, Frost E, Arbeit RD and Michaud S, 2008. Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental water in Quebec. Canada. Journal of Clinical Microbiology, 46, 3404–3411.

Lo Schiavo A, Minniti A, Panebianco A and Iannuzzi L, 1991. Isolamento di Campylobacter jejuni da polli totalmente eviscerati. Archivio Veterinario Italiano, 42, 18–21.

Lucarelli C, Dionisi AM, Trezzi L, Farina C, Passera M, Kärki T, Luzzi I, 2016. Molecular and epidemiological analysis of a *Campylobacter jejuni* outbreak in Northern Italy in November 2013. Foodborne Pathogens and Disease, 13, 490–494.

Man SM, 2011. The clinical importance of emerging *Campylobacter* species. Nature Reviews Gastroenterology & Hepatology, 8, 669–685.

Manfreda G, Parisi A, De Cesare A, Mion D, Piva S and Zanoni RG, 2016. Typing of *Campylobacter jejuni* isolated from turkey by genotypic methods, antimicrobial susceptibility, and virulence gene patterns: a retrospective study. Foodborne Pathogens and Disease, 13, 93–100.

Manning G, Dowson CG, Bagnall MC, Ahmed IH, West M and Newell DG, 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Applied and Environmental Microbiology, 69, 6370–6379.

Moore JE, Barton MD, Blair IS, Corcoran D, Dooley JSG, Fanning S, Kempf I, Lastovica AJ, Lowery CJ, Matsuda M, McDowell DA, McMahon A, Millar BC, Rao JR, Rooney PJ, Seal BS, Snelling WJ and Tolba O, 2006. The epidemiology of antibiotic resistance in *Campylobacter*. Microbes and Infection, 8, 1955–1966.

Mughini Gras L, Smid JH, Wagenaar JA, de Boer AG, Havelaar AH, Friesema IHM and van Pelt W, 2012. Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis.

On SL, 2005. Taxonomy, phylogeny, and methods for the identification of *Campylobacter* species. Campylobacter: molecular and cellular biology, 13–42.8-.

Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Barrell BG, 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature, 403, 665–668.

Pendleton S, Hanning I, Biswas D and Ricke SC, 2013. Evaluation of whole-genome sequencing as a genotyping tool for *Campylobacter jejuni* in comparison with pulsed-field gel electrophoresis and flaA typing. Poultry Science, 92, 573–580.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M and Perin R, 2003. Aparición y resistencia a antibióticos de Campylobacter jejuni y Campylobacter coli en animales y carne en el noreste de Italia. Revista Internacional De Microbiología Alimentaria, 82, 281–287.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M and Perin R, 2003. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. International Journal of Food Microbiology, 82, 281–287.

Piantieri G, Mamolo G, Caffarelli A, Bossi G, Bignamini ML and Bo G, 1985. Presenza di *Campylobacter jejuni* nel pollo di allevamento. Ig. Mod, 83, 510–517.

Piccirillo A, Giacomelli M, Salata C, Bettanello S, De Canale E and Palù G, 2014. Multilocus sequence typing of *Campylobacter jejuni* and *Campylobacter coli* from humans and chickens in North-Eastern Italy. New Microbiologica, 37, 557–562.

Ragimbeau C, Schneider F, Losch S, Even J and Mossong J, 2008. Multilocus sequence typing, pulsed-field gel electrophoresis, and fla short variable region typing of clonal complexes of *Campylobacter jejuni* strains of human, bovine, and poultry origins in Luxembourg. Applied and Environmental Microbiology, 74, 7715–7722.

Ruiz-Palacios GM, 2007. The health burden of Campylobacter infection and the impact of antimicrobial resistance: playing chicken.

