National Reference Center for *Campylobacter*

Activity report 2020-2023

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1. Objectives

The national reference center (NRC) *Campylobacter* supports clinical laboratories with its expertise in the identification, typing and analysis of *Campylobacter* resistance profiles.

The NRC also contributes to the epidemiologic surveillance of *Campylobacter* infections, the evaluation of new analytical techniques and the dissemination of recommendations for good analytical practice developed in collaboration with the other European *Campylobacter* NRCs.

Finally, the NRC acts as an advisor to clinical biology laboratories and other healthcare professionals.

This report describes the activities of the *Campylobacter* NRC in the surveillance of both enteric and invasive *Campylobacter* infections.

2. Analysis of the LHUB-ULB data

2.1 Samples

In the absence of systematic annual surveillance of campylobacteriosis at the Belgian level, the data on enteric infections are based on the epidemiologic surveillance carried out in the Brussels region by our lab, the Laboratoire des Hôpitaux universitaires de Bruxelles-Universitair Laboratorium Brussel (LHUB-ULB), that provides clinical biology analyses for five university hospitals. This section of the report reflects the activity regarding *Campylobacter* and related organisms infections of the Porte de Hal site, which combines the activities of UHC Saint-Pierre, Jules Bordet Institute, UHC Brugmann, Queen Fabiola Children's university hospital and Erasme academic hospital.

2.2 Techniques

In our laboratory, stool samples sent for *Campylobacter* culture are analyzed by standard selective culture on Butzler agar incubated at 42°C, but also according to the Cape Town filtration protocol. This protocol recommends that stools be filtered on antibiotic-free culture media and incubated for five days at 37°C in a hydrogen-enriched micro-aerobic atmosphere. Antimicrobial susceptibility to erythromycin, ciprofloxacin, tetracycline, ampicillin, and amoxicillin/clavulanic acid is determined by the diffusion disk method. Clinical categorization of strains (resistant, intermediate or susceptible) according to zones of inhibition is read manually or using an automated disc reader based on EUCAST (where available) and CA-SFM clinical breakpoints. In the case of erythromycin resistance, the Minimal Inhibition Concentration (MIC) of strains to erythromycin, gentamycin and tetracycline is assessed using gradient strips.

2.3 Number of feces cultures & species distribution

The LHUB-ULB performs an average of 9,600 stool feces cultures per year. The distribution of stool samples collected by each partner hospital between 2020 and 2023 is shown in Table 1. The lower numbers of samples received in 2020 and 2023 are explained by the fact that at the time of the COVID pandemic, we asked our prescribers to limit the collection of stool samples, and by the fact that in the last quarter of 2023, some of the stool samples from UHC Brugmann and Queen Fabiola Children's university hospital were not analyzed in our laboratory.

Table 1: Distribution of stool samples collected by each partner hospital (2020-2023)

Hospital	2020	2021	2022	2023
Erasme	2197	2373	2129	2115
Bordet	2204	2016	2403	2524
Brugmann	1981	2196	1964	1556
Saint-Pierre	2028	2561	2481	2167
Huderf	812	944	930	821
Total	**9222**	**10090**	**9907**	**9183**

The average positivity rate was around 8% and the isolated species were as follows: *C. jejuni, C. coli, C. ureolyticus, C. concisus, C. curvus, C. upsaliensis, C. gracilis, C. hyointestinalis, C. showae, C. fetus, C. lari, A. butzleri, A. cryaerophilus, H. pullorum, H. cinaedi.*

The proportions of different species over the years are shown in Figure 1 and the distribution of the different species isolated in 2023 is presented in Figure 2.

