National reference centre for Burkholderia cepacia complex (BCC) and other Gram-negative non-fermenters (GNNF) (except *P. aeruginosa* and *Acinetobacter* spp.): 2021-2022 data report

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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. In this report the NRC-BCC-GNNF data gathered in the two years 2021 to 2022 are shown and compared to the data from previous years described in previous NRC-BCC-GNNF reports (9,10).

Number of BCC and GNNF

The NRC-BCC-GNNF has gathered data for 251 isolates in Belgium between 2021 and 2022, with 126 in 2021 and 125 in 2022 (Figure 1). This number is in accordance with previous years, where we identified an average of 127 BCC and GNNF strains per year [min 70 (2012) and max 163 (2016)]. BCC and other *Burkholderia* species represented only 25% of the total cases, which is also in accordance with the years since 2014, as in 2012 and 2013 this proportion was higher (59% and 42% respectively).

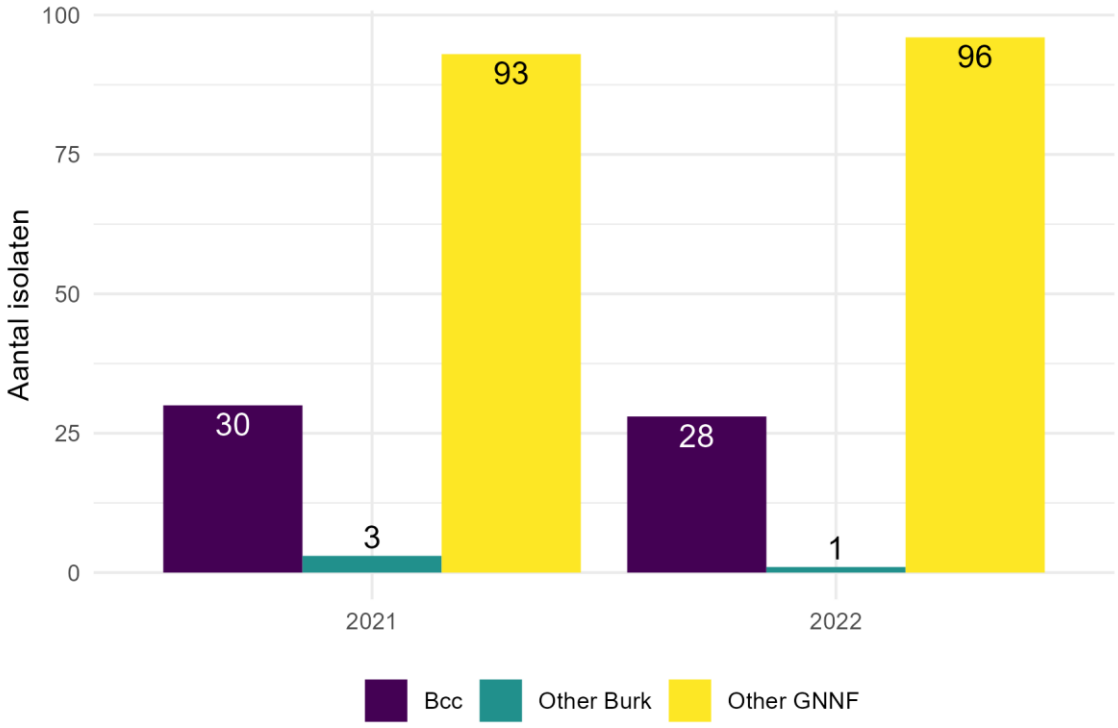

Figure 1. Distribution of *Burkholderia* spp. and GNNF in 2021 to 2022 (Other Burk: other *Burkholderia* spp. than BCC; Other GNNF: other GNNF than *Burkholderia* spp.).

BURKHOLDERIA CEPACIA COMPLEX/OTHER BURKHOLDERIA SPECIES

A total of 62 BCC and other *Burkholderia* species were received during the two year period (2021-2022). Ten different species were found, *B. multivorans* represented 42% followed by *B. cenocepacia* (29%), *B. vietnamiensis* (8%), and the other species at lower proportions (Figure 2). These data are in accordance with the previous years, except for *B. cenocepacia* that represented only 15% of the total *Burkholderia* cases identified in 2012-2020.

