# National reference centre for *Campylobacter*

## Activity report 2024

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## 1. Objectives

The national reference centre (NRC) for *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 microbiological surveillance of campylobacteriosis at the Belgian level, the data on enteric infections are based on the surveillance carried out in the Brussels region by the LHUB-ULB (the University-Hospital Laboratory of Brussels). This section of the report reflects the activity regarding *Campylobacter* infections and related organisms of the Porte de Hal site, which combines the activities of UHC Saint-Pierre, Jules Bordet Institute 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 sensitivity of strains to erythromycin, gentamycin and tetracycline is assessed using gradient strips.

### 2.3 Number of feces cultures & species distribution

The LHUB-ULB performed about 6.000 stool feces cultures in 2024. In 2024, the decrease in the total number of stools analyzed in comparison with the previous year is mostly attributable to the departure of one of our hospital partners (9,183 in 2023 vs. 5,928 in 2024). The distribution of stool samples collected by each partner hospital is shown in Table 1.

Table 1: Number of stools analyzed for each partner hospital in 2024

| Hospital | n |
|---|---|
| Erasme | 2011 |
| Bordet | 2263 |
| Saint-Pierre | 1654 |
| **Total** | **5928** |

A total of 5,928 stools were analyzed, of which, 524 (8.8%) were positive for *Campylobacter* or related organisms. After deduplication, 455 episodes were considered. The distribution of the different species isolated in 2024 is presented in Figure 1. The main species identified were *C. concisus* (167; 36.7%), *C. jejuni* (114; 25.1%) and *C. ureolyticus* (81; 17.8%). *C. coli*, recognized as the most important *Campylobacter* responsible for bacterial gastroenteritis after *C. jejuni*, accounted for only 3.3% 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 [1]. In 2024, there was a slight decrease in *C. ureolyticus*, whilst *C. curvus* increased (51; 11.2%). The progression of the proportion of each species between 2019 and 2024 is delineated in Figure 2.