Figure 1: proportions of *Campylobacter* sp over years

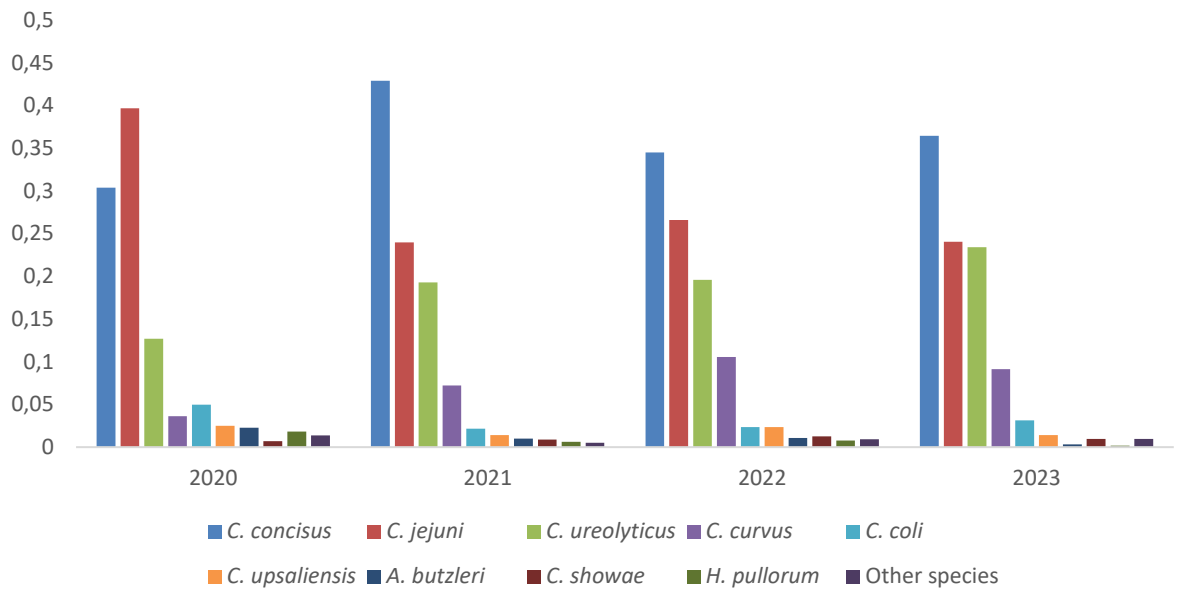
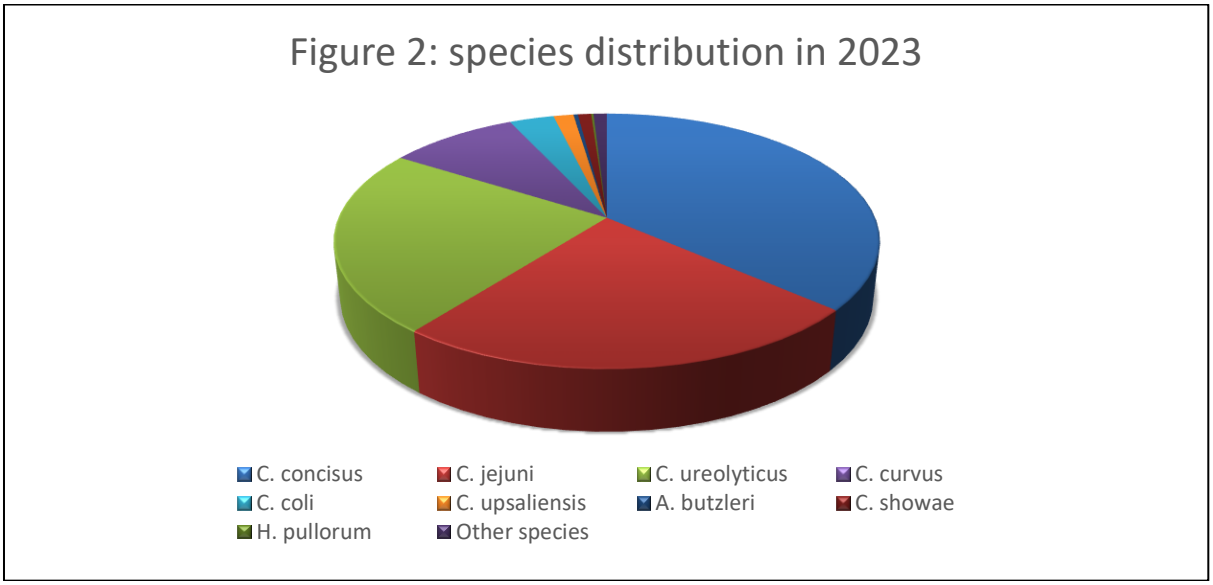