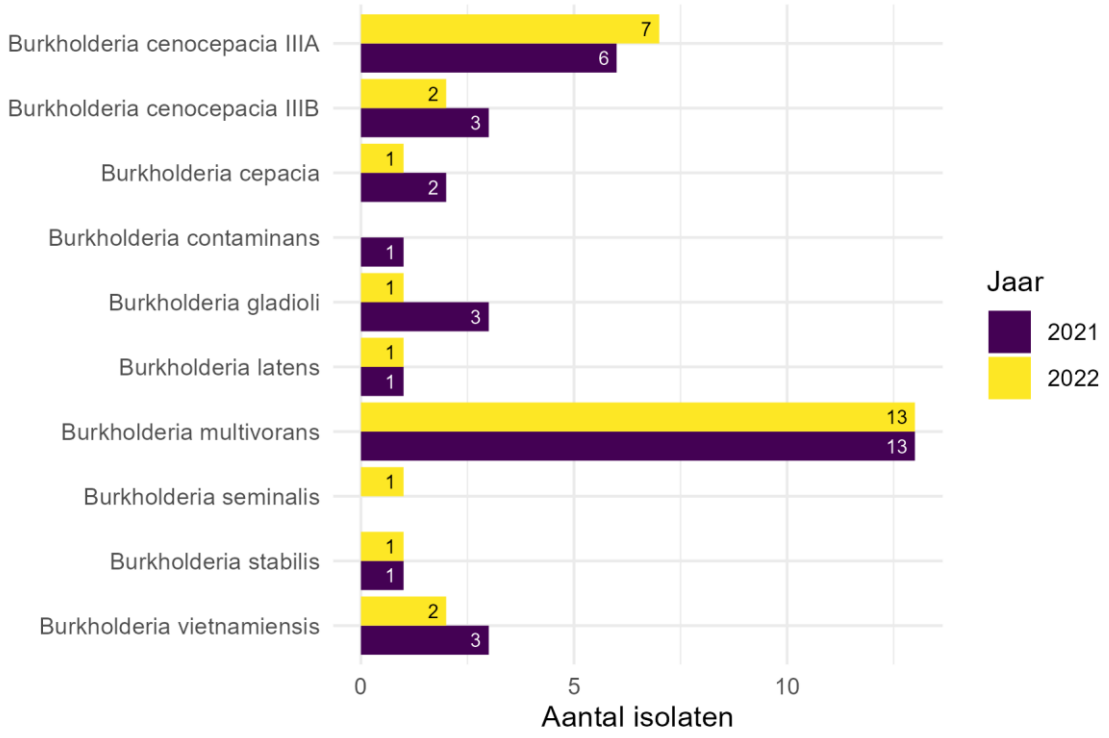

Figure 2. Distribution of BCC and other *Burkholderia* species in 2021–2022.

OTHER GNNF

A total of 189 GNNF species were received in the two year period (2021–2022). *Achromobacter* spp. represented 42% of the total number of GNNF isolates followed by *Stenotrophomonas maltophilia* (17%) and the other GNNF species at lower proportions (Figure 3). Table 1 gives an overview of the remaining other GNNF species for 2012–2022.