Figure 1: species distribution in 2024

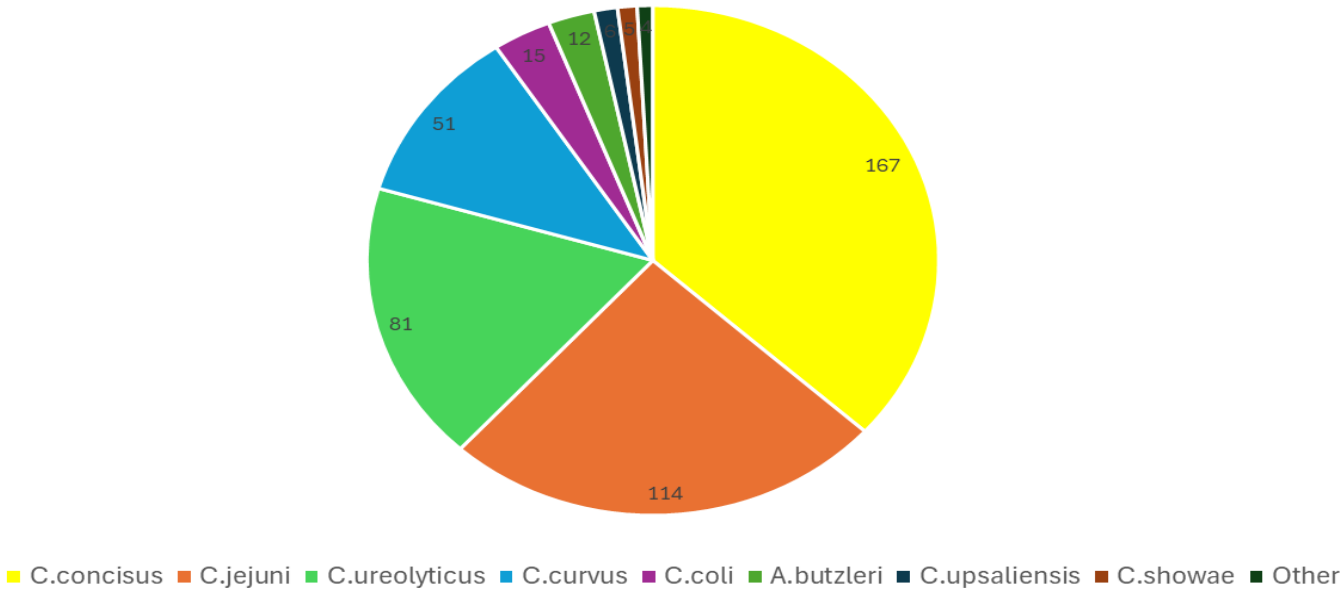
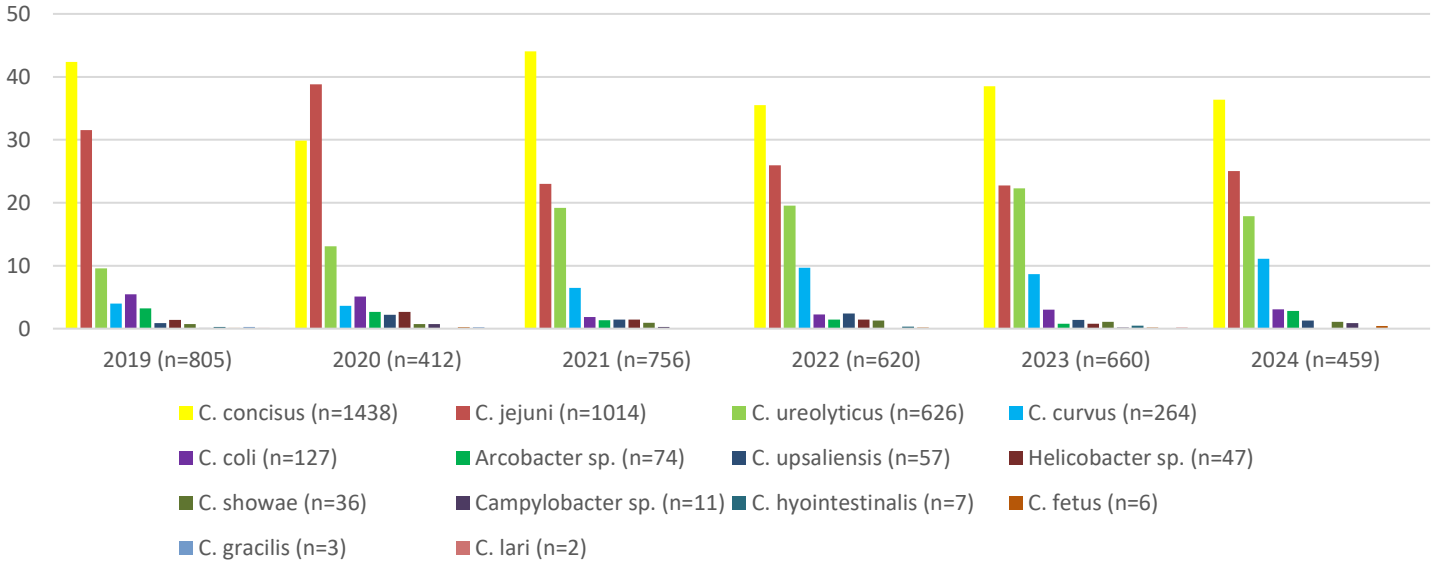



Figure 2: Proportion (%) of *Campylobacter* species isolated at the LHUB-ULB over years: 2019 et 2024



## 2.3 Antibiotic resistance

Antibiotic susceptibility testing using the disk diffusion method demonstrated that fewer than 2% of *C. jejuni* exhibited resistance to azithromycin, as opposed to 8% for *C. coli* (n = 13) and 15% for *C. ureolyticus*. This high rate of azythromycin resistance in *C. ureolyticus* has been previously documented in 2023.