Figure 2: species distribution in 2023



In 2023, 9,183 stools were analyzed, of which, 694 (8%) were positive for *Campylobacter* or related organisms. After deduplication, 636 episodes were considered. The main species isolated were *C. concisus* (36.5%), *C. jejuni* (24%) and *C. ureolyticus* (23.4%). *C. coli*, recognized as the most important Campylobacter responsible for bacterial gastroenteritis after *C. jejuni*, accounted for only 3% of cases. Interestingly, the mean numbers of *C. jejuni* isolates decreased significantly between the pre- and post-COVID-19 periods, while *C. ureolyticus* showed a marked increase after the pandemic (under investigation).

2.4 Antibiotic resistance

Antibiotic susceptibility testing using the disk diffusion method showed that over 99% of *C. jejuni* remained susceptible to erythromycin (n=466), compared to only 85% for *C. coli* (n=17). Susceptibility to quinolones was only 43% and 30% for *C. jejuni* and *C. coli*, respectively. For *C. ureolyticus*, 85, 30 and 30% of the strains were susceptible to erythromycin, ciprofloxacin and tetracycline, respectively. These data are based on all strains isolated between 2021 and 2023 and are presented in Table 2. The year 2020 was excluded due to its non-representativeness (COVID-19) and the limited number of strains isolated.

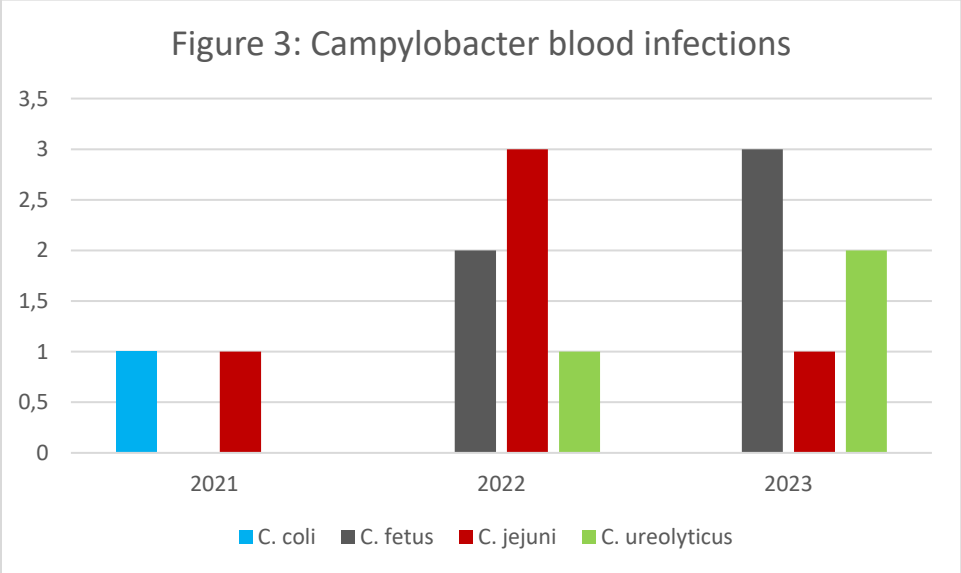
Table 2: Mean proportion of resistant strains among the most commonly isolated species (2021-2023)

Resistance (%)	Erythromycin	Ciprofloxacin	Tetracycline
C. jejuni (n=469)	0.6%	57%	43%
C. ureolyticus (n=369)	12%	44%	6%
C. coli (n=47)	15%	70%	70%

With the exception of *C. coli* resistance rates to erythromycin (33% in 2023) and ciprofloxacin (88% in 2023), which appear to be increasing over time, the resistance levels observed appear to be stable over time. The limited number of *C. coli* strains per year (2021 n=15; 2022 n=14; 2023 n=18) may explain these fluctuations, and these observations remain subject to caution.