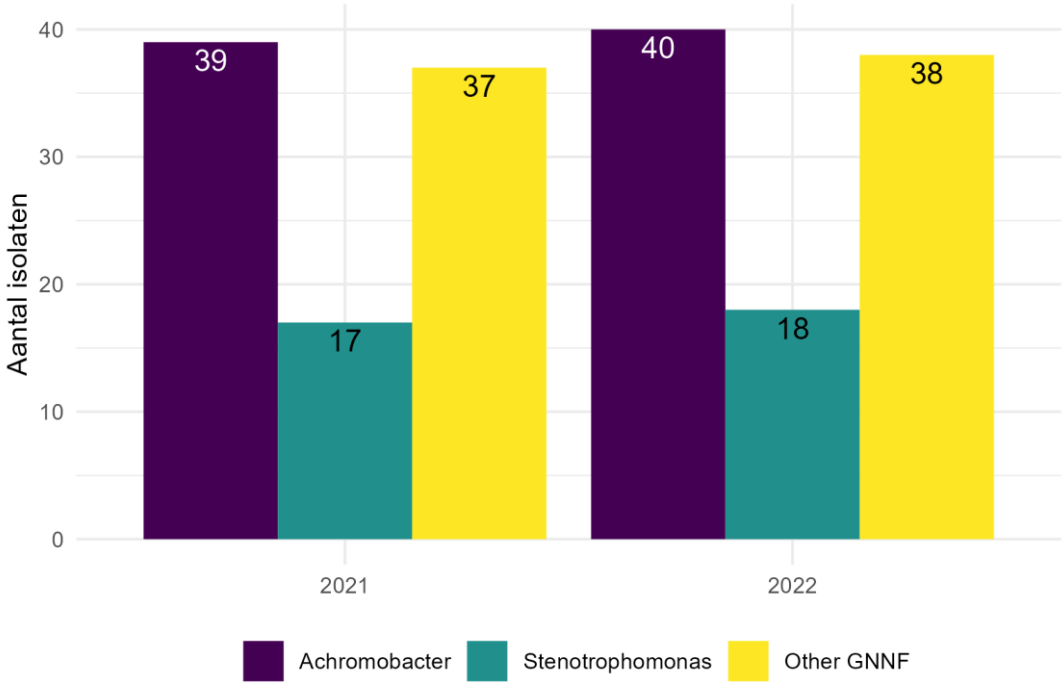

Figure 3. Distribution of GNNF species other than *Burkholderia* spp. in 2021–2022

ACHROMOBACTER SPP.

Nine different *Achromobacter* species were identified in 2021–2022 (Figure 4); with *Achromobacter xylosoxidans* being the most predominant (59%) followed by *Achromobacter insuavis* (19%). Other *Achromobacter* spp. were found at very low proportions.

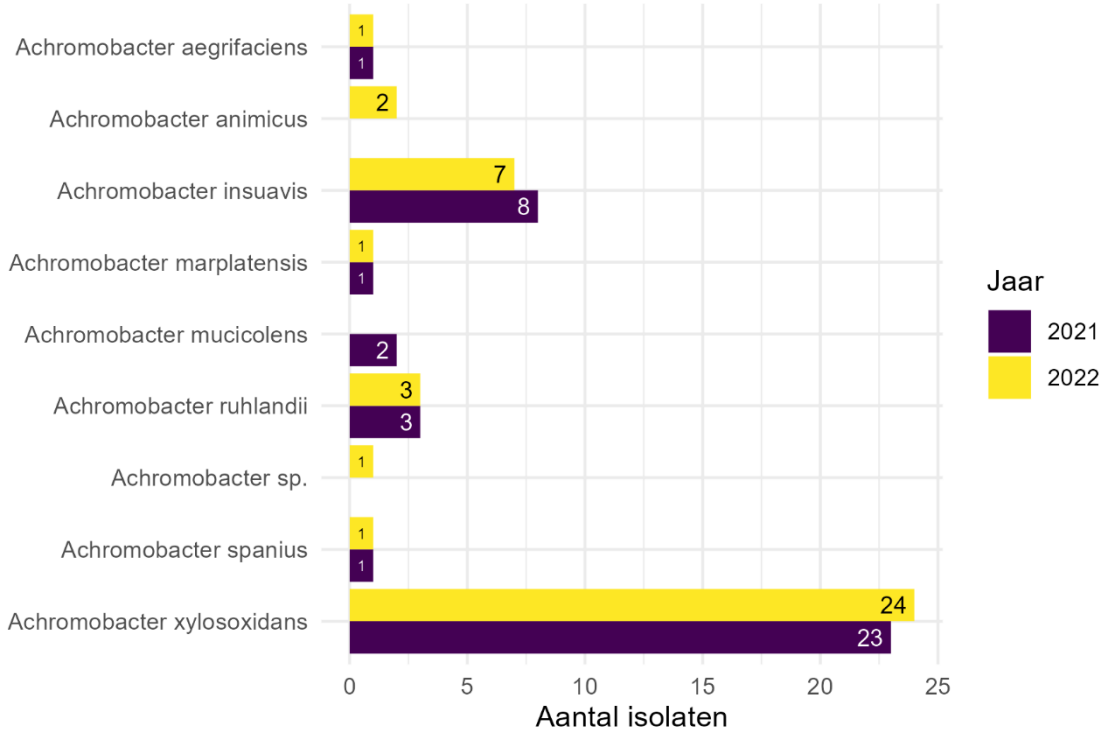

Figure 4. Distribution of the different *Achromobacter* spp. Isolated in 2021 to 2022.