A decline in susceptibility to quinolones has been observed since 2023, with only 33% of *C. jejuni* and 15% of *C. coli* exhibiting susceptibility in 2024 (compared to 43% and 30%, respectively, in 2023). *C. ureolyticus* demonstrated resistance to ciprofloxacin in approximately 40% of cases, which aligns with the figures observed in 2023 (38% vs. 44%).

The resistance rate to tetracyclin exhibited stability in comparison to 2023, with the exception of *C. coli* (46% versus 70% in 2023). The limited number of *C. coli* strains per year (2023 n=18, 2024 n=13) may serve as a potential explanation for these fluctuations, and these observations therefore remain subject to caution.

The data presented herein are derived from all strains isolated in 2024 for which an AST was performed. These data are presented in Table 2.

Table 2: Proportion of resistant strains among *C. jejuni*, *C. coli* and *C. ureolyticus* (2024)

| Resistance (number of tested isolates) | Azithromycin (%) | Ciprofloxacin (%) | Tetracycline (%) |
|---|---|---|---|
| *C. jejuni (n=109)* | 1.8 | 67 | 42 |
| *C. ureolyticus (n=60)* | 15 | 38 | 5 |
| *C. coli (n=13)* | 8 | 85 | 46 |

Antimicrobial susceptibility testing for *C. ureolyticus* was performed using the CA-SFM breakpoints, without taking into account the recommendations to consider it as an anaerobic bacterium. Indeed, despite it has long been considered as part of Bacteroides genus, the bacteria is unable to grow under anaerobic conditions. An evaluation is currently underway to establish reliable ECOFF breakpoints.

## 2.4. Invasive infections

In 2024, three cases of *Campylobacter* bacteremia were identified within our network, with the causative agents identified as *C. fetus, C. jejuni*, and *C. coli*. All of the patients concerned are aged 60 and over. In previous years, the predominant species were *C. jejuni* and *C. fetus*, with *C. ureolyticus* emerging as a significant pathogen since 2022. Notably, no cases of *C. ureolyticus* infection were observed in 2024.

## 3. NRC data analysis

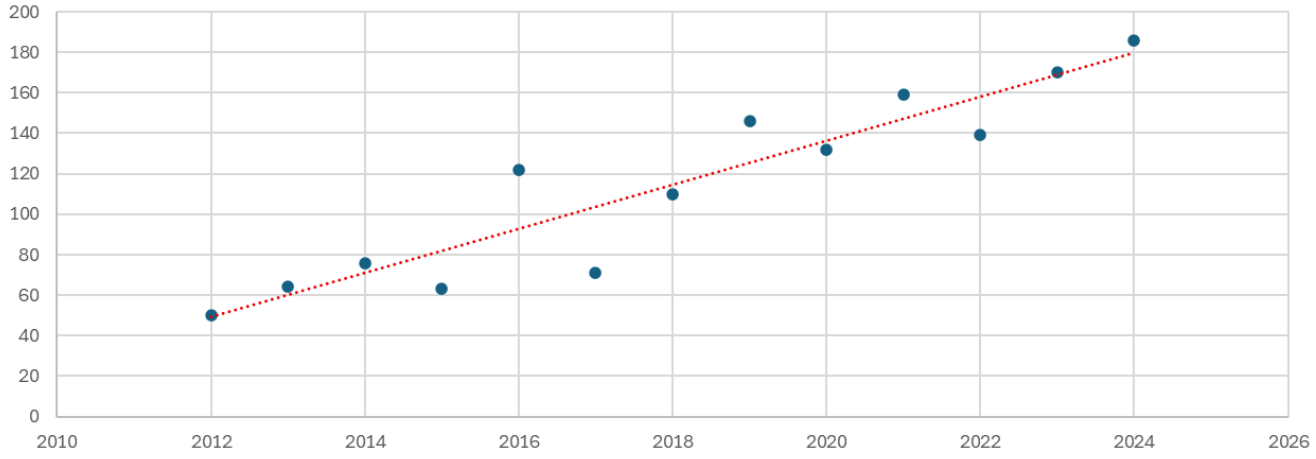
### 3.1 Participating laboratories

More than 40 laboratories sent a total of 186 strains to the NRC in 2024. 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 2024, primarily due to an increase in strains being sent from the province of Hainaut. 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 3.

