Centrum voor Onderzoek in Diergeneeskunde en Agrochemie
Centre d'Etude et des Recherches Vétérinaires et Agrochimiques

CODA - CERVA

Antimicrobial resistance in *Campylobacter* spp. from broiler chickens in 2016 in Belgium

Report on the occurrence of antimicrobial resistance in *Campylobacter* from broiler chickens in 2016 in Belgium.

Summary

Overall, the levels of antimicrobial resistance in *Campylobacter jejuni* from broiler chickens were slightly lower than those from 2014 (EFSA, 2015). Yet, still more than half of the *Campylobacter jejuni* strains showed resistance to ciprofloxacin and nalidixic acid. Antimicrobial resistance to tetracycline reached 40%. Resistance to erythromycin was not present in *C. jejuni* from Belgian broiler chickens, whereas on average 5.9% erythromycin resistance has been found over 25 European countries (EFSA, 2015). 43.2% of *Campylobacter jejuni* showed full susceptibility and 37.5% showed resistance to at least 2 antimicrobial classes (quinolones and tetracyclines). Multi-resistance to quinolones, tetracycline and streptomycin was seen in one *Campylobacter jejuni* strain. Clinical resistance to both ciprofloxacin and tetracycline was seen in 38.1% of de strains.

Introduction

Campylobacteriosis is the most commonly reported gastrointestinal bacterial pathogen in humans in Belgium since 2005. In 2015, 6097 human cases have been reported (WIV-ISP, 2016). The species most commonly associated with human infection are *C. jejuni* followed by *C. coli* and *C. lari*, but other *Campylobacter* species are also known to cause human infections. The principal reservoir is the alimentary tract of wild and domesticated birds and mammals (poultry, cattle, pigs, sheep, pets, including cats and dogs) and in environmental water sources. Animals are mostly asymptomatic carriers. The main source of contamination is insufficiently cooked food, mainly chicken meat. The bacteria can also contaminate raw milk and dairy products. Cross-contamination in the kitchen of vegetables may also contain a risk.

Since 2011, the Federal Agency for the Safety of the Food Chain implements a monitoring of antimicrobial resistance in certain pathogenic and indicator bacteria from cattle, pigs and poultry. In order to get an overview of the occurrence of antimicrobial resistance in the European Union (EU), antimicrobial resistance monitoring became mandatory in 2014 in all Member States for *Escherichia coli*, *Salmonella* and *Campylobacter jejuni* in the major food-producing animal populations (broilers, laying hens, fattening turkeys, fattening pigs, calves) and their derived meat (Decision 2013/652/EU). In the future, data on the antimicrobial resistance monitoring will ideally be combined with those on exposure to antimicrobials.

In this report information is presented on the prevalence of antimicrobial resistance in *Campylobacter* spp. in broiler chickens in Belgium. Antimicrobial resistance data of *Campylobacter* spp. from food products from animals, as potential sources for distribution to humans via the food chain, are reported by the Institute for Public Health (WIV-ISP).

Materials and methods

Sampling

For the isolation of *Campylobacter* spp., fresh fecal samples were collected from broiler chickens. A representative sampling was performed according to general provisions of the legislation and to detailed technical specifications issued by EFSA (2014).

The sample collection was approximately evenly distributed over the year 2016.

Caecal content of broiler chickens was taken at slaughter. Caeca from 10 animals were collected and pooled. One sample originated from one farm to account for clustering.

Isolation and identification

Campylobacter isolates were identified by MALDI-TOF following a standard extraction procedure recommended by the manufacturer (BRUKER DALTONICS).

Antimicrobial susceptibility testing

Upon arrival at CODA-CERVA, strains were tested for their antimicrobial susceptibility using a micro broth dilution method (Trek Diagnostics). To this end, 1 to 3 colonies were suspended in sterile physiological water to an optical density of 0.5 McFarland. Ten microliter of this suspension is inoculated in 11 ml cation adjusted Mueller Hinton broth with TES buffer.