Table 1. Distribution of GNNF other than *Achromobacter* spp. and *S. maltophilia* (2012–2022).

Species	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	Total
Pseudomonas sp.	0	6	8	9	12	4	2	6	3	5	4	59
Ochrobactrum sp.	0	0	5	8	5	5	6	5	3	5	6	48
Rhizobium sp.	0	1	8	7	1	0	0	0	0	0	0	17
Rhizobium pusense	0	0	0	0	0	1	3	4	5	0	0	13
Bordetella sp. (genogroup 6)	0	1	2	1	1	1	1	2	1	1	1	12
Delftia sp.	0	1	0	1	0	3	2	0	1	3	1	12
Inquilinus limosus	1	1	1	1	1	1	1	1	1	2	1	12
Agrobacterium pusense	0	0	0	0	0	0	0	0	0	7	3	10
Bordetella bronchiseptica	0	0	0	2	0	0	0	2	2	2	1	9
Elizabethkingia miricola	0	0	1	0	0	1	1	1	0	0	1	5
Delftia lacuris	0	0	3	0	1	0	0	0	0	0	0	4
Elizabethkingia ursingii	0	0	0	0	0	0	2	1	1	0	0	4
Pandoraea sp.	2	0	1	0	1	0	0	0	0	0	0	4
Stenotrophomonas rhizophila	0	0	0	1	0	0	0	1	1	1	0	4
Bordetella sp.	0	0	1	0	0	1	0	1	0	0	0	3
Brevundimonas diminuta	0	0	1	0	1	0	0	0	0	1	0	3
Chryseobacterium indologenes	0	0	0	0	1	0	0	1	1	0	0	3
Chryseobacterium sp.	0	0	1	1	0	0	0	0	0	1	0	3
Delftia acidovorans	0	0	0	0	1	0	0	0	0	0	2	3
Paracoccus yeei	1	1	1	0	0	0	0	0	0	0	0	3
Pseudomonas juntendi	0	0	0	0	0	0	0	1	2	0	0	3
Pseudomonas lactis	0	0	0	0	0	0	0	0	1	0	2	3
Pseudomonas paralactis	0	0	0	0	0	1	1	1	0	0	0	3
Ralstonia mannitolilytica	2	0	0	1	0	0	0	0	0	0	0	3
Rhizobium massiliae	0	0	0	0	3	0	0	0	0	0	0	3
Rhizobium radiobacter	0	1	0	0	0	0	0	1	1	0	0	3

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

MOLECULAR TYPING OF BCC AND ACHROMOBACTER

BCC and *Achromobacter* spp. isolates from CF patients were systematically typed 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 period 2021-2022.