Sánchez de la Barquera RM and Herrera BIF, 2002. Diarrea por Campylobacter. In: En Romero CR and Herrera BIF (eds.), Síndrome diarreico infeccioso. México-España. Ed. Médica Panamericana, pp. 204–205.
Sarhangi M, Bakhshi B and Peeraeyeh SN, 2021. High prevalence of *Campylobacter jejuni* CC21 and CC257 clonal complexes in children with gastroenteritis in Tehran. Iran. BMC Infectious Diseases, 21, 1–13.
Sebald M and Veron M, 1963. Base DNA content and classification of vibrios. In Annales de l'Institut Pasteur (Vol. 105, pp. 897–910).
Sheppard SK, Dallas JF, MacRae M, McCarthy ND, Sproston EL, Gormley FJ, Strachan NJC, Ogden ID, Maiden MCJ and Forbes KJ, 2009a. *Campylobacter* genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6. International Journal of Food Microbiology, 134, 96–103.
Sheppard SK, Dallas JF, Strachan NJ, MacRae M, McCarthy ND, Wilson DJ, Forbes KJ, 2009b. *Campylobacter* genotyping to determine the source of human infection. Clinical Infectious Diseases, 48, 1072–1078.
Stahl M and Stintzi A, 2011. Identification of essential genes in C. jejuni genome highlights hyper-variable plasticity regions. Functional & Integrative Genomics, 11, 241–257.
Troso O, Gallo T, Gallo G, Crescente MD and Flora ME, 1997. A surveillance system for childhood gastroenteritis. Igiene Moderna, 107, 411–420.
Véron M and Chatelain R, 1973. Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter* fetus (Smith and Taylor) Sebald and Véron. International Journal of Systematic and Evolutionary Microbiology, 23, 122–134.
Young KT, Davis LM and DiRita VJ, 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nature Rev. Microbiol, 5, 665–679.
Zanetti F, Varoli O, Stampi S and De Luca G, 1996. Prevalence of thermophilic *Campylobacter* and *Arcobacter butzleri* in food of animal origin. International Journal of Food Microbiology, 33, 315–321.
Zhang MJ, Xiao D, Zhao F, Gu YX, Meng FL, He LH, Zhang JZ, 2009. Comparative proteomic analysis. of *Campylobacter jejuni* cultured at 37 C and 42 C. Japanese Journal of Infectious Diseases, 62, 356–361.

Abbreviations

AMR	antimicrobial resistance
CC	clonal complex
CIFIV	Centre for Veterinary Training and Information
ECDC	European Centre for Disease Prevention and Control
EMA	European Medicines Agency
IZSAM	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise
LEGO	Learning Genomics for Food Safety
MDR	multidrug resistance
MLST	multilocus sequence typing
NGS	next-generation sequencing
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
WGS	whole genome sequencing

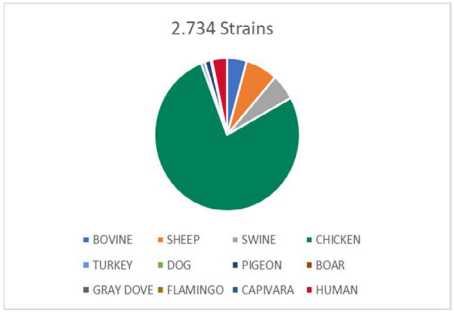
Appendix A – Secondary Activities

Additional relevant activities and learning opportunities completed by the fellow:

- 'Induction training of the European Food Risk Assessment Fellowship Programme' (EFSA) (11– 29 January 2021).
- Content Reviewing LEGO Project In the framework of the Erasmus+ Programme Unit 1. eLearning course for Food Microbial Bioinformatician (1 February–29 March 2021).
- 'Module 1 training of the European Food Risk Assessment Fellowship Programme' (EFSA) (22– 26 March 2021).
- eLearning course for Food Microbial Bioinformatician LEGO Project In the framework of the Erasmus+ Programme (29 March–31 December 2021) https://www.learngenomics.eu/2021/02/04/call-for-applications-e-learning course.
- A course in the Italian language (April–July 2021). Provided by CPIA Municipality of Teramo.
- Illumina Practical Training (Provided by Illumina Technical Expert). (31 May–1 June 2021).
- 'Module 2 training of the European Food Risk Assessment Fellowship Programme' (EFSA) (7–14 June 2021).
- 4th European Summer School on Nutrigenomics (virtual edition, 21–25 June 2021) https://www.unicam.it/nutrigenomics/ Food as Medicine: Food and our Genome https://www.futurelearn.com/courses/fam-genome. Scholarship.
- CampyUK. Liverpool International congress. Abstract Submitted 'Monitoring Antibiotic Resistance in Campylobacter jejuni from Italy in the last 10 years (2011–2021)- Conesa, A.; Garofolo, G.; Janowicz, A.; Di Marcantonio, L.; Di Pasquale, A.; Camma, C. LigthTalk Presentation. (8–10 September 2021).
- Parma Summer School 'Food Safety Aspects of Integrated Food Systems'-EFSA. (28–30 September 2021).
- 'Module 3 training of the European Food Risk Assessment Fellowship Programme' (EFSA) (4–7 October 2021)
- 'Module 4 training of the European Food Risk Assessment Fellowship Programme' (EFSA) (22–26 November)
- Online Course- Introduction to programming for Bioinformatics with Python. October 2021. Udemy.
- Online Course- Practical Bioinformatics: Play with Genes On Your Screens. October 2021. Udemy.
- Antimicrobial Resistance- Theory and Methods. 5 weeks course. Authorized by the Technological University of Denmark (DTU) Trough Coursera. Grade: 90.61%. October–November 2021 https://coursera.org/share/5a1c35b77a615678e17657a88c786cca
- Cohesive Symposium One Health EJP- European Joint Programme Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance, and emerging microbiological hazards. 8–10 November 2021. The Netherlands (On remote).
- Short Training Workshop EFSA Partnering Grants GP/EFSA/ENCO/2020/03 GA3- Basic Bioinformatics Skills Relating to Using Genotypic/Phenotypic techniques for Risk Assessment/ Predictive Microbiology – Online 'virtual' workshop from 9 to 11 November 2021. Dublin, Ireland (On remote).
- Giornata di studio del Centro di Referenza Nazionale per Sequenze Genomiche di microrganismi patogeni: banca dati e analisi di bioinformatica (GENPAT). by IZSAM G. Caporale, Teramo, IT. 30/11/2011. 4.5 Credits.

