NATIONAL REFERENCE CENTRE FOR BURKHOLDERIA CEPACIA COMPLEX (BCC) AND OTHER GRAM NEGATIVE NON FERMENTERS (GNNF) (EXCEPT *P. AERUGINOSA* AND *ACINETOBACTER SPP.*): UPDATE WITH DATA 2012-2020

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INTRODUCTION

BCC bacteria, formerly known as *Pseudomonas cepacia,* were first described as plant pathogens causing onion skin rot (1). Since the 1970s, bacteria now classified as BCC have been described as human pathogens. Today, they are mainly known as infectious agents in cystic fibrosis (CF) patients as well as hospitalized immunocompromised patients (2, 3). BCC is composed of at least 20 different but phylogenetically closely related bacterial species (4). Beside BCC, CF pathogens are mainly *S. aureus* and *P. aeruginosa* but also a number of additional pathogens including GNNF such as *Stenotrophomonas maltophilia* and *Achromobacter* spp. (5-8). These pathogens cause frequent and recurrent infective exacerbations in CF patients that can lead to premature death. The main purpose of our NRC is the surveillance of BCC and GNNF (except *P. aeruginosa* and *Acinetobacter* spp.) in CF patients. This report is a survey of NRC-BCC-GNNF data over the period from 2012 to 2020.

NUMBER OF BCC AND GNNF

From 2012 to 2020, we identified an average of 127 BCC and GNNF strains per year (min 70 (2012) and max 163 (2016)), see Figure 1. During the year 2016, the number of strains received was the highest among the total period because one of the Belgian centers sending the strains to the NRC conducted a study of *Achromobacter* spp. in CF patients, and therefore, included more patients colonized by these microorganisms and referred more strains to our NRC. For the year 2020, the number of strains received was lower in comparison to the previous years, since 2014. This could be explained by the lock-down period due to SARS-COVID-19 pandemic, where the NRC activity has been stopped and only extremely highly urgent cases were processed.

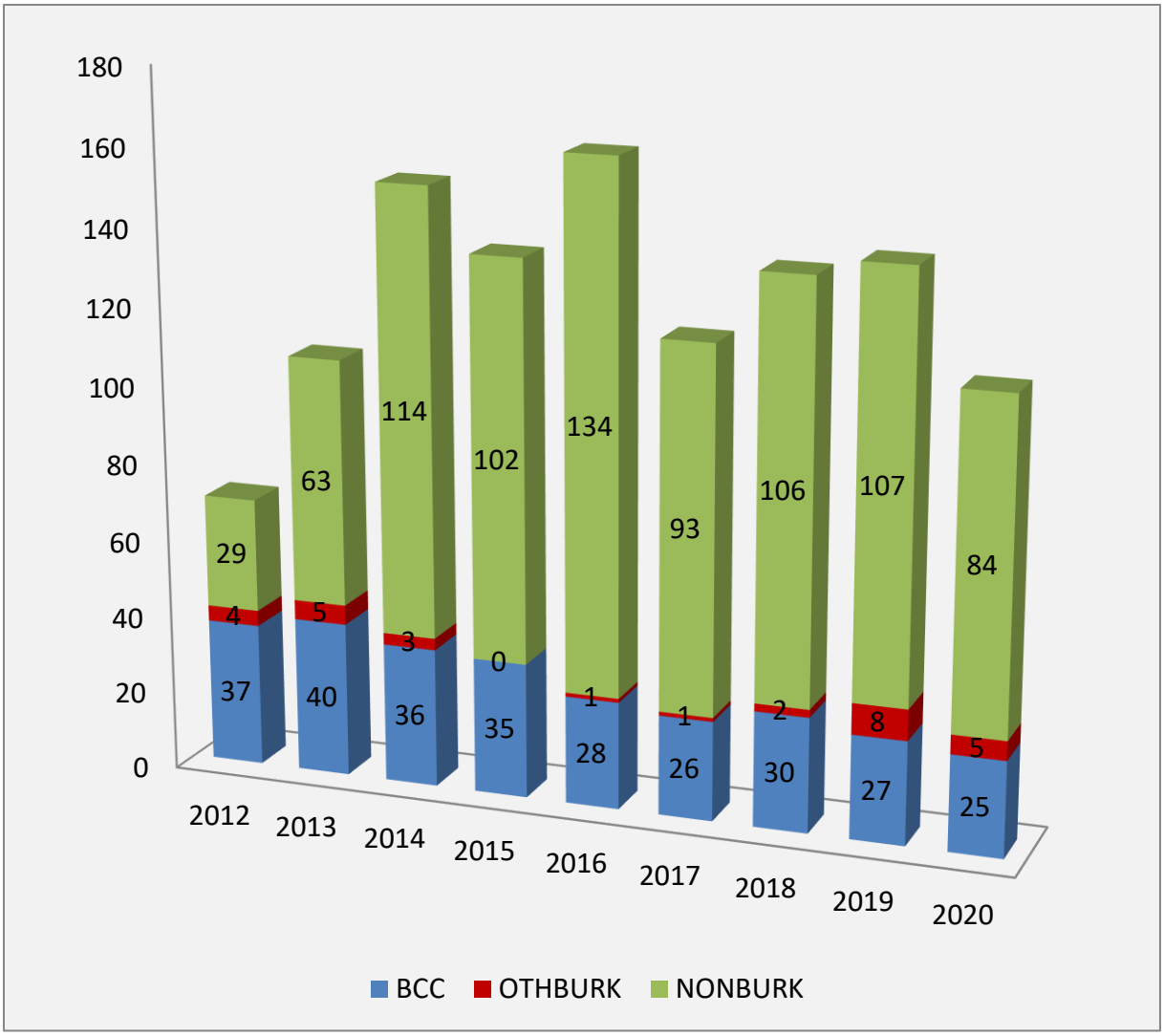


Figure 1. Distribution of *Burkholderia* spp. and GNNF from 2012 to 2020 (OTHBURK: other Burkholderia spp. than BCC; NONBURK: GNNF)

BURKHOLDERIA CEPACIA COMPLEX/OTHER BURKHOLDERIA SPECIES

A total of 313 BCC and other *Burkholderia* species were received from 2012 to 2020. See Figure 2 for the distribution of the *Burkholderia* spp. *B. multivorans* represented 47% of the total number of *Burkholderia* isolates followed by *B. cenocepacia* (15%), *B. vietnamiensis* (12%), and the other species at lower proportions (see Figure 2). The yearly number of received microorganisms slightly increased from 2012 to 2013, but since 2013, this number decreased continuously in favor of other GNNF. *Burkholderia* spp. represented in 2012 59% of the total isolated strains, to go down to 26% in 2020 (see Figure 1).