Fifty microliter of the Mueller-Hinton broth with bacteria was brought on a micro-titer plate containing freeze-dried antimicrobials produced by Trek Diagnostics, using the auto-inoculating system of Trek Diagnostics. The antimicrobial substances incorporated in the antimicrobial susceptibility testing were recommended by the European Food Safety Agency (EFSA) and included in Commission decision 2013/652/EU. They were selected based on their public health relevance and as representatives of different antimicrobial classes (EFSA, 2012). Table 1 shows the antimicrobial substances tested, their abbreviations, the dilutions used, the epidemiological cut-off's (ECOFFs) and clinical breakpoints (CBP), established by the European Committee on Antimicrobial Susceptibility (EUCAST) or as defined by the EU reference laboratory on antimicrobial resistance (DTU) (EUCAST, 2017). No CBP are available for gentamicin, nalidixic acid and streptomycine. Plates were incubated 18-24 hours at 35°C and read. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration by which no visible growth could be detected. MICs were semi-automatically recorded by the Trek Vision system using the SWIN software. Results were automatically exported to an Excel file.

Table 1 : Panel of antimicrobial substances included in antimicrobial susceptibility testing, EUCAST epidemiological cut-off's (ECOFFs) and clinical breakpoints (CBPs), and concentration ranges tested in *Campylobacter* spp.

Antimicrobial (Abbreviation)	Concentration range, mg/l	EUCAST ECOFF	EUCAST CBP
Ciprofloxacin (CIP)	0.12–16	> 0.5	> 0.5
Erythromycin (ERY)		> 4	> 4 (*C. jejuni*) / > 8 (*C. coli*)
Gentamicin (GEN)		> 2	NA
Nalidixic acid (NAL)	1–64	> 16	NA
Streptomycin (STR)		> 4	NA
Tetracycline (TET)		> 1	> 2

EUCAST: European Committee on Antimicrobial Susceptibility Testing
NA: not available

Data analysis and description

Data from the Excel file generated by the software of the semi-automated susceptibility equipment (sensivision, Trek Diagnostics) were merged to the administrative data from the LIMS system at CODA-CERVA. These files were validated for consistency.

Isolates with a MIC value above the ECOFF value were considered not to belong to the wild type population and percentages of isolates with a reduced susceptibility, i.e. non-wild type, were calculated. Throughout the report, isolates with a reduced susceptibility will be referred to as 'resistant isolates', whereas when the clinical interpretative criterion was used, the term 'clinical resistance' will be used.

The number of antimicrobials to which a strain was resistant was counted and cumulative percentages or percentiles were calculated. Graphical representations were prepared in Excel.

Throughout the report, terms used to describe the levels or occurrence of antimicrobial resistance are those proposed by EFSA. Rare: <0.1 %', 'very low: >0.1 % to 1.0 %', 'low: >1 % to 10.0 %', 'moderate: >10.0 % to 20.0 %', 'high: >20.0 % to 50.0 %', 'very high: >50.0 % to 70.0 %', 'extremely high: >70.0 %'. Although these terms are applied to all antimicrobials, the significance of a given level of resistance will depend on the particular antimicrobial and its importance in human and veterinary medicine.

A multi-resistant isolate is one defined as resistant to at least three different antimicrobial substances, belonging to any three antimicrobial classes represented by the antimicrobials included in the analysis (Table 1). Resistance to nalidixic acid and resistance to ciprofloxacin are addressed together when considering multi-resistance.

Results

Antimicrobial resistance

Antimicrobial resistance was very high for ciprofloxacin and high for nalidixic acid and tetracycline. Antimicrobial resistance was only very low to streptomycin and not detected for erythromycin and gentamicin (Figure 1).