2.5 Invasive infections

During the period under review, 18 cases of *Campylobacter* blood infections were detected in our network: none in 2020, 2 in 2021, 6 in 2022 and 6 in 2023. The main species involved are *C. jejuni* and *C. fetus* and, since 2022, *C. ureolyticus* (ongoing publication). The distribution of the strains is shown in figure 3. While the all cases in 2021 concerned children, 50% of the cases in 2022 and 2023 concerned immunocompromised patients.



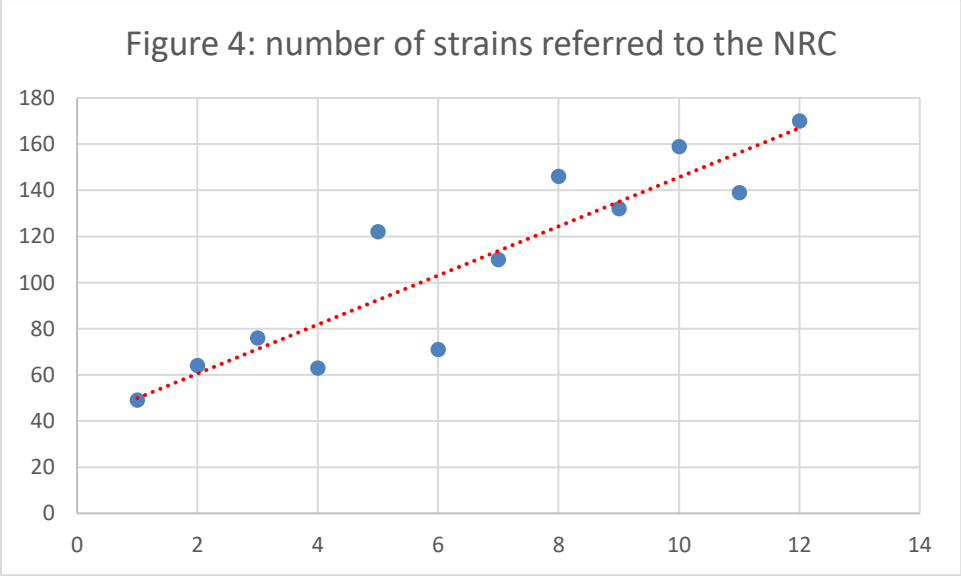
3. NRC data analysis

3.1 Participating laboratories

Sixty laboratories sent a total of 601 strains to the NRC between 1 January 2020 and 31 December 2023. The distribution of strains per year and per province (based on the patient's postal code) is shown in Table 3. The number of strains referred to the NRC increased slowly in 2023, mainly as a result of more strains being sent from Antwerp, Namur and Limbourg provinces. This total number of strains is significantly higher than in the early days of the NRC, when the average number of strains received was around 50 per year. The changes over time in the number of strains referred to the NRC are shown in Figure 4.

Table 3: Distribution of strains by year and by province (2020-2023)

	2020	**2021**	**2022**	**2023**
Hainaut	64	76	66	75
Antwerp	13	13	15	27
West Flanders	7	13	15	17
Namur	1	3	1	14
Flemish Brabant	17	10	11	11
East Flanders	11	14	10	11
Limburg	7	6	4	10
Walloon Brabant	2		3	2
Brussels	8	5	6	2
Liege	1	4	3	
Foreign country		2	1	1
Unknown	2	13	4	
Total général	**132**	**159**	**139**	**170**



3.2 Distribution of strains according to isolation site and age groups

The origin of the strains sent is shown in Table 4. In about 8% of cases the origin was not specified. The vast majority of strains received were isolated from stool followed by blood. The number of strains isolated from blood increased significantly from 24 in 2022 to 44 in 2023.