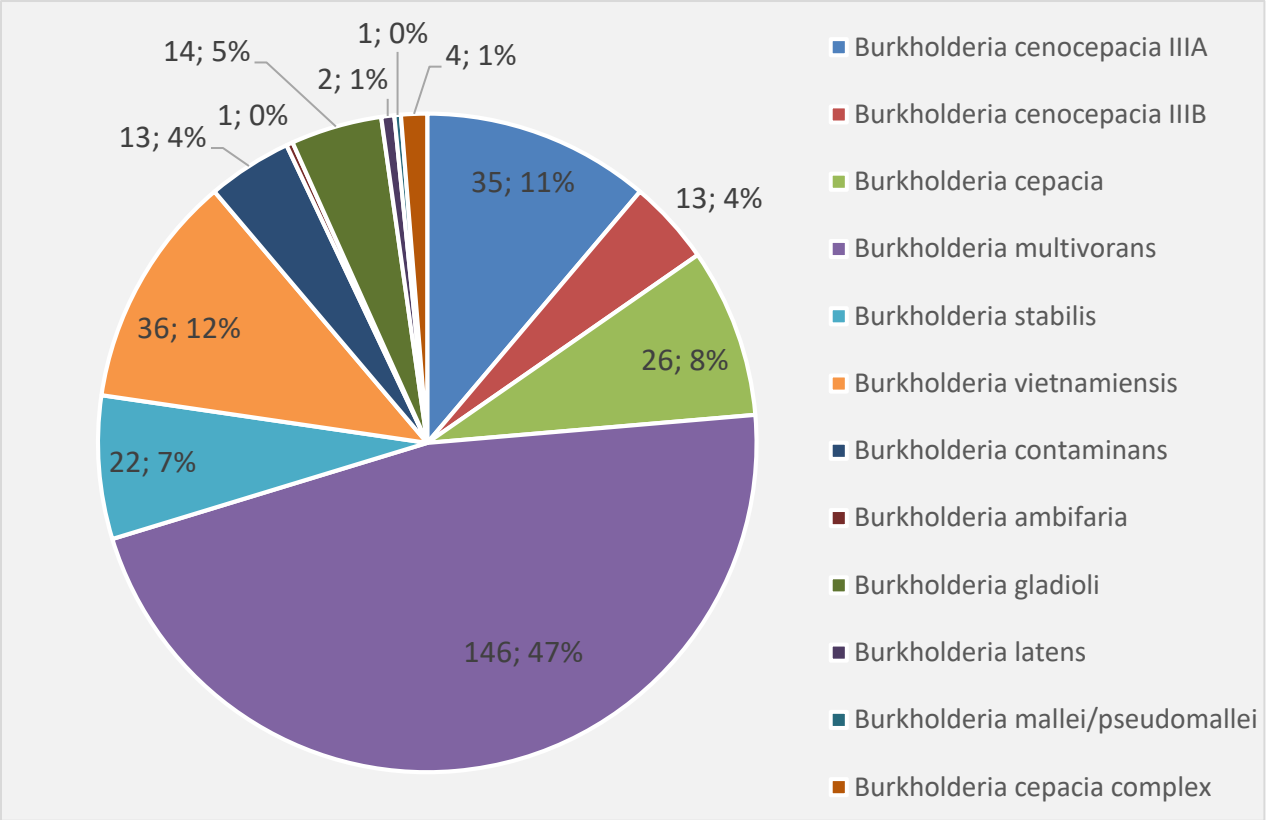


Figure 2. Distribution of BCC and other *Burkholderia* species from 2012 to 2020

OTHER GNNF

A total of 832 GNNF species were received from 2012 to 2020. See Figure 3 for the distribution of the GNNF. *Achromobacte*r spp. represented 48% of the total number of GNNF isolates followed by *Stenotrophomonas maltophilia* (22%) and the other GNNF species at lower proportions. The yearly number of these microorganisms increased highly from 2012 to 2014 (29 to 114), this number decreased slightly in 2015 and 2017. In 2016, the total number of these microorganisms reached a peak of 134 isolates because of the above-mentioned *Achromobacter* study. In 2020, the number of GNNF received was also lower than in previous six years because of the reduced activity at the NRC due to SARS-COVID-19 pandemic as mentioned above.

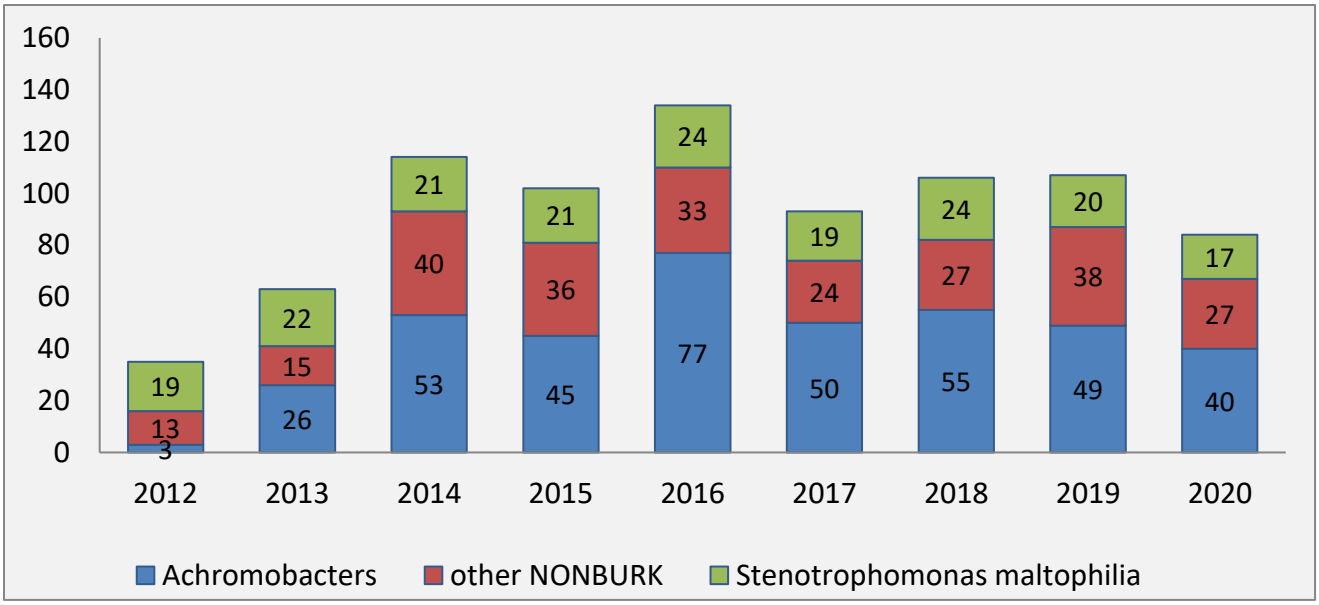


Figure 3. Distribution of GNNF species from 2012 to 2020

Table 1. Distribution of GNNF other than *Achromobacter* spp. and *S. maltophilia* (2012–2020)