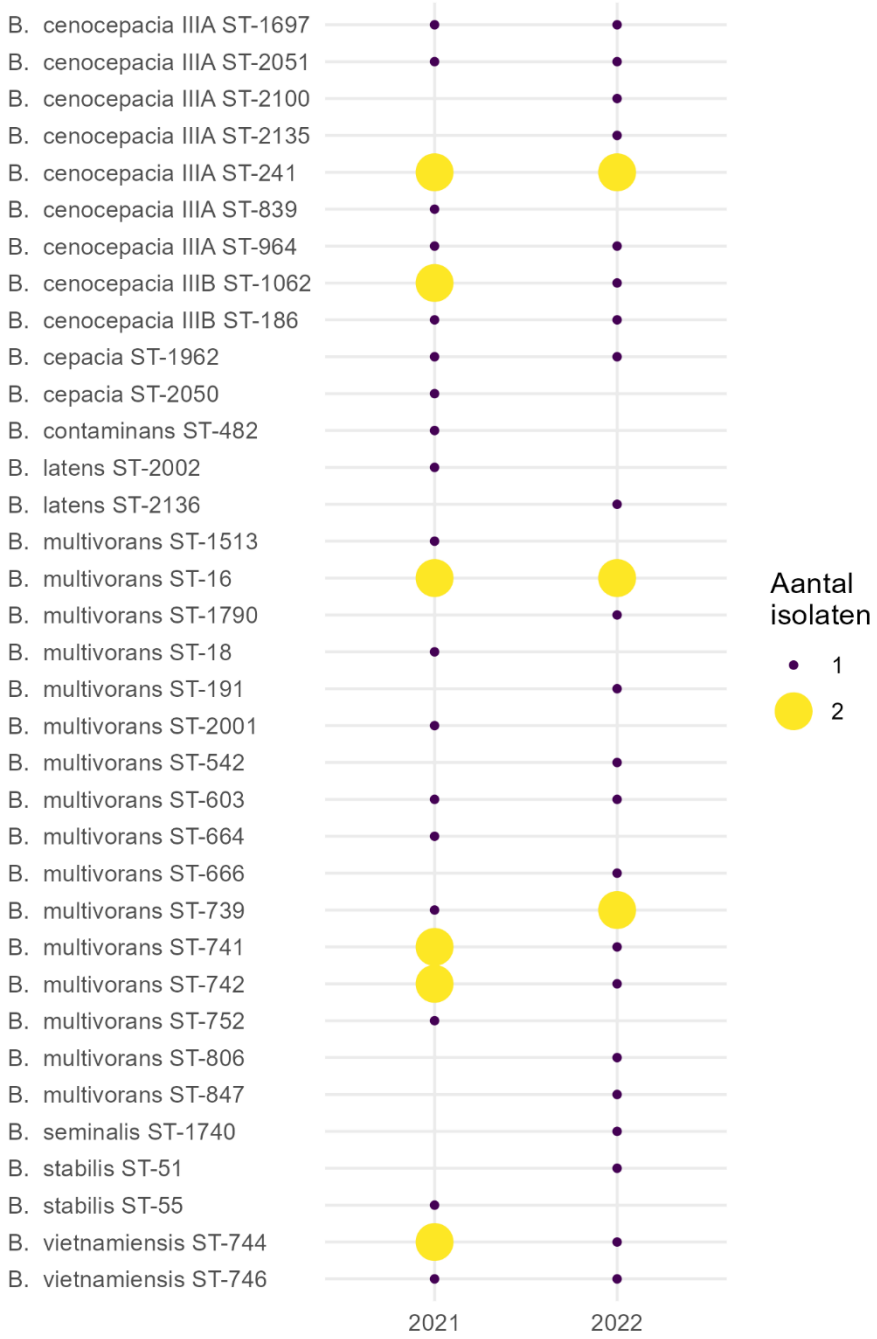

Figure 5. BCC sequence types (ST) among the isolates in 2021-2022.