Table 4: Origin of the strains referred to the NRC

	2020	2021	2022	2023
Stool	108	120	101	100
Blood	24	29	24	44
Unknown	1	7	12	18
Other		3	2	8
Total	133	159	139	170

The distribution of invasive and enteric strains by age group is shown in Table 5. Most invasive strains were isolated from patients aged over 45 years (77.6%), whereas the distribution of enteric strains was more homogenous according to age group: 26.3% <15 years, 27.5% 16 to 45 years, 22.1% 45 to 65 years and 22.6% over 65 years.

Table 5: Distribution of strains referred to the NRC according to age groups

	2020	2021	2022	2023	Total	Percentage
Blood	**24**	**29**	**24**	**44**	**121**	**%**
<1 y	1			1	2	**1,7**
1-5 y	2	1			3	**2,5**
6-15 y		2	2	1	5	**4,1**
16-25 y	1	1	1	1	4	**3,3**
26-45 y	2	2	2	5	11	**9,1**
46-65 y	7	5	8	12	32	**26,4**
> 65 y	11	18	11	22	62	**51,2**
Stool	**108**	**120**	**101**	**100**	**429**	
<1 an	8	2	2	1	13	**3,0**
1-5 y	18	21	6	10	55	**12,8**
6-15 y	12	14	11	8	45	**10,5**
16-25 y	10	9	13	8	40	**9,3**
26-45 y	17	20	23	18	78	**18,2**
46-65 y	22	28	20	25	95	**22,1**
> 65 y	21	25	22	29	97	**22,6**
Total	**133**	**159**	**139**	**170**	**601**	

3.3 Species distribution

The distribution of species of invasive and enteric strains that could be revived and were indeed *Campylobacter* or related organisms is shown in Table 6. Among invasive strains, *C. jejuni* remained the most frequently isolated species, generally followed by *C. fetus.*
As described in the literature, and given that most laboratories only use a selective medium for *Campylobacter* culture, *C. jejuni* and *C. coli* together represent 80% of the strains responsible for enteric infections at the national level. The proportion of non-thermophilic strains transmitted to the NRC remains anecdotal, and the main non-*jejuni*/*coli* species referred to the NRC for identification belong to related genera such as *Helicobacter* or *Arcobacter*.

Antibiotic resistance in the main species responsible for invasive infections is shown in table 7. As enteric strains are sent on a voluntary basis, they are not used to draw any epidemiological conclusions.

Table 6: Species distribution of invasive and enteric strains transmitted to the NRC (2020-2023)

	2020	**2021**	**2022**	**2023**	**Total**
Blood	24	25	24	43	116
C. jejuni	13	18	13	27	71 (61,2%)
C. fetus	5	3	8	6	22 (18,9%)
C. coli	5	2	3	8	18 (15.5%)
C. lari		1		1	2 (1,7%)
A. butzleri		1			1 (0,8%)
Campylobacter sp				1	1 (0,8%)
C. ureolyticus	1				1 (0,8%)
Stool	106	117	100	96	419
C. jejuni	55	74	57	55	241 (57,5%)
C. coli	36	31	31	33	131 (31,3%)
H. pullorum	14	9	10	4	37 (8,8%)
A. butzleri	1	3	1	3	8 (1,9%)
C. concisus			1		1 (0,2%)
C. upsaliensis				1	1 (0,2%)

Table 7: Mean proportion of resistant *C. jejuni* and *C. fetus* isolated from blood specimen (2020-2023)

Resistance (%)	Erythromycin	Ciprofloxacin	Tetracycline
C. jejuni (n=71)	0	0	33,8
C. fetus (n=22)	4,5	4,5	4,5

4. Validation of new techniques and ongoing studies

The NRC carries out cultures using the filtration technique, which allows isolating a significant proportion of campylobacters not isolated by culture on selective media alone. As a result, our average positivity rate of stool cultures is around 8%. About ten non-*jejuni*/*coli* species are isolated in this way, the pathogenicity of which is sometimes controversial. In this context, we observed an increase of *C. ureolyticus* in the post-COVID-19 period, which is currently under investigation (epidemiology and clinical implications).