Species	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total
Pseudomonas sp.	0	6	8	9	12	4	2	6	3	50
Ochrobactrum sp.	0	0	5	8	5	5	6	5	3	37
Rhizobium sp.	0	1	8	7	1	0	0	0	0	17
Rhizobium pusense	0	0	0	0	0	1	3	4	5	13
Bordetella genogroup 6	0	1	2	1	1	1	1	2	1	10
Inquilinus limosus	1	1	1	1	1	1	1	1	1	9
Delftia sp.	0	1	0	1	0	3	2	0	1	8
Bordetella bronchiseptica	0	0	0	2	0	0	0	2	2	6
Delftia lacuris	0	0	3	0	1	0	0	0	0	4
Elizabethkingia miricola	0	0	1	0	0	1	1	1	0	4
Elizabethkingia ursingii	0	0	0	0	0	0	2	1	1	4
Pandoraea sp.	2	0	1	0	1	0	0	0	0	4
Bordetella sp.	0	0	1	0	0	1	0	1	0	3
Chryseobacterium indologenes	0	0	0	0	1	0	0	1	1	3
Paracoccus yeei	1	1	1	0	0	0	0	0	0	3
Pseudomonas juntendi	0	0	0	0	0	0	0	1	2	3
Pseudomonas paralactis	0	0	0	0	0	1	1	1	0	3
Ralstonia mannitolilytica	2	0	0	1	0	0	0	0	0	3
Rhizobium massiliae	0	0	0	0	3	0	0	0	0	3
Rhizobium radiobacter	0	1	0	0	0	0	0	1	1	3
Stenotrophomonas rhizophila	0	0	0	1	0	0	0	1	1	3
Bordetella genogroup 2	0	0	1	1	0	0	0	0	0	2
Brevundimonas diminuta	0	0	1	0	1	0	0	0	0	2
Chryseobacterium sp.	0	0	1	1	0	0	0	0	0	2
Pseudochrobactrum sp.	1	0	0	0	0	0	1	0	0	2
Pseudomonas koreensis	0	0	1	0	0	0	0	0	1	2
Pseudomonas putida	0	0	0	0	0	0	1	0	1	2

*: Only species found more than once are shown in this table.

ACHROMOBACTER SPP.

At the beginning of the NRC in 2012, the number of *Achromobacter* isolates represented only 10% of the total GNNF isolates. However, in the following years, the total yearly number of *Achromobacter* spp. was fluctuating around half of the total number of GNNF (i.e. non *Burkholderia* isolates) (41% in 2013, 48% in 2020). Figure 4 shows the distribution of the different *Achromobacter* spp. isolated from 2012 to 2020. Thirteen different *Achromobacter* species were identified; with *Achromobacter xylosoxidans* being the most predominant over the whole 2012-2020 period (68%) followed by *Achromobacter insuavis* (17%). Other *Achromobacter* spp. were found at very low proportions.

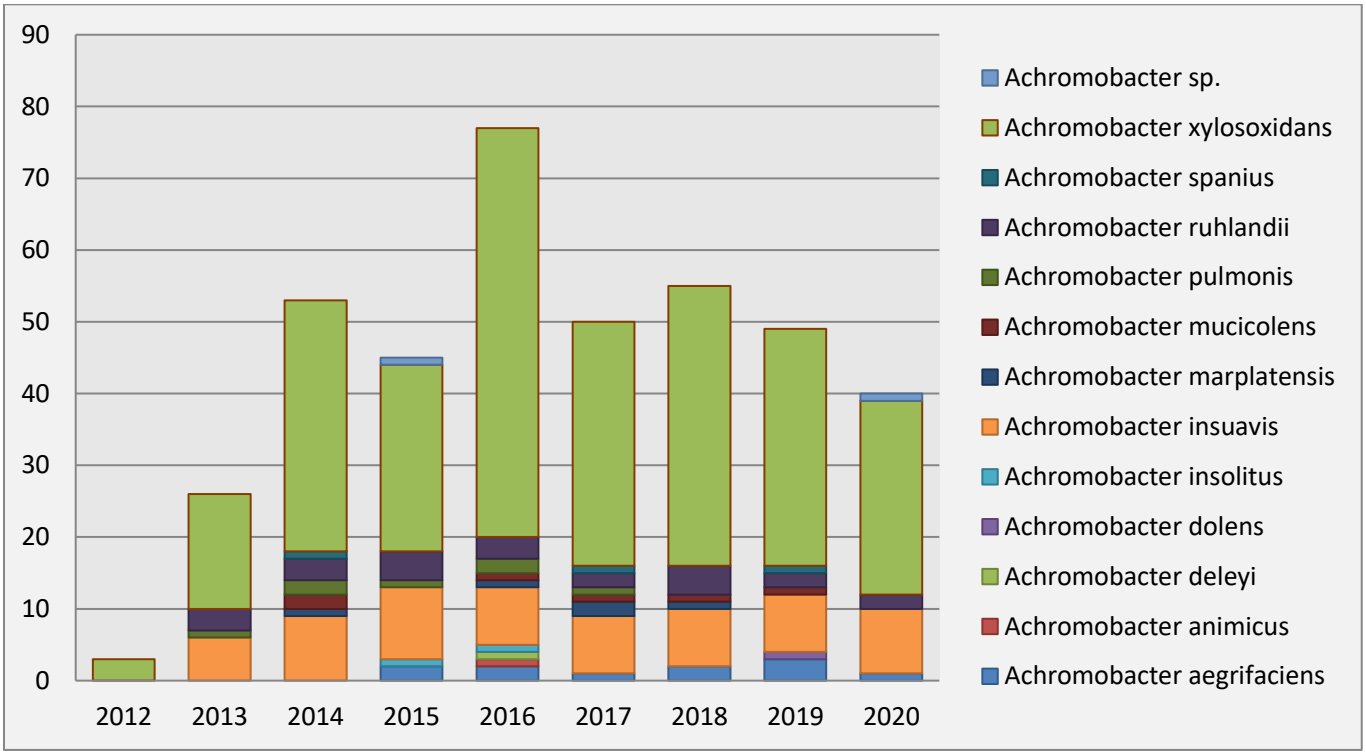


Figure 4. Distribution of the different *Achromobacter* spp. Isolated from 2012 to 2020.