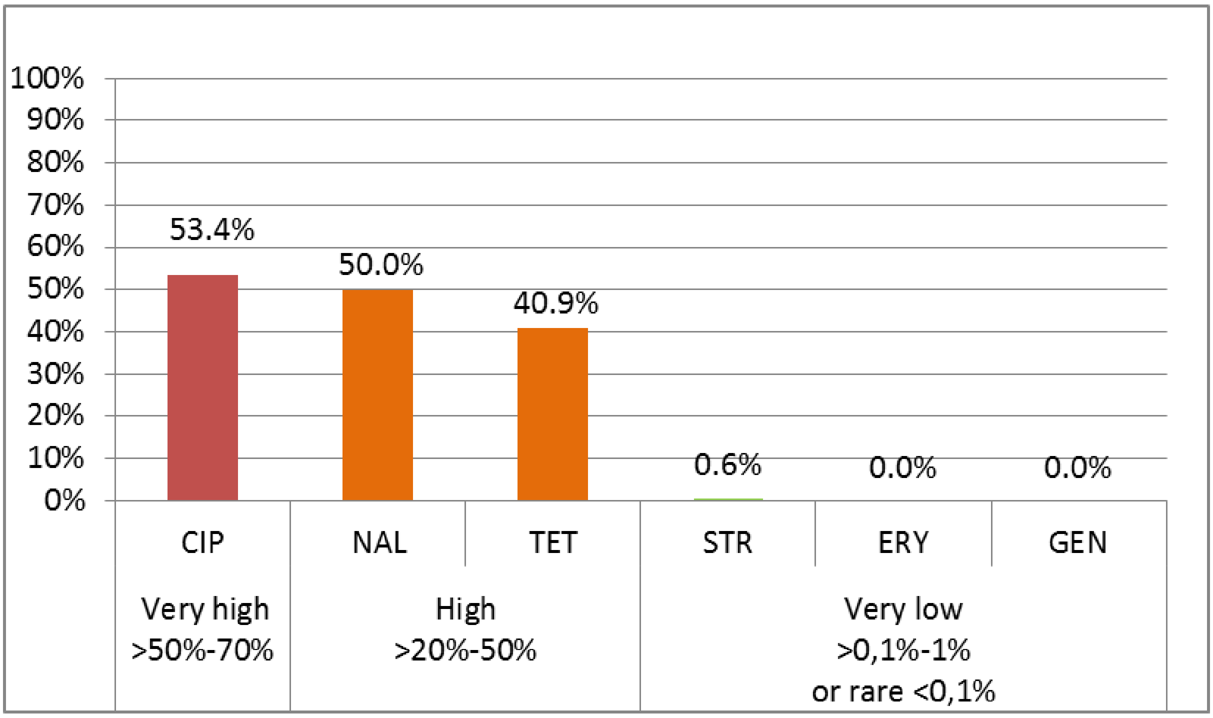

Figure 1 : Antimicrobial resistance prevalence for *Campylobacter jejuni* (n= 176), isolated from broiler chickens at slaughter, based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for ciprofloxacin (CIP), nalidixic acid (NAL), tetracycline (TET), streptomycin (STR), erythromycin (ERY) and gentamicin (GEN).

Multiple antimicrobial resistance patterns

In broiler chickens, 43.2% of *Campylobacter jejuni* showed full susceptibility and 37.5% showed resistance to at least 2 antimicrobial classes (quinolones (ciprofloxacin and nalidixic acid) and tetracyclines). Multi-resistance to quinolones, tetracycline and streptomycin was seen in one *Campylobacter jejuni* strain. Clinical resistance to both ciprofloxacin and tetracycline was seen in 38.1% of de strains.