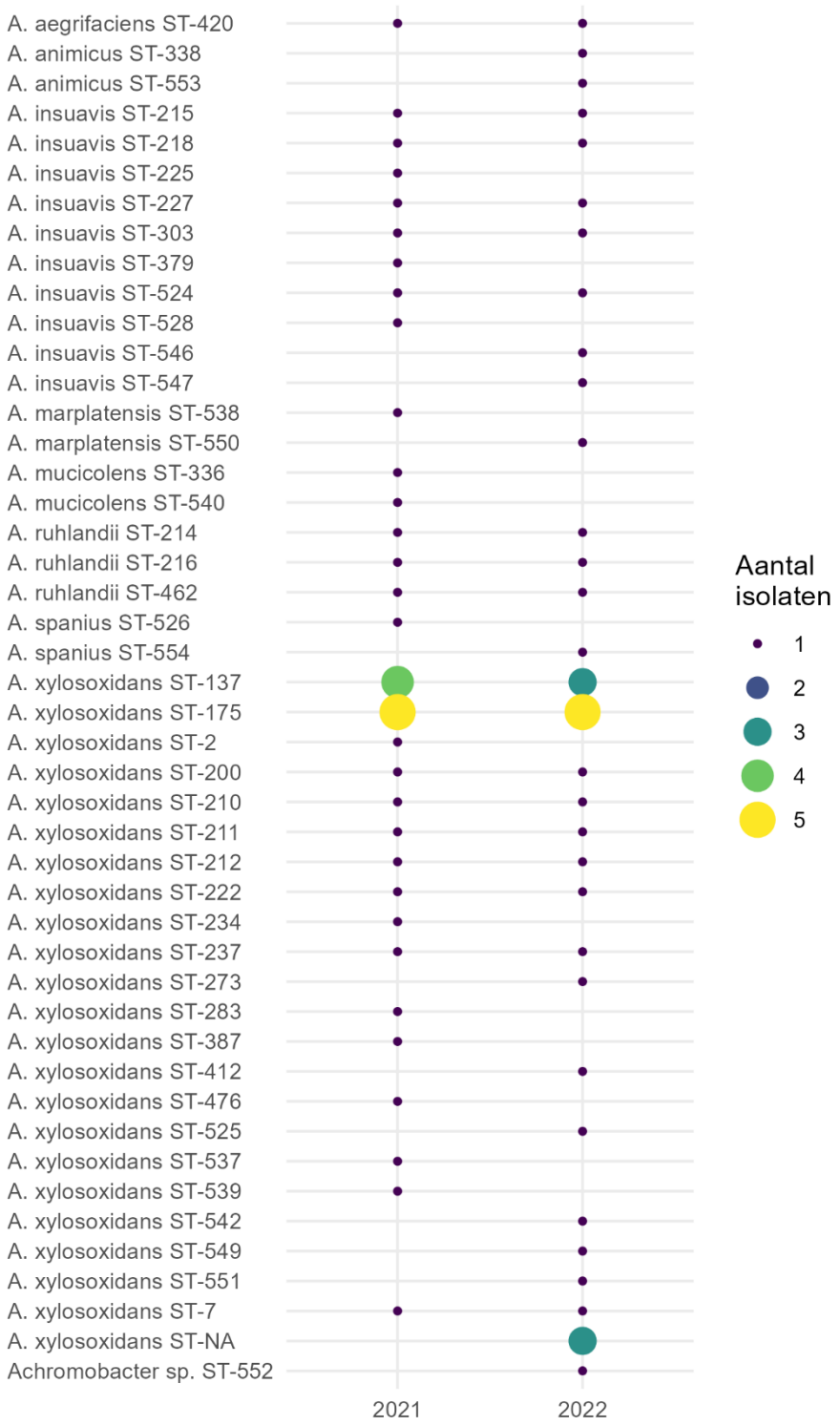

Figure 6. *Achromobacter* spp. ST's among the isolates in 2021-2022.

BCC OUTBREAKS/SMALL CLUSTERS

During 2021-2022, no outbreaks occurred and in the majority of the cases, unique ST's were found. The ST's found in 4 or 3 cases involved maximally 2 known CF patients except *B. cenocepacia IIIB* ST-1062 that was found in 3 different non-CF cases with no clear link between them. This should be further investigated if new cases with this particular ST are received.

ACHROMOBACTER OUTBREAKS/SMALL CLUSTERS

ST-137 and ST-175 accounted for the most frequent ST's among *Achromobacter xylosoxidans* isolates in CF patients in 2021-2022: 17 out of 47 cases (from 33 patients). ST-137 in five different patients and ST-175 in eight different patients. Out of these patients, only two were new for the NRC. These two ST's were frequently isolated also in previous years among CF patients followed mainly in three different CF centers in Belgium as well as in several CF centers in France.

GEOGRAPHICAL DISTRIBUTION

The distribution for the BCC-GNNF cases per Belgian province was based on the patients' postal code data (Figure 7). In addition to the four provinces with the highest number of cases (similar to previous years), more cases were refferd from Hainaut and Liège in 2021-2022.