MOLECULAR TYPING OF BCC AND ACHROMOBACTER

BCC and *Achromobacter* spp. isolates from CF patients are systematically typed by using MLST based on Whole Genome Sequencing data. This characterization of the isolates is performed in order to further investigate the possible association between some subtypes and pulmonary unfavorable disease evolution in CF patients. Other GNNF isolates are not routinely typed. Figure 5 and 6 show the list and the number of different sequence types (ST) found among BCC and *Achromobacter* spp. isolates respectively, and this for the whole period from 2012 to 2020.

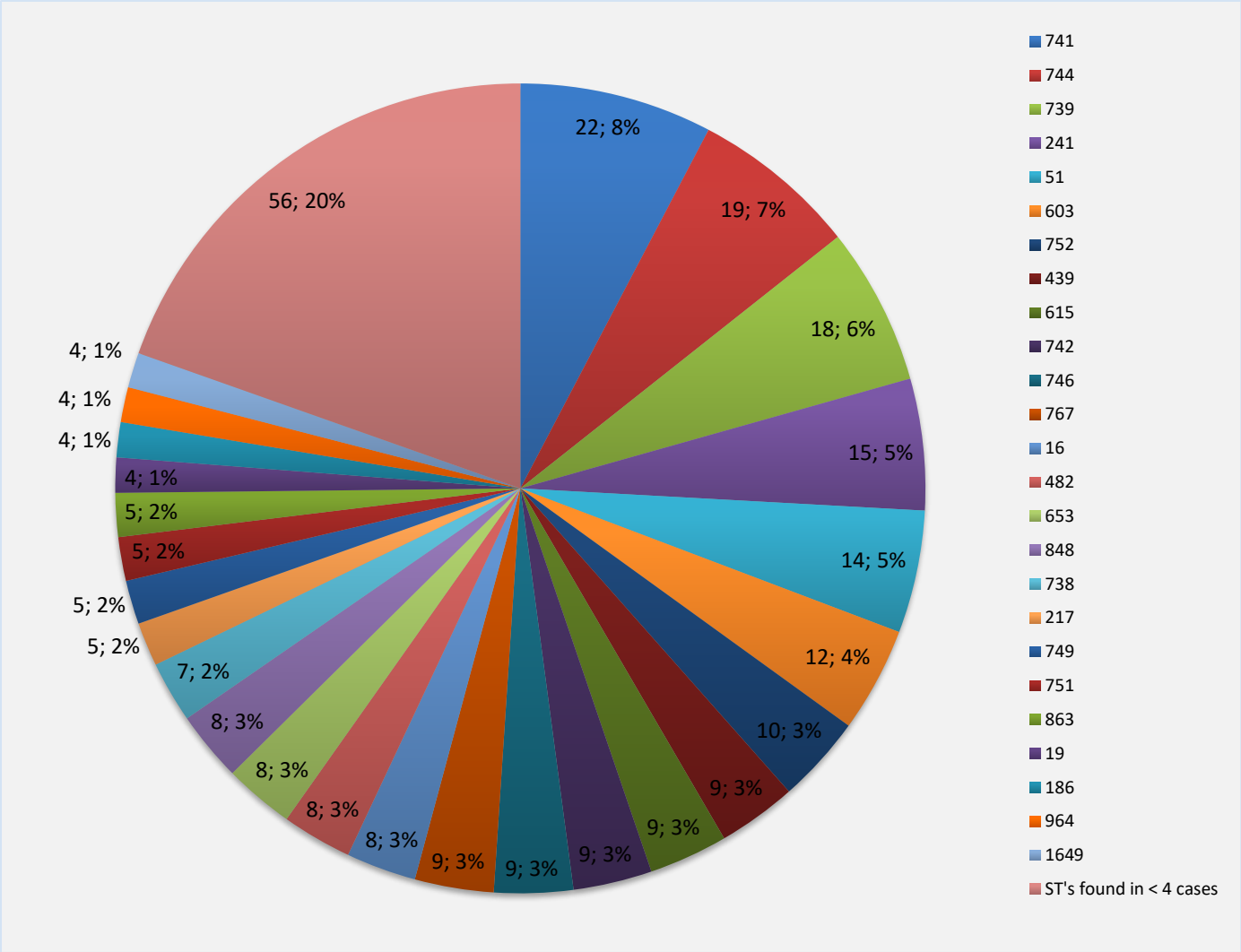


Figure 5. BCC sequence types (ST) among the isolates from 2012 to 2020

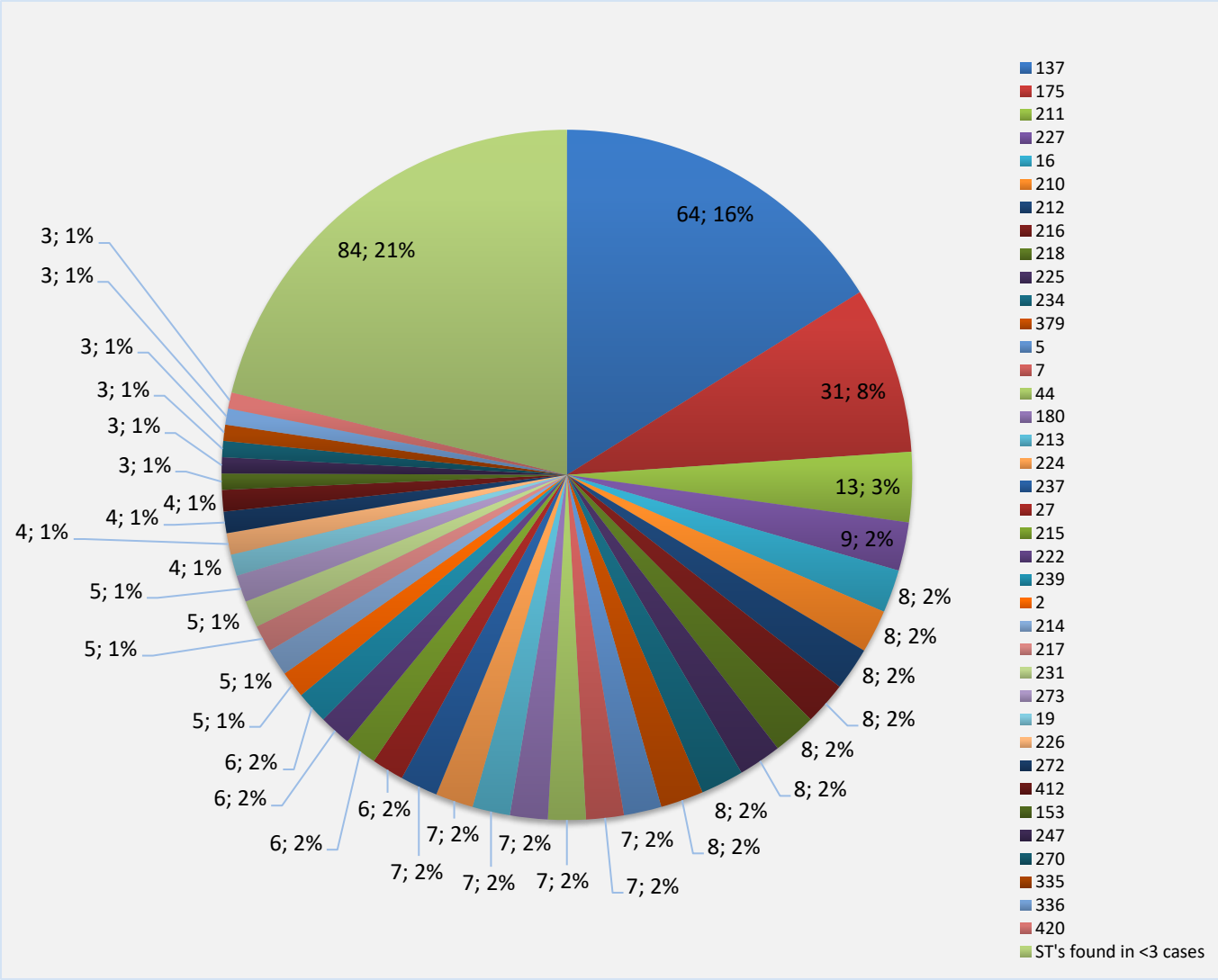


Figure 6. *Achromobacter* spp. ST's among the isolates from 2012 to 2020

OUTBREAKS/SMALL CLUSTERS

OUTBREAKS FROM 2012 TO 2017

Three outbreaks occurred at this period due to *Burkholderia cepacia* MLST type ST-848, *Burkholderia stabilis* MLST ST-653 and *Burkholderia cepacia* MLST ST-767 respectively. More details about these outbreaks were reported at the previous NRC report for the period 2012 to 2017 (published at Sciensano).

BURKHOLDERIA CEPACIA ST-767/ST-1649 OUTBREAK

Two episodes of a polyclonal *B. cepacia* outbreak occurred at a Belgian intensive care unit due to contaminated wash gloves. Five out of seven patient isolates from April-May 2019 were identified as *B. cepacia* (n=5). One of two tested wash glove packages was culture positive and the investigated wash glove isolate was identified as *B. cepacia*. RAPD typing showed an identical pattern for four *B. cepacia* patient isolates and the wash glove isolate, and a different pattern for the remaining *B. cepacia* patient isolate. MLST analysis confirmed the RAPD typing results and identified the two B. cepacia RAPD patterns as ST-1649 (n=5) and ST-767 (n=1). The manufacturer confirmed the contamination of one lot by *Burkholderia* (but provided no cultures) and reported to have taken measures to avoid further contamination.
Three patient isolates from January 2020 were all *B. cepacia* ST-767 (n=three, two isolates from same patient). One out of four tested wash glove lots was culture positive and yielded both *B. cepacia* ST-767 and ST-1649. After abandoning the use of wash gloves from this manufacturer, no new cases were recorded.
SNP analysis showed a maximum of 12 and 6 SNPs among *B. cepacia* patient and wash glove genomes of ST-767 and ST-1649, respectively.
Recovery of *B. cepacia* from several ICU patients led to a small-scale outbreak investigation and identified contaminated wash gloves as the outbreak common source. Remarkably, in the course of the present study, *B. cepacia* ST-767 and ST-1649 contaminated wash gloves from the same manufacturer were reported as the cause of an outbreak in a heart clinic in Switzerland (10).

BURKHOLDERIA MULTIVORANS CLONES

See figure 5 for the distribution of MLST types in BCC. Beside the ST's of the above-mentioned outbreaks, the 5 most frequent *B. multivorans* ST's are shown in table 2. These have been found not only in samples from the same patients but also in a small number of patients that do not necessarily have a link between each other.
