Belgian National Reference Center for *Vibrio cholerae* and *Vibrio parahaemolyticus*

Activity report

2012 – 2021

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1 Introduction

One of the main mission of the national reference center for *Vibrio* sp. is to contribute to confirmation, to the epidemiological surveillance of cholera and to the evolution of non-cholera *Vibrio* of medical interest on the Belgian territory.

Regarding that activity, cholera is a notifiable disease to declare to the Belgian health authorities. All Belgian laboratories are invited to send to the national reference center *Vibrio cholerae* and *parahaemolyticus* (NRC-Vib) each strain of *Vibrio cholerae* isolated from stools to confirm the identification and production of toxin. As non-toxigenic *Vibrio cholerae*, *Vibrio parahaemolyticus, Vibrio vulnificus* and other species are sporadically associated to non-cholera vibriosis and suppurative infections, medical laboratories are also invited to send these isolates.

The NRC-Vib also collaborate with specialized laboratories in food microbiology for the surveillance of *Vibrio* contamination. The NRC-Vib is also doing some environmental surveillances of water.

During the period, 2012-2021, laboratories sent 217 *Vibrio* sp. strains to the NRC-Vib, 53 were from human clinical specimens and 164 were from quality control for food industry. Clinical strains were isolated mainly from stools, ears, blood and wound.

2 General and specific missions of the NRC *Vibrio cholerae* and *parahaemolyticus*

2.1 General missions:

- To guarantee and widen its expertise in the field of *Vibrio* sp., prevention strategies, development and evaluation of new reagents or diagnostic tools.
- To support and advise the laboratories informing them about new methods, new culture media or new diagnostics tools available and about the emergence of resistance to antibiotics among *Vibrio* sp.
- To maintain a collection of *Vibrio sp*. strains

2.2 Specific missions *(Sciensano, 2019):*

- To confirm the diagnosis of *V. cholerae* serogroup O1 and O139 and of the toxin production.
- To confirm the diagnosis of *V. cholerae* non-O1, non-O139 and other *Vibrio species* pathogenic for human causing invasive diseases or suppurative infections.
- To confirm the diagnosis of toxigenic *V.parahaemolyticus*.

- To monitor circulating strains by performing epidemiological typing on isolated strains of *Vibrio cholerae*.
- To monitor the antimicrobial susceptibility.
- To collaborate with existing national, European or other international networks.
- To participate in national surveillance, transfer microbiological data (through e-health reporting) and scientifically report the analyzed data for public health concerns.
- To interact with epidemiologists and other NRC's with the aim to sustain/adapt the use of various outputs (with regards to quality of care, recommendations for control/ prevention, …)

- **The NRC must be able to** *(Sciensano, 2019)* **:**

- Confirm the bacteriological diagnosis of cholera by classical methods and/or molecular methods on isolates and from clinical specimens.
- Determine the major serotypes.
- Determine the antimicrobial susceptibility.
- Determine the pathotype of the isolated strains by molecular methods.
- Perform genotyping on confirmed potentially virulent strains.
- Determine the presence of genes coding for toxins produced by *V. cholerae* and *V. parahaemolyticus*.
- Have access to whole genome sequencing and expertise in species specific bioinformatics analysis.

- **Tasks that will be asked in a particular context** *(Sciensano, 2019)* **:**

- To perform or confirm the diagnosis of *V. cholerae*, *V. parahaemolyticus,* and other *Vibrio spp.* on isolates and from clinical specimens together with environmental specimens, when requested.
- To type all potentially virulent isolated strains.
- To collaborate to the microbiological investigation around a case of cholera or a case of infection with *V. parahaemolyticus.*
- To collaborate with the national and European food safety agencies and participate to a joint output report.
- To participate in surveillance of the presence of *Vibrio spp.* in Belgian recreational waters.
- To organize surveillance of invasive diseases and soft and skin tissue infections caused by *Vibrio spp*. In Belgium.

- For the surveillance of cases, the NRC-Vib requests relevant epidemiological data relating to travel, place of residence, hospitalization, clinical diagnosis including

symptoms, date of onset, evolution, risk factors and outcome, additional epidemiological characteristics such as isolated or grouped cases, food borne, source of contamination, contact, handling or consumption of sea products.

- In addition, among the objectives of the NRC-Vib , there are:
    - To participate to international projects about *Vibrio* sp.
    - To highlight the scientific work with its diffusion at conference / symposium and oral / written communications

3 Quality assurance approach

The NRC-Vib is part of the clinical microbiology service of the CHU of Liège, which has a quality system that meets the obligations of the accreditation of clinical biology laboratories, including participation in national quality control organized by Sciensano.

3.1 Accreditation

The clinical biology laboratories of the CHU of Liège have implemented a quality system for many years. Their accreditation has evolved in particular from standard 17025 to standard 15189. In this quality approach in the microbiology department, the accredited scope specifically concerned certain sectors of activity such as molecular microbiology. At the same time, the same approach was implemented with a view to accrediting the activities of the NRC-Vib: culture, methods for identifying and typing *Vibrio* sp as well as methods for determining their sensitivity to antimicrobial agents or molecular biology methods. The implementation of the NRC-Vib quality system according to the ISO 15189 standard took place from 2011 to mid-2013. It included the drafting of new procedures for each proposed analysis, the formalization of complete validation files for each of these techniques already used or under development and an inventory of existing procedures and protocols to highlight their weak points and establish corrective measures to remedy it. The approach also consisted of bringing the premises and equipment dedicated to NRC-Vib activities into compliance and organizing internal audits. The accreditation audit with acquisition of the BELAC certificate took place in May 2013. Surveillance audits took place in 2016, 2017, 2018, 2020, and 2021 without any particular remarks for the NRC-*Vib* confirming the continued quality and dynamism of the NRC-Vib for updating current procedures and the introduction of news analyzes to its scope.

3.2 External quality controls

In connection with the implementation of the quality system, the NRC-Vib has organized and set-up inter-laboratory ring tests with the French NRC (Pasteur's Institute, M-L Quilici) to which the NRC-Vib participates annually in order to validate the analyzes for which there is no control offered by an external organization. These controls cover identification techniques, typing methods and virulence determination.

3.3 Scope of analysis

Following, are the different analysis which can be performed on *Vibrio sp* isolates. The main procedures are available on request to the NRC-Vib.