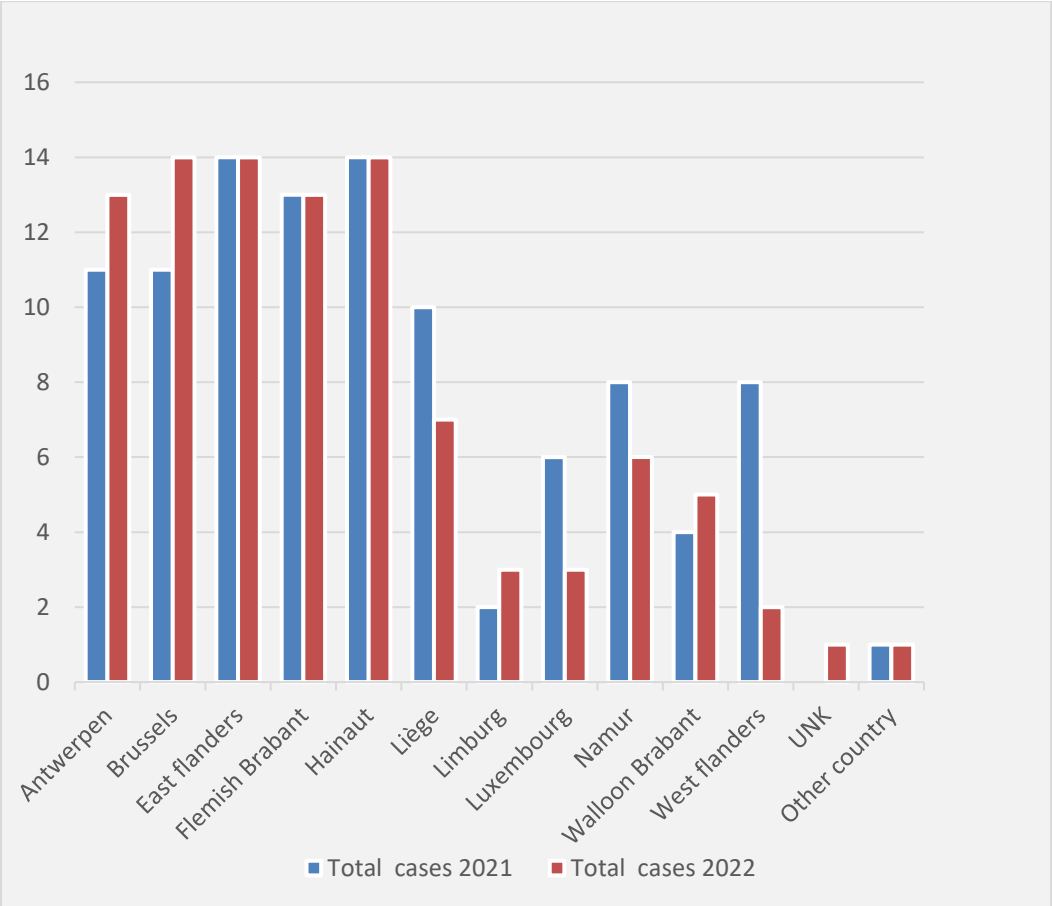

Figure 7. BCC-GNNF cases distribution per province for 2021-2022.

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 two hospitals: UZ Brussel, UCL Saint Luc (Brussels-capital region). In previous years, UZ Leuven (Flemish Brabant) was also contributing for a higher number of the cases.