For ST-742, a genomic analysis was performed for thirteen isolates from an endemic *B. multivorans* strain infecting four cystic fibrosis patients treated in different pediatric cystic fibrosis centers in Belgium, with no evidence of cross-infection. The study showed limited within patient *B. multivorans* evolution but high between-patient strain diversity, indicating that an environmental micro diverse reservoir must be present for this endemic strain (11).

Table 2. *Burkholderia multivorans* frequent ST's

	ST-741	ST-739	ST-603	ST-752	ST-742
Number of isolates	22	18	12	10	9
Number of patients	11	9	6	4	4

BURKHOLDERIA STABILIS ST-51

A total of 14 isolates found in 9 different patients over the period from 2012 to 2020. Only one isolate was found during the last three years.

BURKHOLDERIA VIETNAMIENSIS ST-744

A total of 19 isolates found in three different patients over the period from 2012 to 2020. Two out of the three patients are brother/sister. No isolate belonging to the third patient was received during the last three years.

BURKHOLDERIA CENOCEPACIA IIIA ST-241

A total of 15 isolates found in 6 different patients over the period from 2012 to 2020. In the last three years, isolates from only two patients were received. As we do not have the data about the outcome of the patients, we do not know the reason of this decline in number of patients contaminated with this particular *B. cenocepacia* ST at our NRC.

ACHROMOBACTER CLONES

ST-137 and ST-175 accounted for the most frequent ST's among *Achromobacter xylosoxidans* isolates in CF patients from 2012 to 2020 (see table 3). These two ST's are frequently isolated among CF patients followed mainly in three different CF centers in Belgium. In a comparative study, the Belgian ST-137 (n=25) clones shared the same PFGE profile with the French ones (n=23), suggesting a Belgian origin, and most have the same multi-resistant profile to the majority of the 26 antibiotics tested (14). It would be interesting to further study the clinical effect of harboring these resistant strains. In addition, it would be interesting to investigate whether current infection control measures in CF wards would still need improvement or would CF patients acquire this infection from multiple environmental reservoirs.

Table 3. *A. xylosoxidans* frequent ST's

	ST-137	ST-175
Number of isolates	64	31
Number of patients	29	17

GEOGRAPHICAL DISTRIBUTION

See Figure 7 for the BCC-GNNF cases distribution per Belgian province. This distribution is based on the patients' postal code data. According to these data, we can observe that Antwerp, Flemish Brabant, East Flanders and Brussels-capital region are the most represented. Namur Walloon Brabant and Luxembourg are the less represented provinces. For the distribution of the cases according to the centers attended by the patients for their follow-up see Figure 8. The centers contributing for the most cases are in the following hospitals: UZ Brussel, UCL Saint Luc (Brussels-capital region) and UZ Leuven (Flemish Brabant).