- **Culture**
  - of received isolate
  - of feces with a selective enrichment
  - of water samples including an enrichment step
- **PCR**
  - on feces
  - of water samples
- **Identification by MALDI-TOF** mass spectrometry (Bruker Biotyper)
- **Antimicrobial susceptibility testing**
- **Determination of serogroup and serotype** by agglutination with sera anti-O1, anti-O139, anti-Inaba and anti-Ogawa.
- **PCR for the detection of genes coding for virulence factors**
  - *Ctx*A and *Tcp*A for *Vibrio cholerae*
  - *Tdh* and *Trh* for *Vibrio parahaemolyticus*
- **Whole genome sequencing**, dendrograms and phylogeny study

3.4 Followed algorithm by NRC-Vib

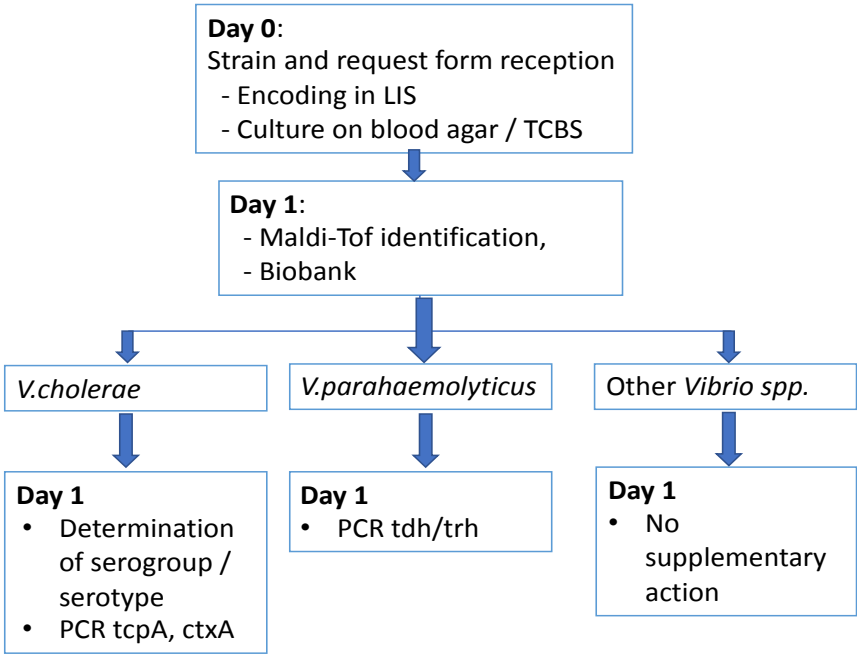
3.4.1 Isolates of *Vibrio spp*

The algorithm that is followed when a strain of *Vibrio* sp. is received at the NRC, is described in **Figure 1**. First the request form is encoded in the LIS and the strain is subcultured to confirm the identification by Maldi-Tof and for the biobanking. According to the identification, the follow-up differs. If *V. cholerae* is identified, the serogroup/serotype is then determined by agglutination tests against O1 and O139 serogroups and Inaba/Ogawa serotypes. Then the virulence of the isolate is determined by real time

PCR detecting the CtxA gene coding for the production of the cholera toxin. A classical PCR can also be done detecting the toxin co-regulated pilus gene TcpA gene (facultative). If the identification is *V. parahaemolyticus*, a classical multiplex PCR is done for the detection of two hemolysin *Tdh* (thermostable direct hemolysin) and *Trh* (thermostable related hemolysin) genes. If another *Vibrio* sp. is detected (or another bacteria), no further action is needed.

In case of high suspicion of cholera, the PCR tcpA and ctxA can be performed directly from the isolate.

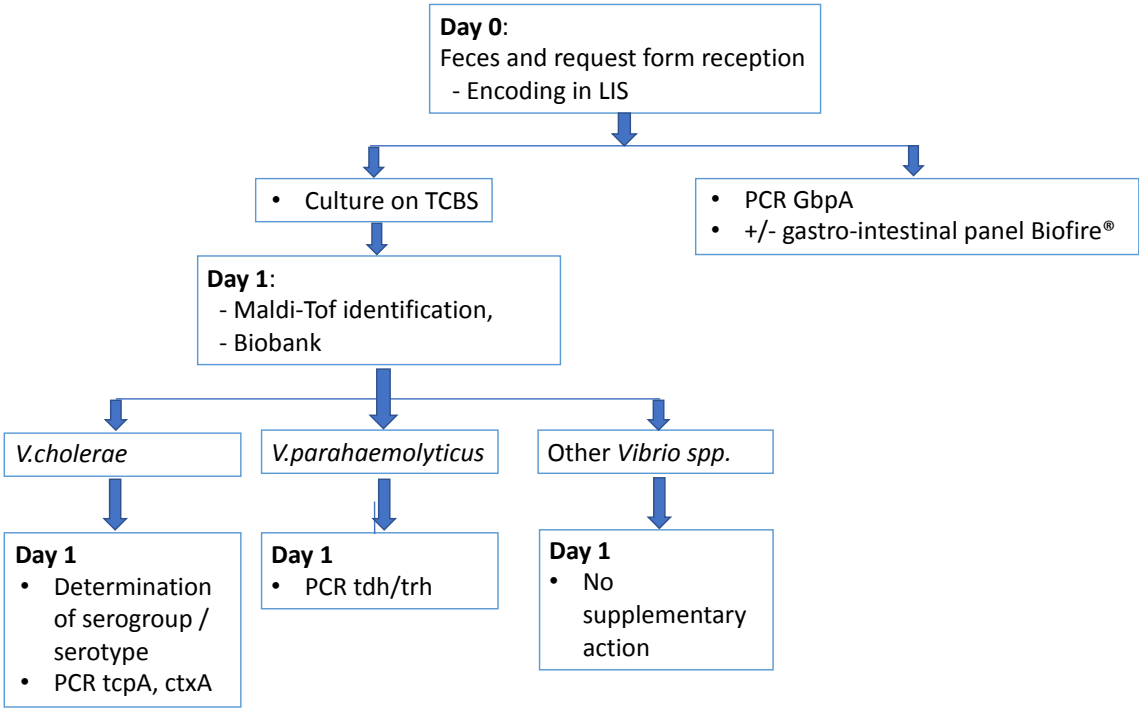
Figure 1: Algorithm followed by the NRC-Vib when a strain is received



3.4.2 Detection of *Vibrio spp* in samples of feces

Stool treatment is illustrated in **Figure 2**. First the request form is encoded in the LIS and the sample is both cultured on TCBS agar (Thiosulfate Citrate Bile Sucrose agar) and directly extracted with the QIAamp DNA stool mini kit. A PCR against the *GbpA* gene is then done to detect all *Vibrio cholerae*, toxigenic and non-toxigenic, and if the need arises or requested, a PCR gastro-intestinal panel Biofire® can also be done. On Day 1, cultures on TCBS are read looking for presumptive *Vibrio cholerae* and other *Vibrio spp* colonies. Identification on presumptive colonies is done by Maldi-Tof. According to the identification, the follow-up differs as described for strain in **Figure 1**. All confirmed vibrio are subcultured and saved for biobanking.