Appendix B – Graphics

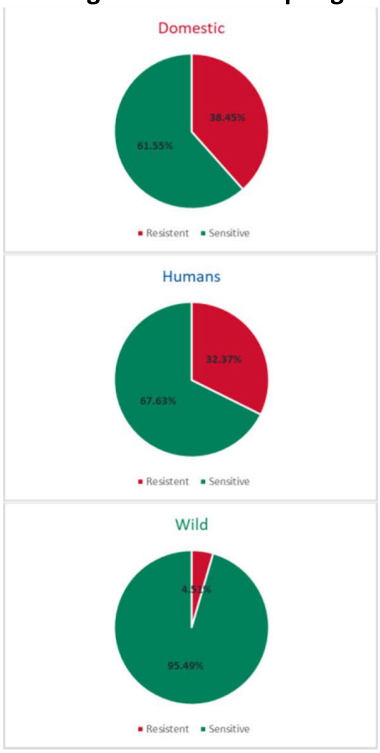
Total Number of Strains in the study and origin of the sample



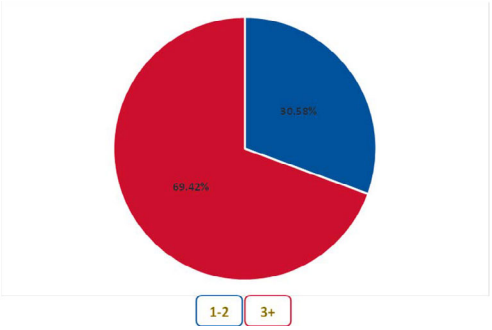
Percentage of total resistance during the decade for the principal antibiotics

HOST	PROFILE	CIP	NAL	TET	ERY
CHICKEN	R	89,49%	87,65%	73,98%	12,78%
	I+S	10,50%	12,34%	26,04%	87,2%
HUMAN	R	77,55%	62,5%	65,30%	6,1%
	I+S	22,44%	37,5%	34,69%	93,87%

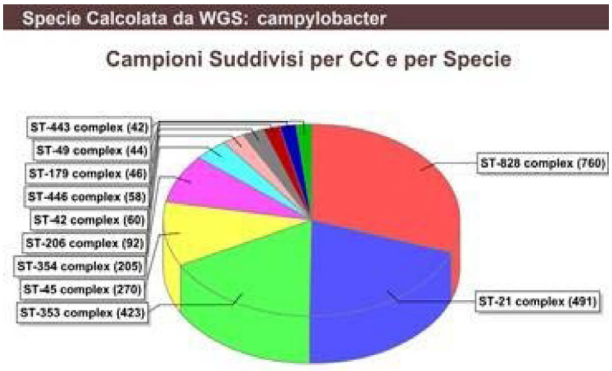
Percentage of Resistance per group



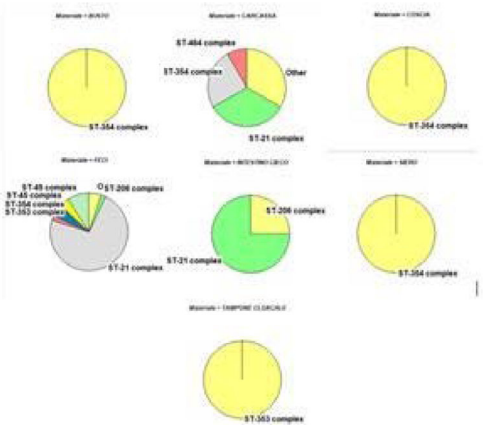
Percentage of total strains resistant to 1-2 vs 3+antibiotics



Total cases of each CC (GenPat platform Report)



Example of some CC per substrate of the sampling (GenPat platform output)



Resistance timeline per antibiotic during the decade