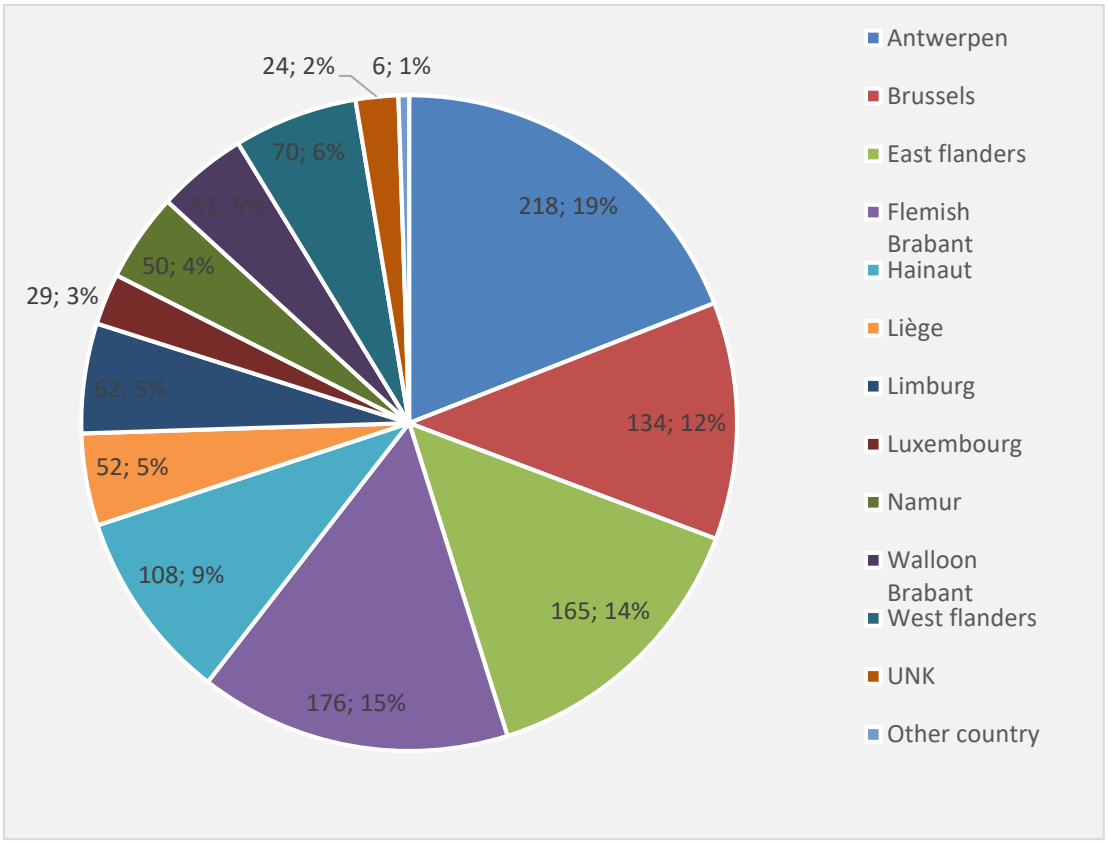


Figure 7. BCC-GNNF cases distribution per province for 2012-2020

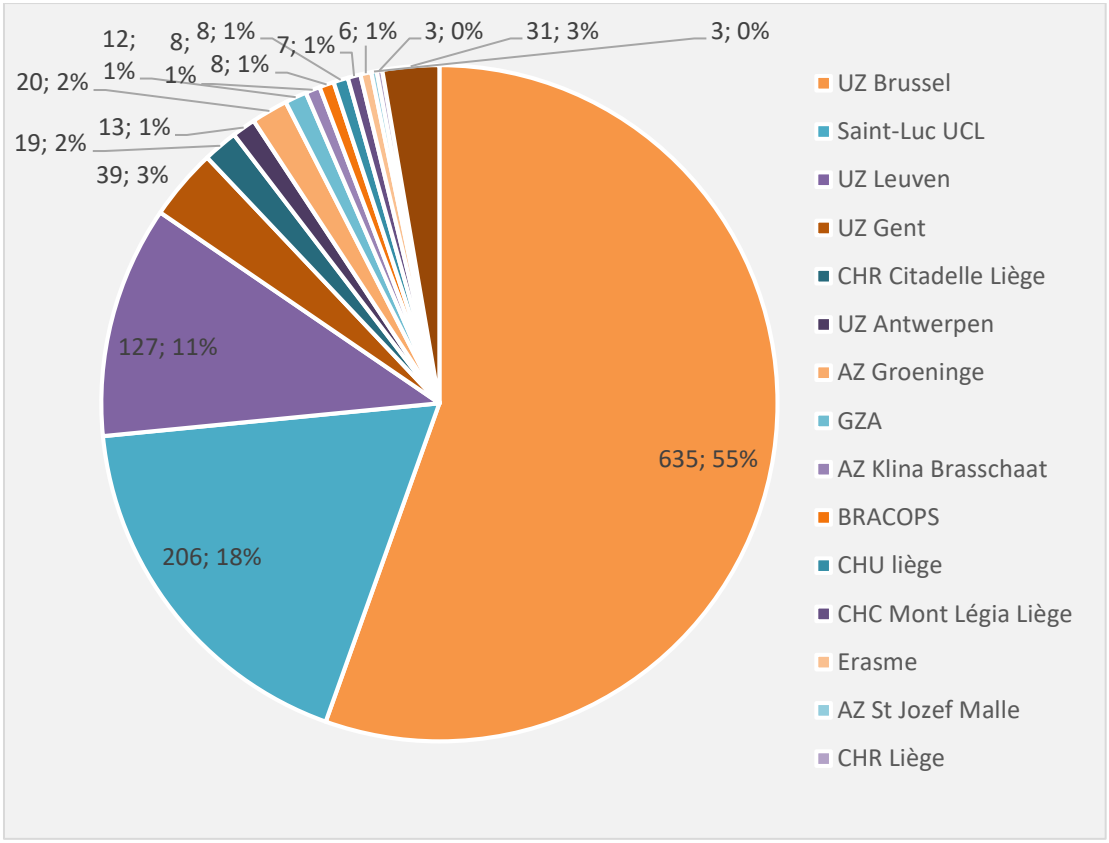


Figure 8. BCC-GNNF cases distribution per reffering center

NRC-BCC-GNNF PATIENTS

TOTAL NUMBER

The average number of patients was 106 (min 69 [2012] and max 141 [2016]), see figure 9. About 17% of the patients were infected by more than one BCC-GNNF microorganism.