Figure 2: Algorithm followed by the NRC-Vib when a stool is received is received



3.4.3 Detection and count of *Vibrio spp* in environmental water

The management of aquatic environmental samples for the detection and count of *Vibrio* spp is illustrated in **Figure 3**. Upon reception, first the request form is recorded and the sample is submitted to a specific procedure of culture. Each sample of water is treated in triplicate. On day 0,

- 10 mL and 100 mL are filtered (0.45 µm) and filters are inoculated in 50 mL of alkaline peptone water (APW) and then incubated.
- 10 µl, 100 µl and 1 mL are directly inoculated in 9 mL of APW
- All inoculated APW are then incubated 18-20h at 41°C

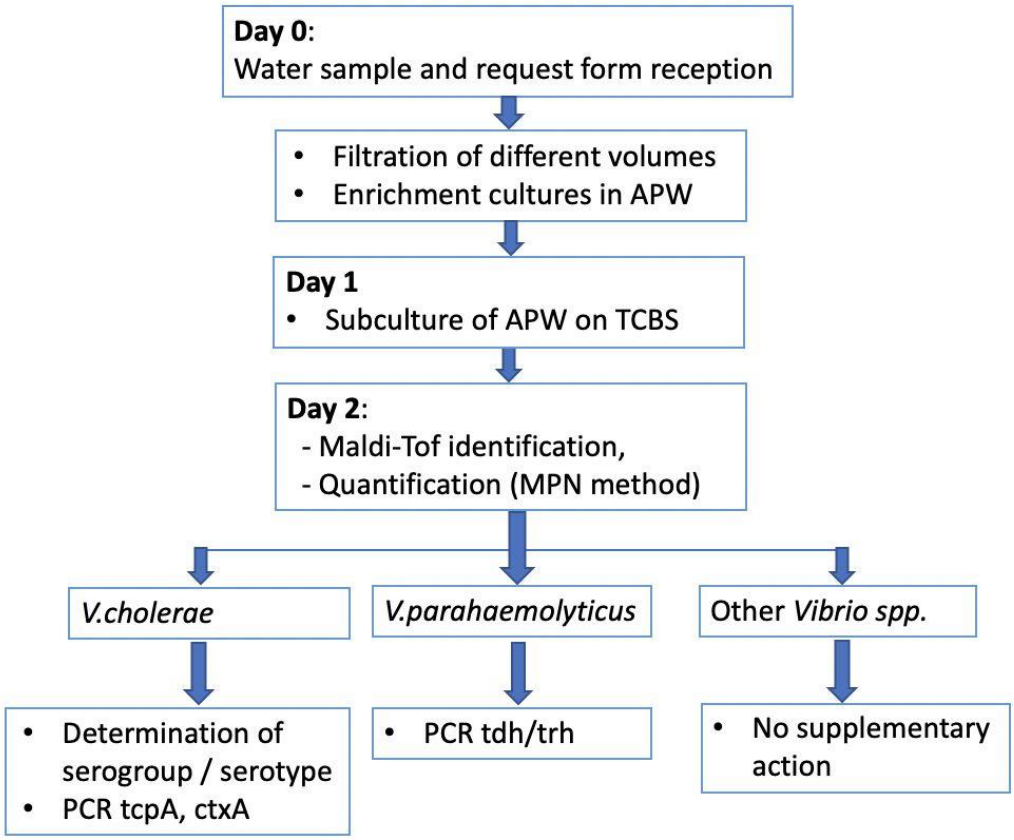
On day 1, all cultures in APW are further sub-cultured (10 µL) on TCBS agar, then incubated 16-20h at 35°C.

On Day 2, cultures on TCBS are read looking for presumptive *Vibrio cholerae* and other *Vibrio spp* colonies, and as soon as possible,

- Identification on presumptive colonies is done by Maldi-Tof. According to the identification, the follow-up differs as described for strain in Figure 1. All confirmed Vibrio are subcultured and saved for biobanking.

- The positive TCBS plates originating from the serial dilutions in APW, allow the estimation of the concentration of *Vibrio spp* in the different samples according to most probable number (**MPN)** method.

- **Figure 3**: Algorithm followed by the NRC-Vib when a water sample is received



4 Ten years summary of NRC activities

4.1 Expertise activity

Between the 2012-2021 study period, 217 strains of *Vibrio* sp. have been addressed to the NRC-Vib: 53 strains were from clinical samples and 164 were isolated from food and are described in a separate analysis (cf 4.3).

4.2 Clinical strains characterization

Between 2012 and 2021, 53 strains of *Vibrio* sp. were isolated from clinical specimens (**Figure 4**): 32 stool, 8 ears, 8 blood, 2 intestinal fluids (bile and ascites fluids) and 2 from wound. It is important to notice the increasing reporting of *Vibrio* sp. bacteremia during these last years. Indeed, during this ten-year period, 8 cases of bacteremia have been reported to the NRC, six concerning *Vibrio cholerae* and

two concerning *Vibrio parahaemolyticus*. All *Vibrio cholerae* isolated from blood were non-O1, non-O139 (6/6). Non-O1, non-O139 *Vibrio cholerae* are increasingly frequently observed in human pathology and occasionally responsible for intestinal and extra-intestinal infections. Most cases involve self-limiting gastroenteritis or ear and wound infections in immunocompetent patients. But these recent years several cases of bacteremia have been described[1,2].

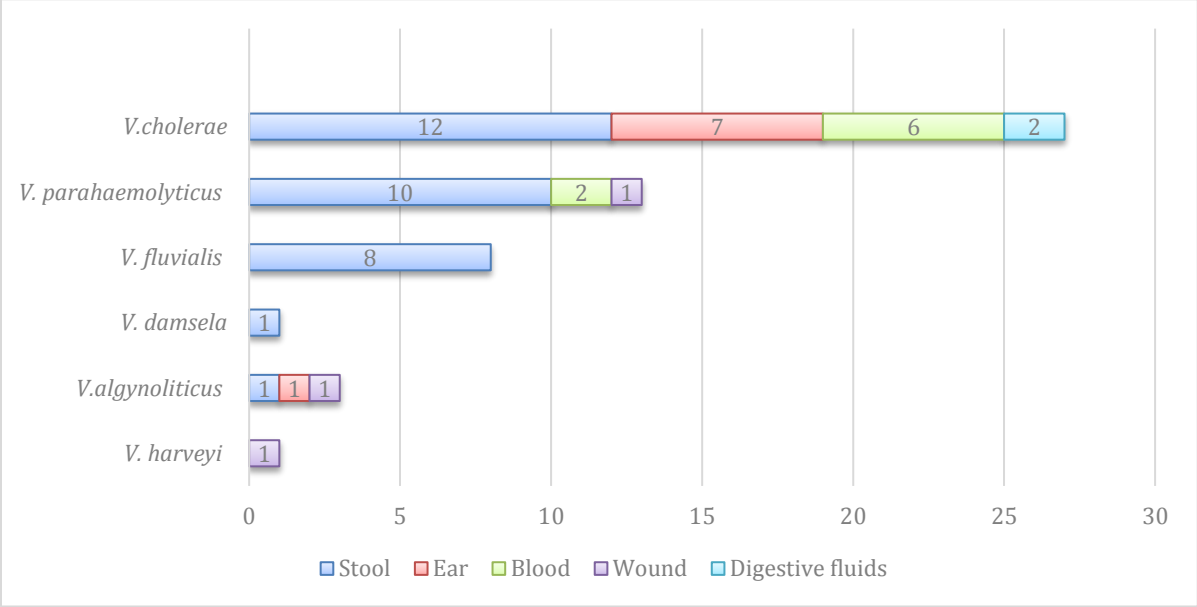


Figure 4: Distribution of *Vibrio* spp. among human clinical samples between 2012 and 2021

Regarding stool samples, among the 32 stools sent to the NRC, 12 were positive for *V. cholerae,* 10 for *V. parahaemolyticus*, 8 for *V. fluvialis*, one for *V. damsela* and one for *V. algynoliticus*.