In addition, the Belgian nomenclature should soon evolve to include molecular diagnostics on stool samples. The performance of the tools available on the market is variable, which raises a number of questions about the results obtained: how should a "*Campylobacter sp*." result be interpreted? Is it necessary to perform a reflex culture? In this context, the NRC has revalidated a *Campylobacter* sp. PCR (not much in demand so far) and put it on the BELAC scope, and has applied for a clinical study budget to evaluate several syndromic panels in the field. A student was supervised on the PCR project, which constituted his final technologist dissertation (subject, school).

The NRC also participated in the thesis committee and jury of Mrs. Maureen Feucherolles (Luxembourg Institute of Science and Technology), whose thesis is entitled " *MALDI-TOF-enabled subtyping and antimicrobial resistance screening of the food- and waterborne pathogen Campylobacter ",* defended in June 2022. The following articles have been published in this context:

- Feucherolles M, Nennig M, Becker SL, Martiny D, Losch S, Penny C, Cauchie HM, Ragimbeau C. *Combination of MALDI-TOF Mass Spectrometry and Machine Learning for Rapid Antimicrobial Resistance Screening: The Case of Campylobacter spp*. Front Microbiol. 2022 Feb 18;12:804484. doi: 10.3389/fmicb.2021.804484. PMID: 35250909; PMCID: PMC8894766.
- Feucherolles M, Nennig M, Becker SL, Martiny D, Losch S, Penny C, Cauchie HM, Ragimbeau C. *Investigation of MALDI-TOF Mass Spectrometry for Assessing the Molecular Diversity of Campylobacter jejuni and Comparison with MLST and cgMLST: A Luxembourg One-Health Study. Diagnostics* (Basel). 2021 Oct 20;11(11):1949. doi: 10.3390/diagnostics11111949. PMID: 34829296; PMCID: PMC8621691.

Finally, the NRC's activities in 2023 focused on the development of its in-house whole genome sequencing capability with a tender for the acquisition of a sequencer as well as reagents to enable the validation of our pipeline. The tender was completed at the end of the third quarter with the purchase of an Illumina miniSeq. At the request of one of our customers, we also performed by WGS the typing and comparison of strains in the investigation of a *Campylobacter* prosthetic joint infection (ongoing publication).

5. « Take Home » messages

The number of *Campylobacter* and related organisms strains transmitted to the NRC increases year on year.

Selective media allow isolation of thermophilic campylobacters only. Among the species isolated using the filtration technique, *C. ureolyticus* appears to be on the increase since 2020.

Erythromycin remains the treatment of choice for campylobacteriosis, when treatment is indicated. Resistance is less than 1% in *C. jejuni* and around 10-15% in *C. coli* and *C. ureolyticus*. Resistance to quinolones, on the other hand, ranges from 44 to 70%, depending on the species.

The number of invasive strains referred to the NRC increased significantly in 2023. *C. jejuni* remains the leading species responsible for invasive infections, followed by *C. fetus* and *C. coli*. The sensitivity of invasive strains (*C. jejuni* and *C. fetus*) to erythromycin and ciprofloxacin remains above 95%.

Attention should be paid to the introduction of syndromic panels for the diagnosis of enteric infections: test performance varies, and results are sometimes difficult to interpret. Reflex culture seems essential, but due to the fragility of non-*jejuni*/*coli* species will probably require the collection of a new sample.

6. Team contact details

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