Table 3: Distribution of strains by year and by province (2024)

| | **2020** | **2021** | **2022** | **2023** | **2024** |
|---|---|---|---|---|---|
| *Hainaut* | 64 | 76 | 66 | 75 | 102 |
| *Antwerp* | 13 | 13 | 15 | 27 | 28 |
| *West Flanders* | 7 | 13 | 15 | 17 | 8 |
| *Namur* | 1 | 3 | 1 | 14 | 5 |
| *Flemish Brabant* | 17 | 10 | 11 | 11 | 7 |
| *East Flanders* | 11 | 14 | 10 | 11 | 9 |
| *Limburg* | 7 | 6 | 4 | 10 | 13 |
| *Walloon Brabant* | 2 | | 3 | 2 | 1 |
| *Brussels* | 8 | 5 | 6 | 2 | 6 |
| *Liege* | 1 | 4 | 3 | | 5 |
| *Foreign country* | | 2 | 1 | 1 | 1 |
| *Unknown* | 2 | 13 | 4 | | 0 |
| ***Total général*** | **132** | **159** | **139** | **170** | **186** |

Figure 3: Number of strains referred to the NRC: 2012-2024



3.2 Distribution of strains according to isolation site and age groups

Five out of the 186 strains were no *Campylobacter* or related organisms. The origin of the 181 remaining strains is shown in Table 4. The vast majority of strains received were isolated from stool (n= 139) followed by blood (n=40). The number of strains isolated from blood remained consistent when compared to 2023 (40 vs 44 in 2023).

Table 4: Origin of the strains

| | 2020 | 2021 | 2022 | 2023 | 2024 |
|---|---|---|---|---|---|
| **Stool** | 108 | 120 | 101 | 100 | 139 |
| **Blood** | 24 | 29 | 24 | 44 | 40 |
| **Unknown** | 1 | 7 | 12 | 18 | 0 |
| **Other** | | 3 | 2 | 8 | 2 |
| **Total** | 133 | 159 | 139 | 170 | 181 |

The distribution of invasive and enteric strains by age group is demonstrated in Table 5. The majority of invasive strains were isolated from patients aged over 45 years (>90%), whereas approximately 80% of patients with gastro-enteritis are older than 25 years old.

Table 5: Distribution of strains according to age groups

| | 2020 | 2021 | 2022 | 2023 | 2024 | Percentage |
|---|---|---|---|---|---|---|
| **Blood** | **24** | **29** | **24** | **44** | **40** | |
| **<1 y** | 1 | | | 1 | 1 | 2,5% |
| **1-5 y** | 2 | 1 | | | 0 | 0% |
| **6-15 y** | | 2 | 2 | 1 | 1 | 2,5% |
| **16-25 y** | 1 | 1 | 1 | 1 | 4 | 10,0% |
| **26-45 y** | 2 | 2 | 2 | 5 | 3 | 7,5% |
| **46-65 y** | 7 | 5 | 8 | 12 | 11 | 27,5% |
| **> 65 y** | 11 | 18 | 11 | 22 | 20 | 50,0% |
| **Stool** | **108** | **120** | **101** | **100** | **139** | |
| **<1 an** | 8 | 2 | 2 | 1 | 3 | 2,2% |
| **1-5 y** | 18 | 21 | 6 | 10 | 5 | 3,6% |
| **6-15 y** | 12 | 14 | 11 | 8 | 21 | 15,1% |
| **16-25 y** | 10 | 9 | 13 | 8 | 17 | 12,2% |
| **26-45 y** | 17 | 20 | 23 | 18 | 28 | 20,1% |
| **46-65 y** | 22 | 28 | 20 | 25 | 36 | 25,9% |
| **> 65 y** | 21 | 25 | 22 | 29 | 29 | 20,9% |