4.2.1 V. cholerae

4.2.1.1 V. cholerae from fecal origin

Among the 27 *V. cholera*e strains, 12 were isolated from feces and 4/12 were cholera vibrio: two strains were of O1, Inaba serogroup/serotype and two others were of O1, Ogawa serogroup/serotype. Considering the O1, Inaba strains, these were isolated from patients travelling from Pakistan and Congo. Regarding the O1, Ogawa strains, both cases were isolated from people having travelled to India. **So, we can conclude that all cases of cholera diagnosed in Belgium were imported cases**. The 8/12 remaining strains were non O1, non O139 (see **figure 5**) as determined by agglutinations tests.

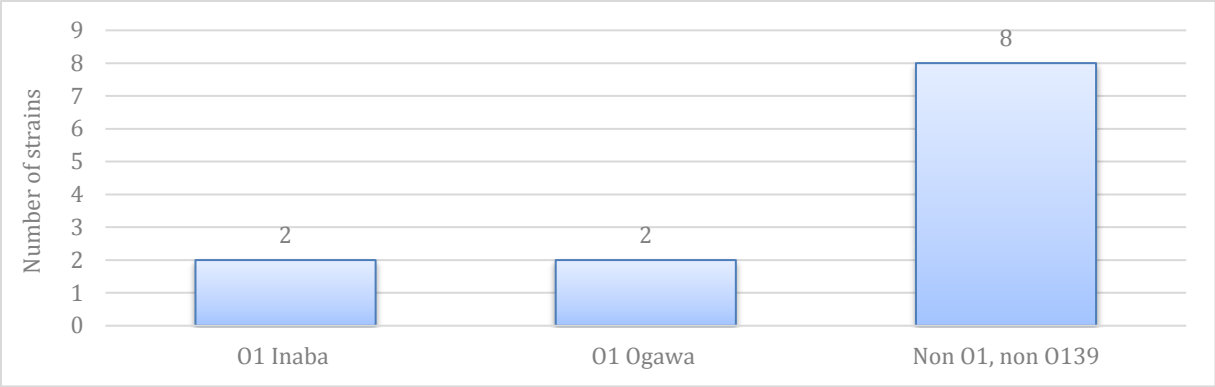


Figure 5: Distribution of the different serotypes identified among 12 *Vibrio cholerae* strains isolated from stools between 2012 and 2021 (Belgian NRC.-Vib)

All cases including both cholera and non-cholera cases occurred lonely.

The virulence of these fecal isolates was determined by real time PCR for the detection of the virulence gene *CtxA* associated with the presence of the CTX prophage (CTXϕ). CTXϕ is a filamentous, lysogenic bacteriophage. Its genome encodes the cholera toxin, the primary virulence factor produced by *Vibrio cholerae*. The *CtxA* and *CtxB* genes encoding cholera toxin, are present within the genome of the bacteriophage. CTXϕ DNA is generally found integrated at either one (El Tor) or two (classical) loci within the *V. cholerae* genome[3,4].

Only four strains (33%), that are the 4 cholera Vibrio, were harboring the *CtxA* gene and so producing the cholera toxin (see **Figure 6**). These 4 cases identified to be cholera cases and were notified in real time to the AVIQ ("Agence pour une vie de qualité", Wallonia, Belgium) according to the recommended procedure for notification of infectious diseases. Only confirmed cholera cases with detection of *CtxA* gene has to be declared to this organization.

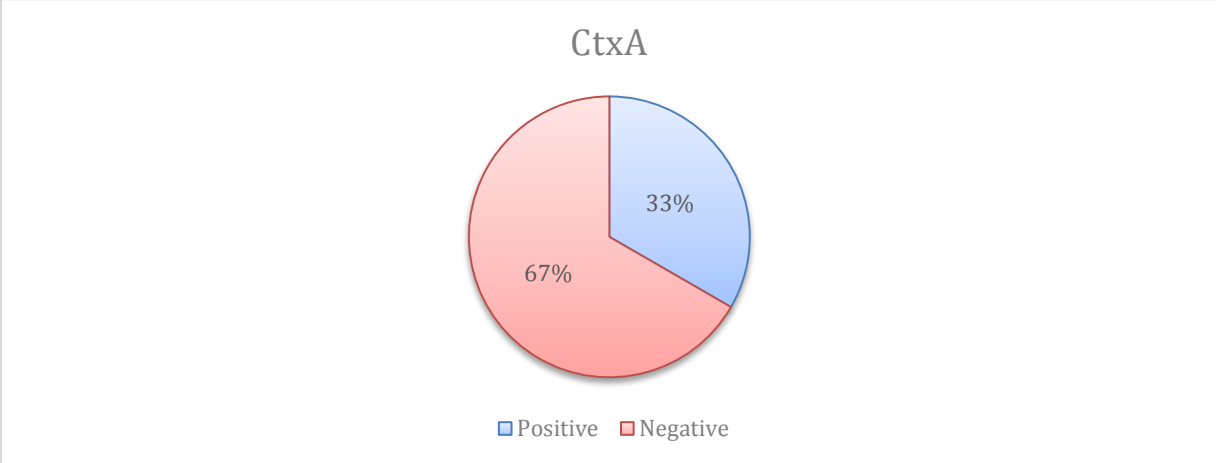


Figure 6: Graph showing the rate of presence of cholera toxin genes among the twelve *V. cholerae* strains isolated from stools.

In 2018, one of the strains was isolated from an 8-year old girl, hospitalized with an acute gastro-enteritis. The link was done with the fact she had swam in a recreational water located in Flanders, Belgium. The NRC-Vib requested large water specimens and cultivated *Vibrio cholerae* from this aquatic environment, 4 to 100 colony forming unit /100 mL of *V. cholerae* were quantified in these water specimens. In collaboration, a risk assessment report was established by Sciensano.

4.2.1.2 Non-fecal V. cholerae

Among non-fecal isolates of *Vibrio cholerae*, 6 were isolated from blood of bacteremic or septic patients, 7 from ear of patients consulting for otitis and 2 from bile and ascites fluids of two patients.

In contrast with fecal isolates, 15/15 strains of *V. cholerae* isolated from non-feces specimen, in particular from ear and blood, were not harboring the cholera toxin genes and were negative for *CtxA* by real time PCR. These 15 strains were *V. cholerae* non-O1, non-0139.