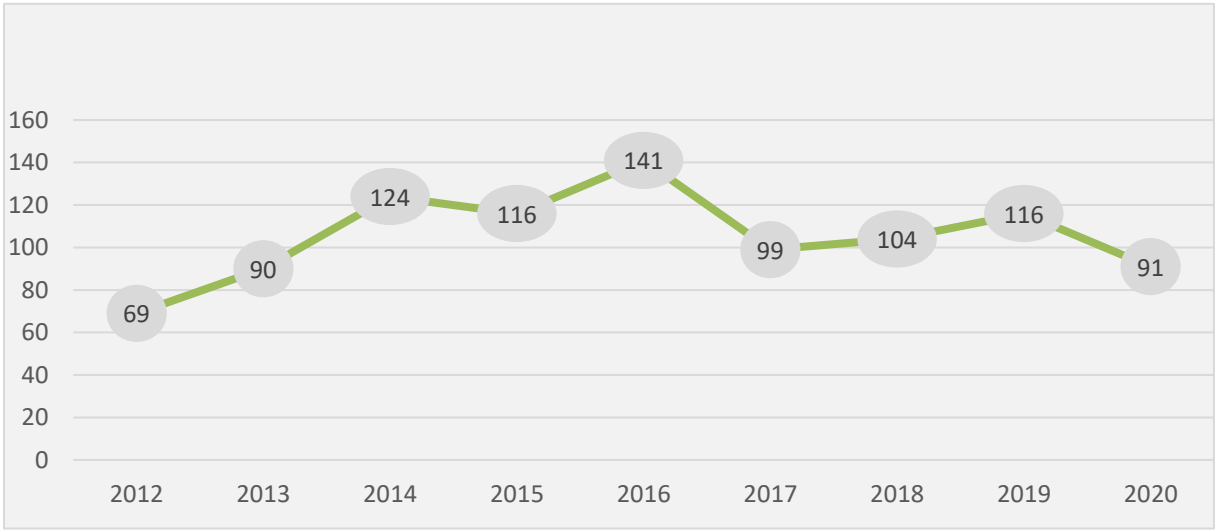


Figure 9. Yearly total number of the patients included at NRC-BCC-GNNF

AGE AND GENDER DISTRIBUTION

The mean age of the patients that have been followed at NRC during the whole period from 2012 to 2020 was 25 years (median: 23). The male/female distribution is shown in figure 10. From 2013, the number of males is yearly slightly higher than the number of females among the patients followed at the NRC-BCC-GNNF.

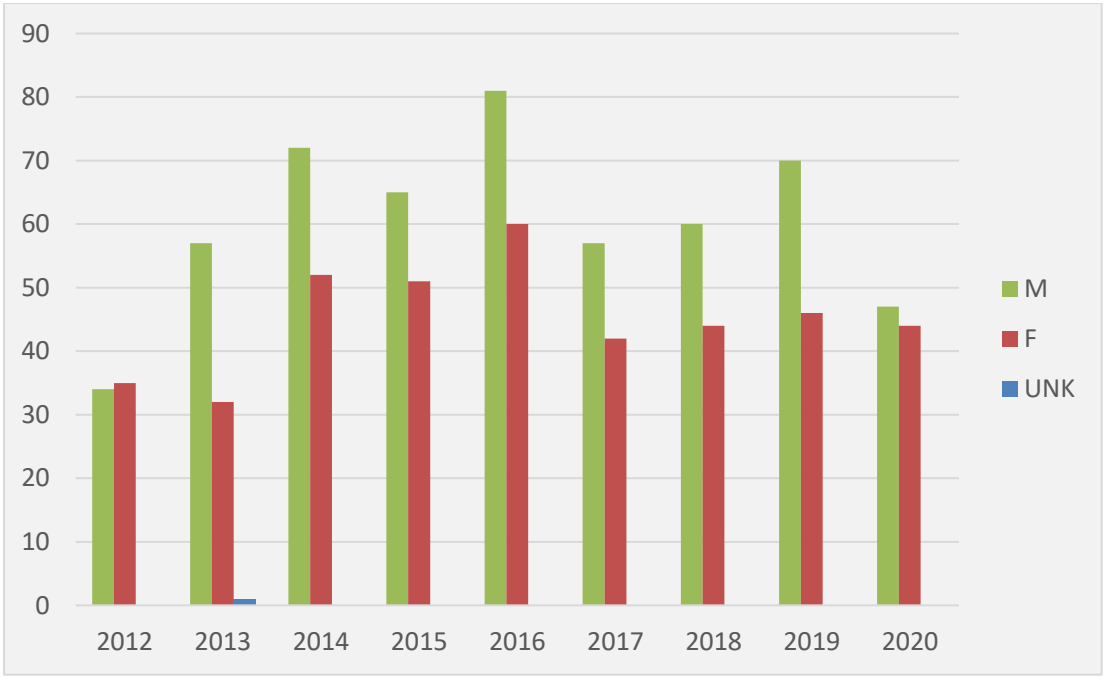


Figure 10. Yearly Male/Female distribution among the patients included at NRC-BCC-GNNF

CF/non-CF Patients

In addition to BCC-GNNF isolates from cystic fibrosis (CF) patients, we yearly receive a very small proportion of isolates from patients affected by other diseases, namely immunocompromised or patients affected by other lung diseases. Analyzing such isolates is also relevant because it may help to detect outbreaks due to BCC-GNNF at a very early stage. See Figure 11 for the distribution of CF/non-CF patients.

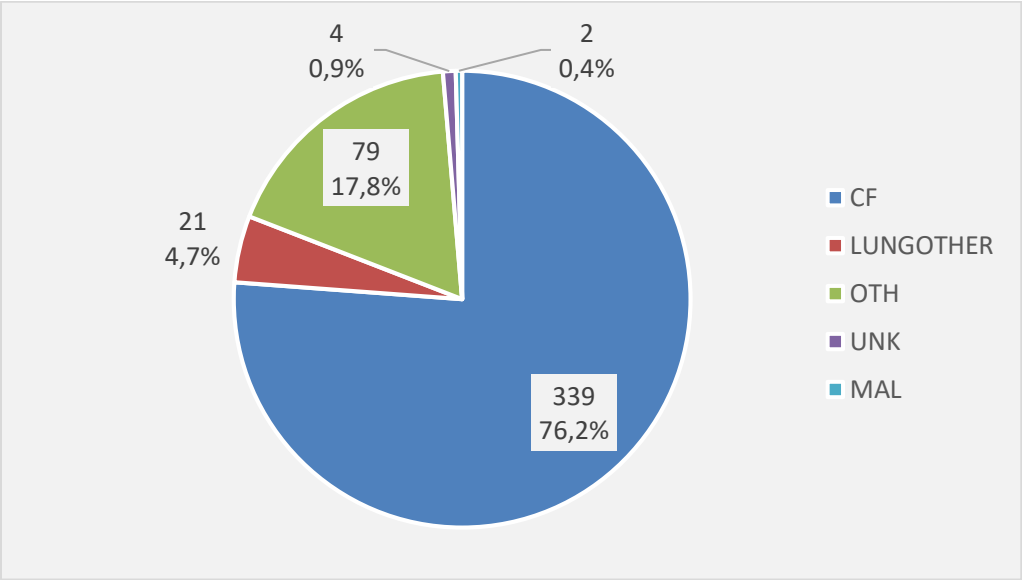


Figure 11. NRC-BCC-GNNF patients' (CF/non CF) distribution according to disease (MAL: malignancy, UNK: unknown, OTH: other, LUNGOTHER: other lung disease)