## 3.3 Species distribution

The distribution of species of invasive and enteric strains is shown in Table 6. Among invasive strains, *C. jejuni* remained the most frequently isolated species, 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 95.7% of the strains responsible for enteric infections at the national level. The proportion of non-thermophilic strains transmitted to the NRC remains anecdotal. It is interesting to note that no Arcobacter or Helicobacter species, which were previously the most frequently identified non-jejuni/coli species, were received in 2024. Three *C. lari*, which were responsible for enteric infections, were all derived from the same laboratory in the Hainaut region. However, no temporal link was identified between them.
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: distribution of species transmitted to the NRC: 2020-2024

| | **2020** | **2021** | **2022** | **2023** | **2024** | **Percentage** |
|---|---|---|---|---|---|---|
| **Blood** | **24** | **25** | **24** | **43** | **40** | |
| *C. jejuni* | 13 | 18 | 13 | 27 | 24 | 60,0% |
| *C. fetus* | 5 | 3 | 8 | 6 | 7 | 17,5% |
| *C. coli* | 5 | 2 | 3 | 8 | 8 | 20,0% |
| *C. lari* | | 1 | | 1 | 1 | 2,5% |
| *A. butzleri* | | 1 | | | | |
| *Campylobacter sp* | | | | 1 | | |
| *C. ureolyticus* | 1 | | | | | |
| **Stools** | **106** | **117** | **100** | **96** | **139** | |
| *C. jejuni* | 55 | 74 | 57 | 55 | 92 | 66,2% |
| *C. coli* | 36 | 31 | 31 | 33 | 41 | 29,5% |
| *H. pullorum* | 14 | 9 | 10 | 4 | | |
| *A. butzleri* | 1 | 3 | 1 | 3 | | |
| *C. concisus* | | | 1 | | | |
| *C. upsaliensis* | | | | | 1 | 0,7% |
| *C. lari* | | | | | 3 | 2,2% |
| *C. hyointestinalis* | | | | | 1 | 0,7% |
| *C. peloridis* | | | | | 1 | 0,7% |
| *Campylobacter sp.* | | | | | 1 (no growth) | |

Table 7: Antimicrobial resistance of *C. jejuni* and *C. fetus* isolated from blood specimen in 2024

| Resistance (%) | Erythromycin | Ciprofloxacin | Tetracycline | Ampicillin |
|---|---|---|---|---|
| *C. jejuni (n=19)* | 0 | 36.8 | 26.3 | 36.8 |
| *C. fetus (n=7)* | 0 | 0 | 0 | 0 |

## 4. Validation of new techniques and ongoing studies

The NRC carries out cultures using the filtration technique, which allows isolating a significant proportion of campylobacter not isolated by culture on selective media alone (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. The prevalence and dynamics of emerging Campylobacter in stool specimens in Brussels have been assessed and published. Preliminary data on the increase of *C. ureolyticus* in stool and blood specimens was also presented at several national and international meetings. The clinical significance of *C. ureolyticus* is currently being evaluated by the NRC, as is the establishment of reliable ECOFF breakpoints, as previously mentioned.