Most of the bacteremic patients had risk factors such as hepatic disorders, cancer, diabetes, immune depression, gastric by-pass and gastrectomy. Among these 6 patients, 2 had travelled to North Africa, 1 to Dominican Republic and 1 to Hungary. One of the six became bacteremic after ingestion of brackish water when paddling in a creek in Belgium. While this last case was explored, the relationship with the brackish water was suggested. The NRC-Vib requested large specimen of the incriminated recreational water; the cultures showed a contamination with *Vibrio cholerae* at a very high level, 5.10^4 to 10^5 colony forming unit / 100 mL. In collaboration, a risk assessment report was established by Sciensano.

4.2.2 V. parahaemolyticus

During the study period 2012-2021, 13 *V. parahaemolyticus* have been isolated from human samples. 10 samples from stools, 2 from blood and one from wound. Regarding isolates from stool samples, the presence of hemolysins has been attested by classical multiplex PCR targeting *tdh* and *trh* genes. Among the 10 stool samples, 9 (90%) harbored one gene coding for one of the two hemolysins: four of them were positive for *Tdh* and the other five were positive for *Trh*. Among the two blood samples, both strains were positive for *Trh*. Among the wound sample the PCR was negative for both *tdh/trh* genes. We have to notice that clinical specimens of *V. parahaemolyticus* received at the Belgian NRC-Vib from humans (13/15, 86.9%), are almost always positive for one of the screened hemolysin genes.

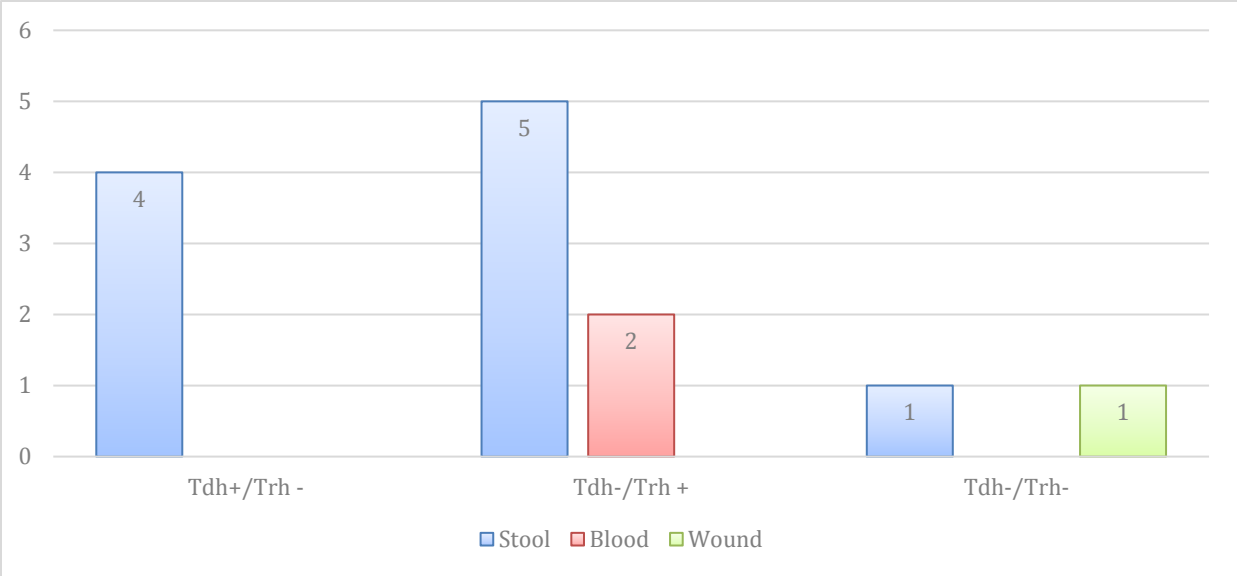


Figure 7: Graphical representation of the hemolysin genes *Tdh/Trh* distribution among *V. parahaemolyticus* strains.

4.2.3 Other *Vibrio spp.*

Eight *V. fluvialis* have been isolated from stools. The distribution of *V. fluvialis* is a global phenomenon[5] and this organism is not only isolated from human diarrheal cases but also from aquatic environments and concerns food poisoning phenomenon's too[6–8]. There are few informations available on the virulence factors associated with *V. fluvialis* infections and its mechanism of pathogenicity remains not well known. However, this species seems to be of increasing importance in terms of public health [9,10], that is why it is important to follow this pathogen even in industrialized countries such as Belgium. This pathogen is easily distinguished from other *Vibrio* species with the Maldi-Tof MS.

One *V. damsela* strain has also been isolated from stool, one *V. harveyi* has been isolated from wound and 3 *V. algynolyticus* have been isolated from stool, ear and wound. These species have already been described to be implicated in human pathologies, *Vibrio damsela* being involved in wound infections and in septic shock for example[11,12].

4.3 *Vibrio* sp. isolated from food controls

Different Belgian laboratories specialized in microbiological quality control throughout the supply chain of food are invited to send strains to the NRC-Vib. Between 2012 and 2021, the NRC received 164 strains isolated from food (mainly sea food). Among them, there were 89 *V. parahaemolyticus* (54.3%), 67 *V. cholerae* (41%), 4 *Vibrio metchnikovii* (2.43%), 3 *V. alginolyticus* (1.83%), and 1 *V.*

vulnificus (0.6%)**. Figure 8** shows the distribution of the different *Vibrio* species isolated from food samples.