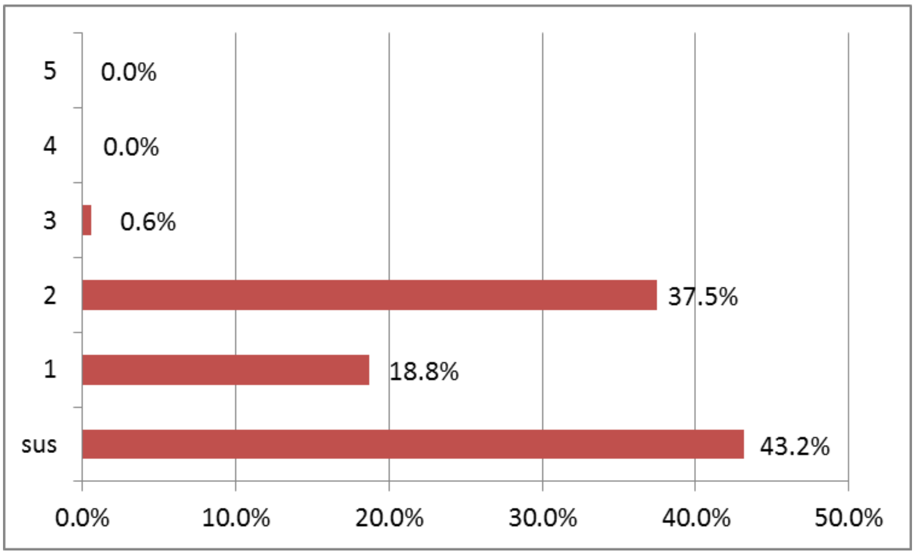

Figure 2 : Percentages of *Campylobacter jejuni* from broiler chickens (n= 176) showing full susceptibility (sus) or resistance to at least 1 antimicrobial. Resistance to ciprofloxacin and nalidixic acid are addressed together.

Discussion

Overall, the levels of antimicrobial resistance in *Campylobacter jejuni* from broiler chickens were slightly lower than those from 2014 (EFSA, 2015). Yet, still more than half of the *Campylobacter jejuni* strains showed resistance to ciprofloxacin and nalidixic acid. Antimicrobial resistance to tetracycline reached 40%. Treatment of enteric infections in humans may involve administration of macrolides, such as erythromycin, or fluoroquinolones (e.g. ciprofloxacin), as the first- and second-choice drugs (ECDC et al., 2009). With ciprofloxacin, resistance may develop rapidly. Resistance to erythromycin was not present in *C. jejuni* from Belgian broiler chickens, whereas on average 5.9% erythromycin resistance has been found over 25 European countries (EFSA, 2015). Resistance to macrolides in *Campylobacter* spp. has generally been the result of mutations in ribosomal RNA or ribosomal proteins and these mutations are thought to have incurred fitness costs, accounting for the low occurrence of erythromycin resistance in many countries (Wang et al., 2015). The recent emergence of transferable macrolide resistance in *Campylobacter* spp. may provide a means whereby macrolide resistance can spread rapidly in *Campylobacter* spp. The situation may be compared to tetracycline resistance, which is frequently plasmid mediated in *Campylobacter* spp., and is frequently detected in Belgium and other European countries.

Tetracycline resistance, frequently detected in Belgium as well as in many European countries, is frequently plasmid mediated in *Campylobacter* spp. The acquisition of the erm(B) gene by successful circulating tetracycline resistance plasmids in C. coli from fattening pigs could provide a rapid means of dissemination of macrolide resistance, since such plasmids would confer resistance to both macrolides and tetracyclines and be subject to co-selection.

Supplementary data

Table 1: Minimum Inhibitory Concentrations for *Campylobacter jejuni strains* (n= 176), isolated from broiler chickens at slaughter, based on epidemiological cut-off's, according to the European Committee on Antimicrobial Susceptibility (EUCAST) for ciprofloxacin (CIP), nalidixic acid (NAL), tetracycline (TET), streptomycin (STR), erythromycin (ERY) and gentamicin (GEN).

	<=0.008	<=0.015	<=0.03	<=0.06	<=0.12	<=0.25	<=0.5	<=1	<=2	<=4	<=8	16	32	64	128	256	512	1024	2048
CIP	-	-	-	-	68	10	4	0	0	32	29	30	3	-	-	-	-	-	-
NAL	-	-	-	-	-	-	-	11	61	7	2	7	27	49	12	-	-	-	-
TET	-	-	-	-	-	-	86	18	1	2	11	5	5	26	22	-	-	-	-
STR	-	-	-	-	-	32	108	32	3	0	0	1	0	0	0	0	0	0	0
ERY	-	-	-	-	-	-	-	169	7	0	0	0	0	0	0	0	0	0	0
GEN	-	-	-	-	92	71	13	0	0	0	0	0	0	0	0	0	-	-	-

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