- Giraudon E, Miendje Deyi VY, Martiny D. Assessing the Prevalence and Dynamics of Emerging Campylobacterales in Human Stool Samples in Brussels by Filtration Culture. Pathogens. 2024 Jun 4;13(6):475. doi: 10.3390/pathogens13060475. PMID: 38921773; PMCID: PMC11206970.
- Giraudon E, Alexandre M, Hing M, Miendje Deyi VY, Martiny D. Assessing the prevalence and dynamics of emerging Campylobacterales in human stool samples in Brussels by filtration culture. Symposium on Diagnostic and surveillance of infectious diseases. Brussels, Belgium 2024 (Poster)
- Giraudon E, Prevost B, Martiny D. Retrospective study on *Campylobacter* spp. bacteremia in Belgium: 2014-2023. Symposium on Diagnostic and surveillance of infectious diseases. Brussels, Belgium 2024 (Poster)
- Giraudon E, Mairesse C, Miendje Deyi VY, Martiny D. Occurence of *C. ureolyticus* in both stool and blood human specimen in Belgium. CHRO Perth, Australia, 2024 (Poster)

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 collaborated with Sciensano and other NRCs that specialise in enteric pathogens in order to organise a national survey on diagnostic practices in Belgium and how these might evolve. In 2025, the NRC will also be carrying out an evaluation of several commercially available instruments for syndromic diagnosis of enteric diseases.

- W. van Dyck W, De Muylder G, Ceyssens PJ, Mattheus W, Crombé F, PierardD, Martiny D, Vandenberg O, Vernelen K, China B, Van den Abeele A, Van Cauteren D. Possible impact of culture-independent diagnostic techniques on surveillance of gastrointestinal pathogens in Belgium. ESCAIDE, Stockholm, Sweden 2024 (Poster)

Finally, in 2024, the NRC focused its activities on developing its in-house whole genome sequencing capability. Whole genome sequencing (WGS) was performed on a C. coli strain isolated from a prosthetic joint infection, and a literature review was conducted in close collaboration with colleagues from Jan Yperman Hospital. We also investigated the genomes of several collection strains to explore the molecular epidemiology, virulome and resistome of enteric Campylobacter in Belgium.

- Jonckheere S, Mairesse C, Vandecandelaere P, Vanbiervliet J, Terryn W, Somers J, Prevost B, Martiny D. *Campylobacter coli* Prosthetic Joint Infection: Case Report and a Review of the Literature. Pathogens. 2024 Sep 27;13(10):838. doi: 10.3390/pathogens13100838. PMID: 39452710; PMCID: PMC11510586.
- Jonckheere S, Mairesse C, Vandecandelaere P, Vanbiervliet J, Somers J, Prevost B, Martiny D. Whole-genome sequencing and analysis of Campylobacter coli isolated from Prosthetic Joint Infection. CHRO Perth, Australia, 2024 (Poster)
- Mairesse C, Giraudon E, Prevost B, Wautier M, Ahajjam F, Yin N, Martiny D Comprehensive epidemiological and genomic analysis of enteric *Campylobacter jejuni* strains in Brussels: Insights into antimicrobial resistance and virulence profiles. Symposium on Diagnostic and surveillance of infectious diseases. Brussels, Belgium 2024 (Poster)

## 5. « Take Home » messages

The number of Campylobacter and related organisms strains received at 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 between 2020 and 2023. This increase is not confirmed in 2024.

Macrolides remain the treatment of choice for campylobacteriosis, when treatment is indicated. Resistance is less than 2% in *C. jejuni* and around 10-15% in *C. coli* and *C. ureolyticus*. Resistance to quinolones, on the other hand, ranges from 38 to 85%, depending on the species.

The number of invasive strains referred to the NRC is comparable to the figure for 2023. *C. jejuni* is still the main species causing invasive infections, followed by *C. fetus* and *C. coli*.

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. The NRC is currently conducting an evaluation of most commercially available assays.

## 6. Team contact details

Head of the NRC: Phn Biol D. Martiny
Scientific manager: Mr Benoit Prevost

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In July 2024, the NRC relocated to the LHUB-ULB Porte de Hal site. The updated contact information is as follows:

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Useful telephone numbers:
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02/4352032 (Phn Biol D. Martiny)
02/4352096 (Mr Prevost)