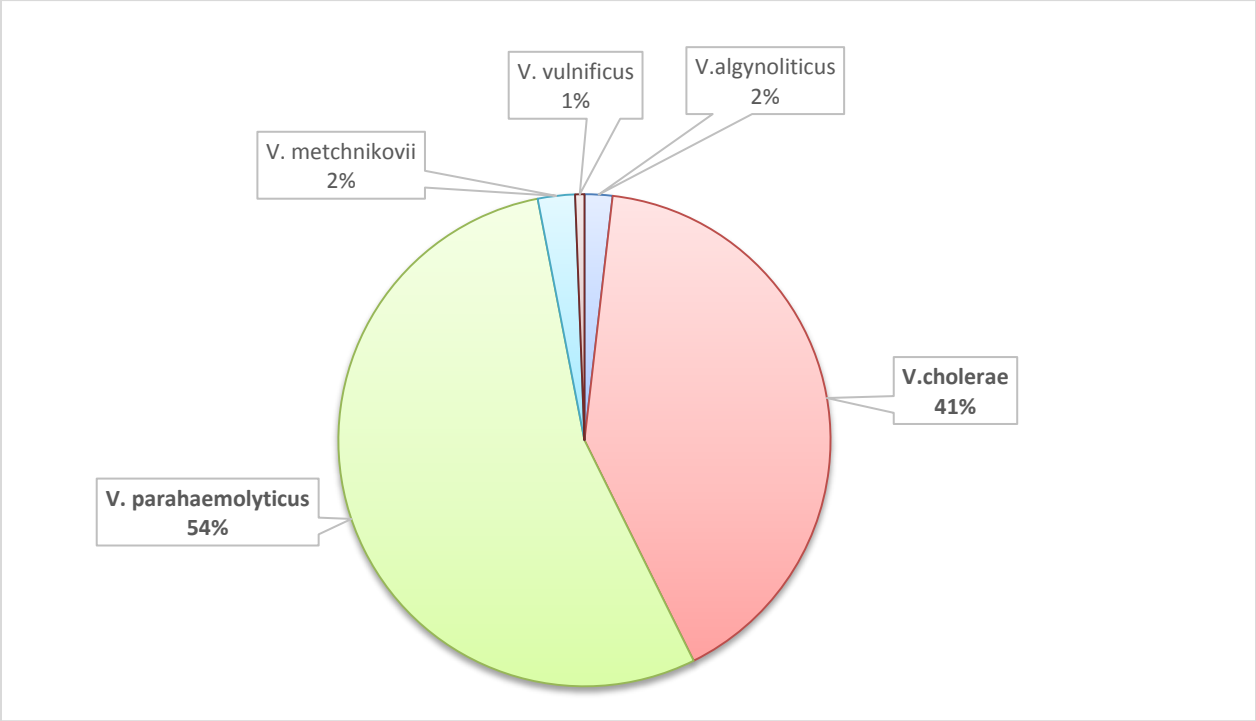


Figure 8: Distribution of *Vibrio* spp. isolated from food samples between 2012 and 2021.

Among strains of *V. cholerae* isolated from food samples, all were non-O1, non-O139 and none of them harbored the cholera toxin genes. Among isolates of *V. parahaemolyticus*, 17/91(18.8%) were positive for the *Trh* hemolysin gene.

4.4 *Vibrio* spp. from aquatic environments in Belgium; results of the national study conducted in 2021

These results were collected during end study thesis done by **Camille Philippe (ULiege)**.

Non-toxigenic *Vibrio cholerae* and most *Vibrio spp.* are found in aquatic environment and are generally non-pathogenic. A few species can cause sporadically illnesses such as wound infections, otitis, bacteremia and gastroenteritis. Rarely, they can cause collective food poisoning events. Invasive clinical cases of vibriosis have been described in Belgium after contact with recreational water[13]. Recently, the number of reports of human infections, which can be life-threatening, involving non-O1, non-O139 *V. cholerae* and other *Vibrio* spp. has increased in Europe and in France[1,14]. In Belgium, aquatic environment for recreational use such as lakes and sea water are not monitored for *Vibrio spp*; it is not yet legally requested.

Among the recommended actions enumerated in the risk assessment report edited by Sciensano in 2018, in response to the 2 cases of vibriosis (2017 and 2018) related to some recreational aquatic environment, there was "a specific study on the contamination of Belgian natural recreational water by *Vibrio cholerae*, which could be performed by the NRC-Vib."

So, the Belgian NRC-Vib conducted a preliminary study, by initiating a cartography of Belgian water points for screening the presence of *Vibrio* spp. A few selected points were screened aiming to evaluate the possible impact of the presence of *Vibrio* spp. on public health.

4.4.1 Design of the study

- According to recent clinical cases of vibriosis and to the distribution of recreational water locations, 8 areas were selected in Wallonia and Flanders including the North Sea. **Figure 9** illustrates the different localizations selected all around Belgium for water analysis.

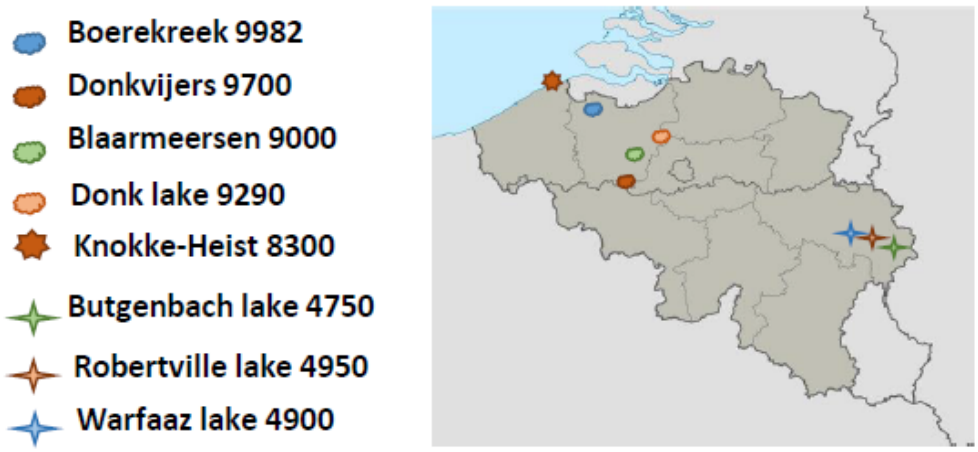


Figure 9: Geographical representation of the 8 water plans that have been analyzed in 2021 by the NRC.

- Five samplings of water were done at each site, once per month between May and September 2021. A telescopic device was used for collection of 1 liter of sample. Water was poured in a sterile bottle, transported on ice and kept at 4°C until analysis within 24 hours. Temperature and pH of water was measured and recorded at time of each sampling.

Culture method

- The Most Probable Number (MPN) culture method was used for bacterial quantification that consists of serial dilutions in alcaline peptone water (APW). Upon reception in the laboratory, for each sample of water, in triplicate: 10 mL and 100 mL were filtered (filters with porosity of 0.45 µm) and filters were then inoculated in 50 mL of APW.10 µl, 100 µl and 1 mL of the collected water were directly inoculated in 9 mL of APW. All inoculated APW were incubated

18-20h at 41°C and further 10µl were sub-cultured on thiosulfate citrate bile sucrose agar medium (TCBS), then incubated 16-20h at 35°C. Identification of growing colonies on TCBS was done by Maldi-Tof mass spectrometry + agglutination tests (serogroup/serovar) and PCR for *V. cholerae CtxA* gene. The positive TCBS plates originating from the serial dilutions in APW, allowed the estimation of the concentration of *Vibrio spp* in the different samples according to MPN interpretation [15,16]. **Figure 10** illustrates the culture method and bacterial quantification used in our study.