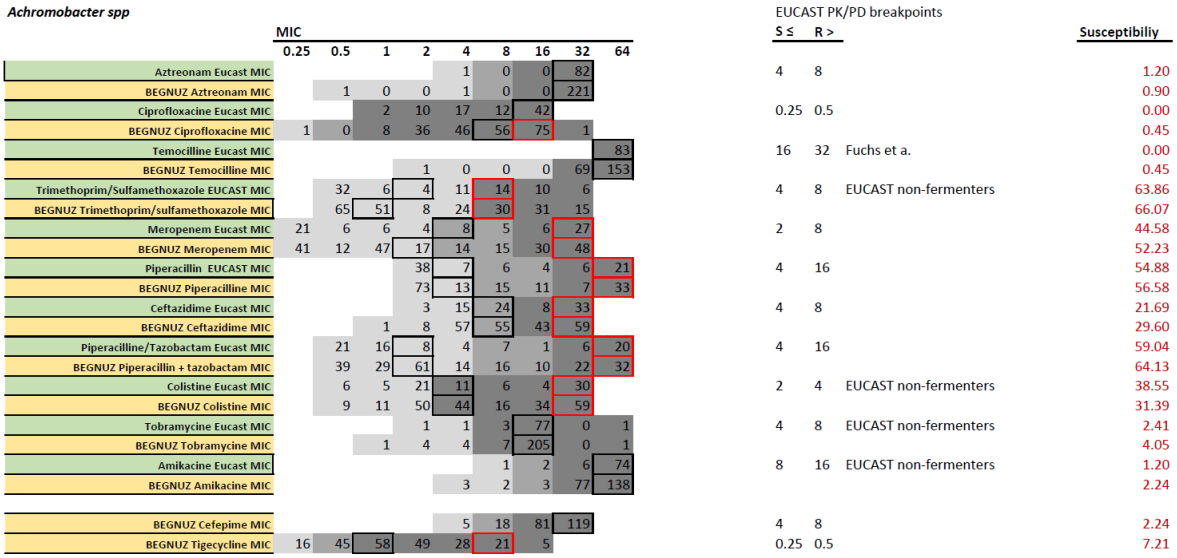
MIC distribution of BCC and GNNF strains for the 13 tested antibiotics

BCC

See the following publication (15)

Achromobacter spp.

We performed the *in vitro* antimicrobial susceptibility of *Achromobacter* species to 13 antimicrobial agents by microdilution in microtiter plates, with the Sensititre Antimicrobial Susceptibility Testing System (Thermofischer). A total of 222 clinical samples were isolated from Belgian cystic fibrosis patients (2016-2019). Since there are no species-specific EUCAST or CLSI breakpoints available for *Achromobacter* species, the EUCAST PK/PD (non-species related) breakpoints were applied for most antibiotics. Breakpoints for aminoglycosides, colistin, and trimethoprim-sulfamethoxazole were based on those from EUCAST for non-fermenters. For temocillin, breakpoints described by Fuchs et al. (1985) were used.
See figure 12 for the results obtained. These results were presented at the 43rd European Cystic Fibrosis Conference, 2020 and the abstracts of this conference were published as supplement in the Journal of Cystic Fibrosis (16).



Achromobacter spp

	MIC									EUCAST PK/PD breakpoints			Susceptibiliy
	0.25	0.5	1	2	4	8	16	32	64	S ≤	R >		
Aztreonam Eucast MIC					1	0	0	82		4	8		1.20
BEGNUZ Aztreonam MIC		1	0	0	1	0	0	221					0.90
Ciprofloxacine Eucast MIC			2	10	17	12	42			0.25	0.5		0.00
BEGNUZ Ciprofloxacine MIC	1	0	8	36	46	56	75	1					0.45
Temocilline Eucast MIC									83	16	32	Fuchs et a.	0.00
BEGNUZ Temocilline MIC				1	0	0	0	69	153				0.45
Trimethoprim/Sulfamethoxazole EUCAST MIC		32	6	4	11	14	10	6		4	8	EUCAST non-fermenters	63.86
BEGNUZ Trimethoprim/sulfamethoxazole MIC		65	51	8	24	30	31	15					66.07
Meropenem Eucast MIC	21	6	6	4	8	5	6	27		2	8		44.58
BEGNUZ Meropenem MIC	41	12	47	17	14	15	30	48					52.23
Piperacillin EUCAST MIC				38	7	6	4	6	21	4	16		54.88
BEGNUZ Piperacilline MIC				73	13	15	11	7	33				56.58
Ceftazidime Eucast MIC				3	15	24	8	33		4	8		21.69
BEGNUZ Ceftazidime MIC			1	8	57	55	43	59					29.60
Piperacilline/Tazobactam Eucast MIC		21	16	8	4	7	1	6	20	4	16		59.04
BEGNUZ Piperacillin + tazobactam MIC		39	29	61	14	16	10	22	32				64.13
Colistine Eucast MIC		6	5	21	11	6	4	30		2	4	EUCAST non-fermenters	38.55
BEGNUZ Colistine MIC		9	11	50	44	16	34	59					31.39
Tobramycine Eucast MIC				1	1	3	77	0	1	4	8	EUCAST non-fermenters	2.41
BEGNUZ Tobramycine MIC			1	4	4	7	205	0	1				4.05
Amikacine Eucast MIC						1	2	6	74	8	16	EUCAST non-fermenters	1.20
BEGNUZ Amikacine MIC					3	2	3	77	138				2.24
BEGNUZ Cefepime MIC					5	18	81	119		4	8		2.24
BEGNUZ Tigecycline MIC	16	45	58	49	28	21	5			0.25	0.5		7.21

Figure 12. In vitro susceptibility of *Achromobacter* species isolated from cystic fibrosis patients

CONCLUSIONS

- A slight decline in the number of the referred samples to BCC GNNF NRC during the year 2020 was observed. This decline is due to NRC activity stop during few months at the beginning of the COVID-19 pandemic.
- As reported before, the same three Belgian centers are contributing to a large proportion of the referred isolates. Other Belgian centers should include more cases in order to obtain a better representation of the distribution of cases over the whole country.
- The recent availability of whole genome sequencing data for the analysed BCC and *Achromobacter* spp. isolates has allowed for deeper investigations of frequent sequence types among CF patients. These data could further help in a better understanding of acquisition ways of infection and subsequently would contribute to the improvement of infection control measures.

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