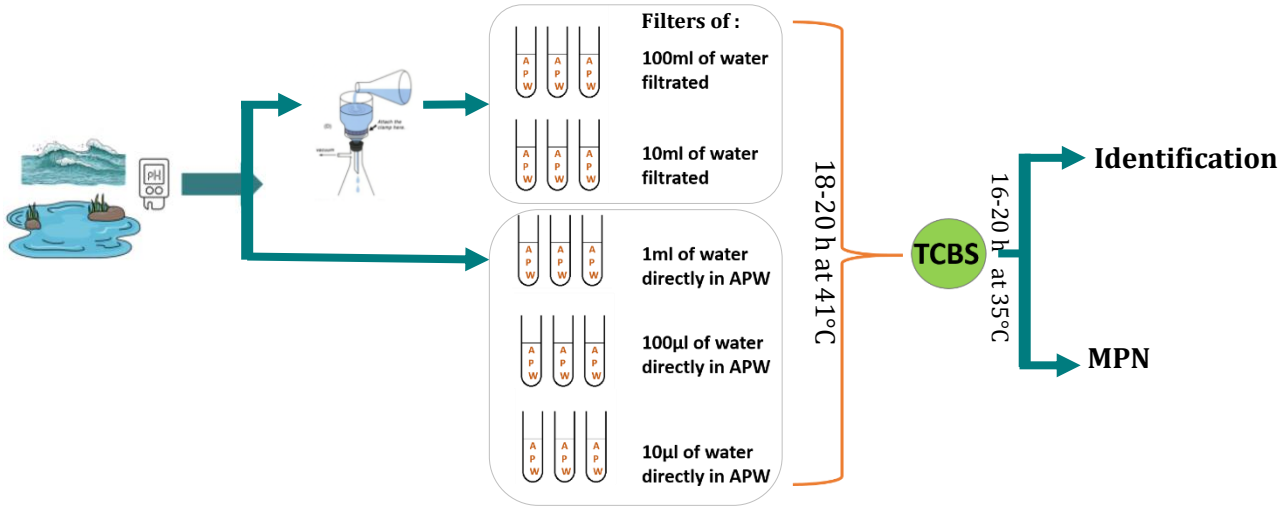


Figure 10: illustration of the Most Probable Number (MPN) method and the culture procedure applied for bacterial quantification of *Vibrio* spp.

4.4.2 Results

4.4.2.1 PH and T° monitoring

The pH of each water sample has been measured in each location at time of sampling. Results are represented in **figure 11**. The average pH value in Wallonia was 7.8 while it was 8.6 in Flanders. No impact of the pH variation was correlated with bacterial growth (see **table 1**)

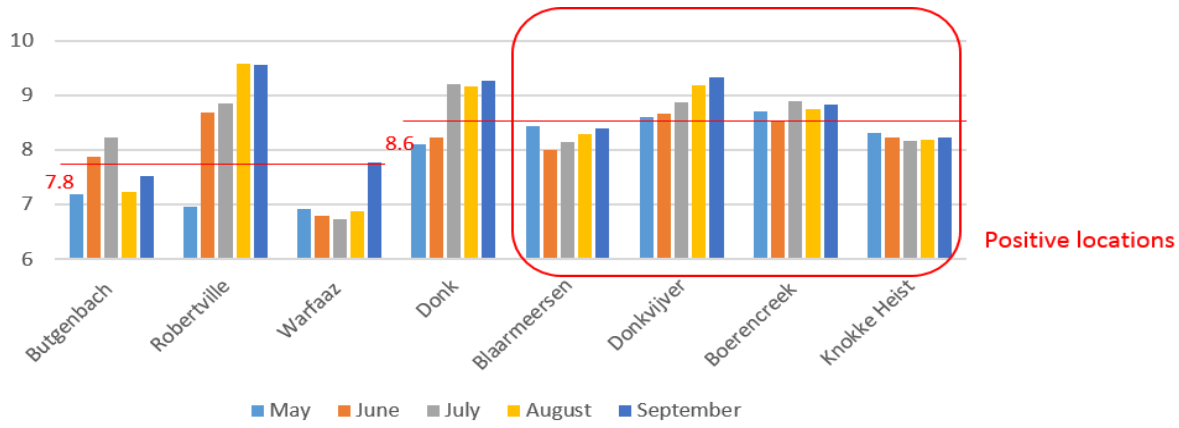


Figure 11: pH monitoring in each water points between May and September 2021. The highest pH were measured in Flanders. Positive locations for *Vibrio* spp. are surrounded.

Temperature of collected waters were also recorded and results are illustrated in **figure 12**. The average temperature in Wallonia was 18.6°C while it was 21.4°C in Flanders. In general, an increase of the T° in summer months was observed with the highest T° in Flanders.

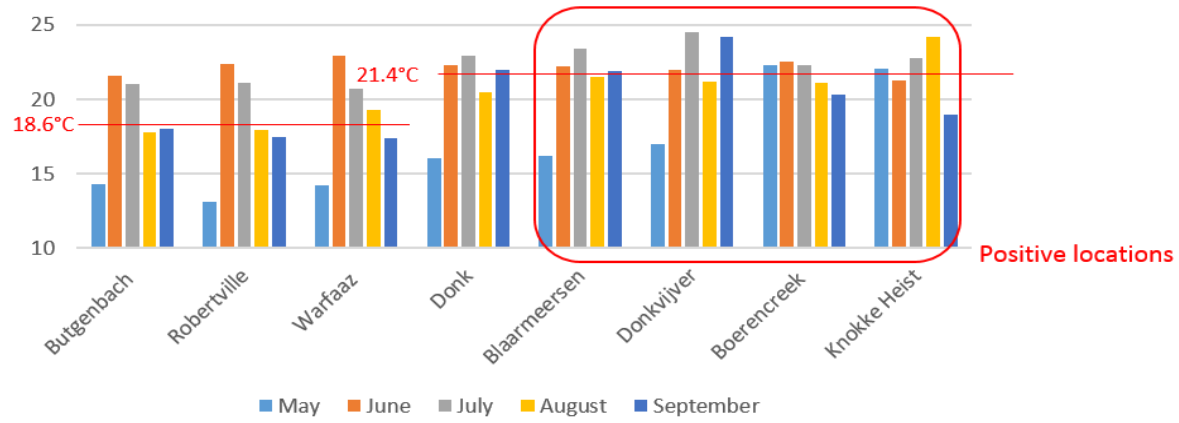


Figure 12: T° monitoring in each water points between May and September 2021. Positive locations for *Vibrio* spp. are surrounded.

4.4.2.2 Bacterial detection and quantification

No *Vibrio* spp. were detected in the Walloon lakes while some were detected in four water points in Flanders. The calculated concentration with MPN method is described in **table 1**. In the North sea, the monthly concentration of *Vibrio* spp. seemed to be correlated with an increase of the water temperature (see **figure 12**) as for example, in June (21.3°C) the *Vibrio cholerae*

concentration was evaluated at 110 CFU/ml while in August (24.2°C), the concentration reached >11.000 CFU /ml. This tendency was also observed in Boerenkreek.

	BLAARMEERSEN	DOMEIN DONK	BOERENKREEK	SEA (KNOKKE)
MAY	/	110 CFU/ml	7.5 CFU/ml	/
JUNE	2.3 CFU/ml	/	>110 CFU/ml	110 CFU/ml
JULY	/	/	210 CFU/ml	110 CFU/ml
AUGUST	/	46 CFU/ml	1100 CFU/ml	>11000 CFU/ml
SEPTEMBER	/	/	460 CFU/ml	>110 CFU/ml

Table 1: Estimation of the concentration of *Vibrio cholerae* (non-01, non-0139) and *Vibrio spp* (by MPN) in four water points in Flanders.

Regarding identification (done by Maldi-Tof MS) of the positive cultures, *Vibrio cholerae* (non-01, non-0139 determined by agglutination) was found in three lakes in Flanders and in the North Sea. Other *Vibrio* spp. as *V. alginolyticus* and *V. parahaemolyticus* were also found in the North Sea. All *Vibrio* strains have been characterized for the presence of cholera toxin but all were negative. **Table 2** summarize all the identifications obtained from positive cultures in different waterpoints.

	Blaarmeersen	Donkvijers lake	Boerekreek	Knokke-Heist
MAY	/	*V. cholerae*	*V. cholerae*	/
JUNE	/	/	*V. cholerae*	*V. cholerae*
				V. alginolyticus
JULY	*V. cholerae*	/	*V. cholerae*	*V. cholerae*
				V. alginolyticus
				V. parahaemolyticus
AUGUST	/	*V. cholerae*	*V. cholerae*	*V. cholerae*
				V. alginolyticus
				V. parahaemolyticus
SEPTEMBER	/	/	*V. cholerae*	*V. cholerae*
				V. alginolyticus

Table 2*:* Results of culture on TCBS and Maldi-Tof MS identification for positive cultures. No *Vibrio* spp. were found in Walloon lakes.

4.4.3 Conclusion

Our study demonstrates the presence of *Vibrio cholerae* (non-O1, non-O139) and other *Vibrio spp*. at concentrations able to cause human infections in different water points mostly in the North of Belgium. Mean temperatures and pH were higher in Flemish selected locations than in Walloon selected lakes. They can be favorable factors for the growth of *Vibrio spp*. Other factors such as salinity should be also included in future surveillance. This study supports the recommendation to include *Vibrio* spp. in water quality controls from aquatic natural environment in order to define if water recreational activities may be harmless for humans in Belgium.

4.5 Training activities

- Conferences given at regional and national level, educational trainings, LOK-GLEM (Local Group for Medical Evaluation), symposium. The NRC is often consulted for clinical and therapeutic advices.
- Specific training for professionals involved in microbiological quality assurance and controls of food:"*Vibrio* infections in Belgium" in collaboration with Sciensano (Marie Polet) in December 2016.

4.6 Reception of trainees and doctoral students

End study thesis (university degree or graduate degree)

- **2020**: Supervision of end study master thesis of Philippe Camille, student in 2nd master in biomedical sciences (ULiege). Thesis: Monitoring and characterization of *Vibrio* spp. present in coastal waters and in surface waters for recreational use in Belgium and development of a qPCR method for detection of *V. cholerae* in stool and water samples.
- **2017**: Supervision of end study thesis of Uwineza Claudine student in 2nd master of pharmacy (ULiege). Thesis: Validation de deux PCR pour la detection du gene CtxA chez *Vibrio cholerae* et la détection des gènes tdh/trh et ToxR chez *Vibrio parahaemolyticus* selon la norme ISO 15189.
- **2014**: Supervision of end study graduate thesis of Duveau Elodie, student in 3rd baccalauréat in chemistry, biochemostry purpose. Thesis: Mise au point et validation de PCR d'identification et de détection de gènes de virulence pour *Vibrio cholerae* et *Vibrio parahaemolyticus.*

PhD students

- **2019-2022:** Collaboration with Abomey Calavi University, Benin, the student stayed 3 months in Belgium and all her collected strains from Benin were characterized with molecular biology methods in our laboratory. Eliane AKPO de l'Ecole Doctorale Sciences de la Vie et de la Terre de l'Université d'Abomey-Calavi, Bénin, thesis object : Le choléra au bénin : état des lieux, antibiorésistance, caractéristiques phénotypique et génétique moleculaire des souches de *Vibrio cholerae*, 2022

5 Research, communication & publication

5.1 Research projects

We try to continuously improve our knowledge about *Vibrio* sp. Recently Whole genome sequencing has been applied to *Vibrio* strains and a specific bioinformatic tool has been developed and acquired. A lot of different characterizations can be done with this tool.

We plan to characterize all the human isolates from Belgian collection of the NRC-Vib and also strains isolated from aquatic environment to deeply study the genome of these strains and make comparative phylogenetic analyses with strains isolated in other contexts such as clinical strains or strains isolated in food. Genome analyses will identify gene families that are important to our understanding of how *Vibrio spp.* can cause invasive human infections, and how they respond to the host immune response. Moreover, WGS-based method would reveal a greater genomic diversity of *Vibrio spp.* Increasing sequencing capacity combined with a dramatic decrease in costs made these sequencing techniques available for microbiological labs for both research and diagnostic purposes. The aim of our genomic study is to compare the strains found in natural aquatic environment of different geographical regions in Belgium and see if they share genetic diversity or similarities. The genetic comparison can also be done comparing *Vibrio spp*. isolated from waters with *Vibrio spp.* causing cholera diseases (present in the collection of the National Reference Center since 2010). The comparison can further be done with *Vibrio spp.* isolated in seafood thanks to a collaboration with Sciensano (Dr. Marie Polet). As other countries such as Holland and Germany have monitored *Vibrio spp*. in water, we have also plan to compare these European profiles with what we hope to find in Belgium. On this purpose, we plan to evaluate the WGS typer bioinformatics tool to easily interpret our WGS data. The evaluation of such an

interface allowing an easy interpretation of WGS data for typing and characterizing *Vibrio spp*. has a cost. Thanks to this tool, the obtained draft sequences, should be compared with currently available *Vibrio spp.* genomes. The WGS is of interest to characterize the virulence of isolated strains of *Vibrio spp.* several factors can be targeted such as *CtxA* gene coding for the cholera toxin and associated with cholera disease. The *TcpA* gene coding for the toxin coregulated pillin is also associated with high virulence of *V. cholerae* strains. Molecular markers of resistance to antibiotics can also be targeted.

We also plan to develop a surveillance of the resistance to antimicrobial agents among the different *Vibrio* species as several resistance have already been described to nalidixic acid for example, a reduced susceptibility to fluoroquinolones has also been observed. A multiresistance to frequently used antibiotics for treating cholera cases, such as tetracyclin, doxycycline, erythromycin and chloramphenicol for example, has been reported among *Vibrio cholerae* including cholera strains [17–22].

5.2 Publications and communications

5.2.1 Publications

Vibrio cholerae (Cholera), JACQUINET Stéphanie; **SACHELI Rosalie ; MELIN Pierrette** 2018, In *Maladies infectieuses liées à la consommation des aliments et de l'eau Surveillance épidémiologique 2015-2016 (Sciensano)*

Eliane Assiba Olorun-Kosun Akpo, Tamegnon Victorien Dougnon, **Rosalie Sacheli**, Alidehou Jerrold Agbankpe, Olivia Mariette Yégbandé Houngbégnon, **Pierrette Melin**, Honoré Sourou Bankole *Microbiology Research Journal International*, Page 1-13

DOI: 10.9734/mrji/2021/v31i630322

Published: 20 August 2021

5.2.2 Posters

National cartography of water points for the presence of Vibrio spp. in Belgium

PHILIPPE Camille; **SACHELI Rosalie; MELIN Pierrette**, HAYETTE Marie-Pierre 2022 • 32nd ECCMID European congress of clinical microbiology and infectious diseases

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