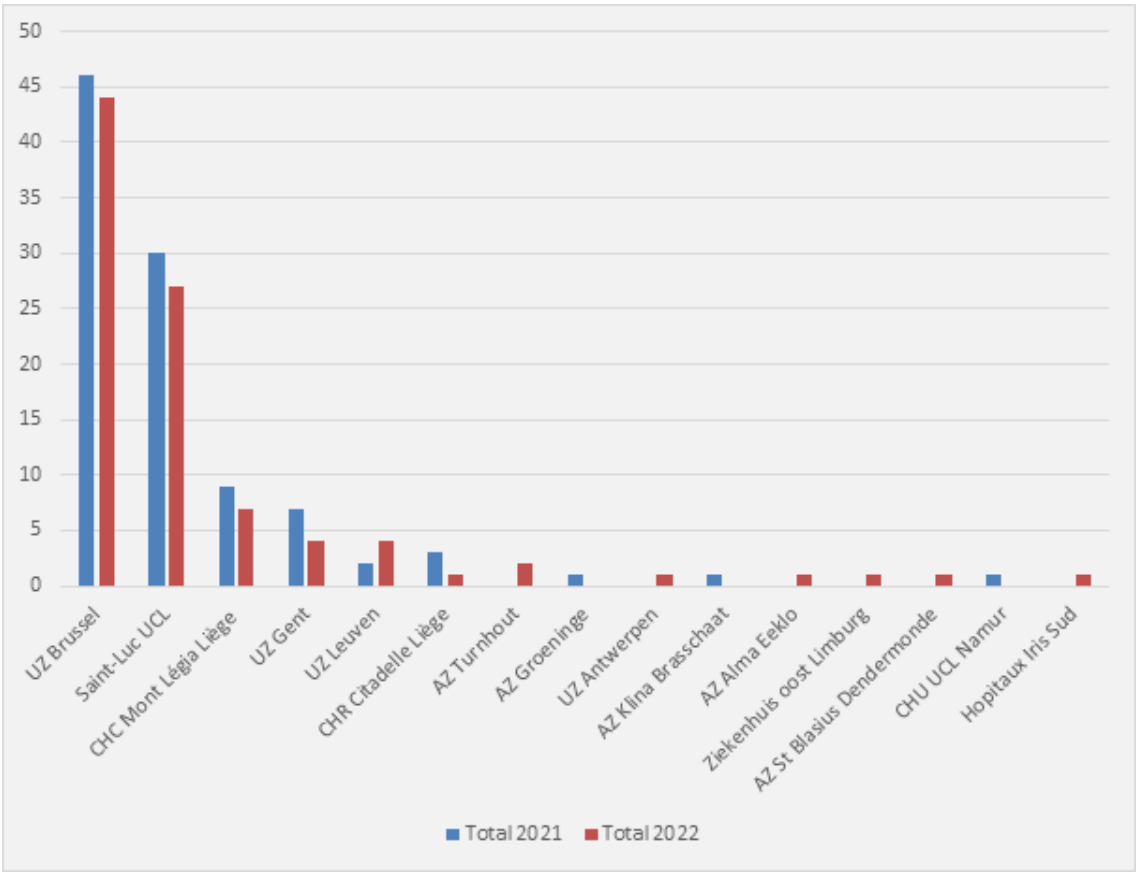

Figure 8. BCC-GNNF cases distribution per reffering center.

NRC-BCC-GNNF PATIENTS

TOTAL NUMBER

The number of patients remained around one hundred each year during 2021-2022, but the number of newly included CF patients has declined in the last six years (Figure 9).

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

AGE AND GENDER DISTRIBUTION

The gender distribution was similar for the two year period (Figure 10).

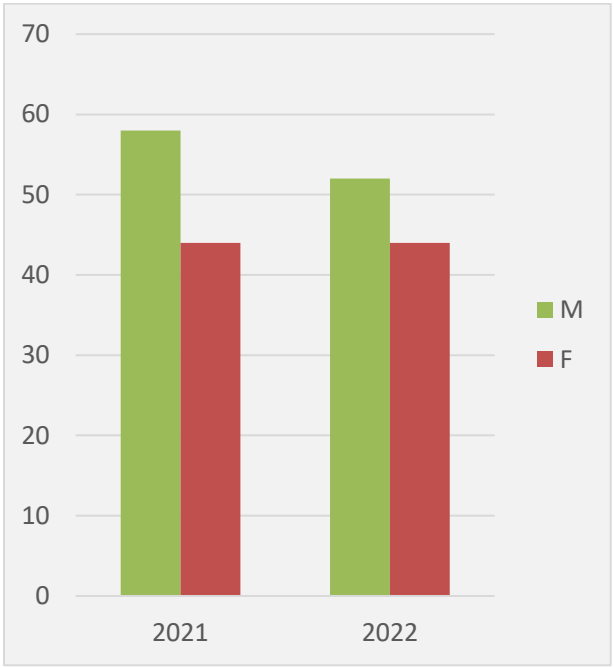

Figure 10. Male/Female distribution among the patients included at NRC-BCC-GNNF.

CF/NON-CF PATIENTS

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

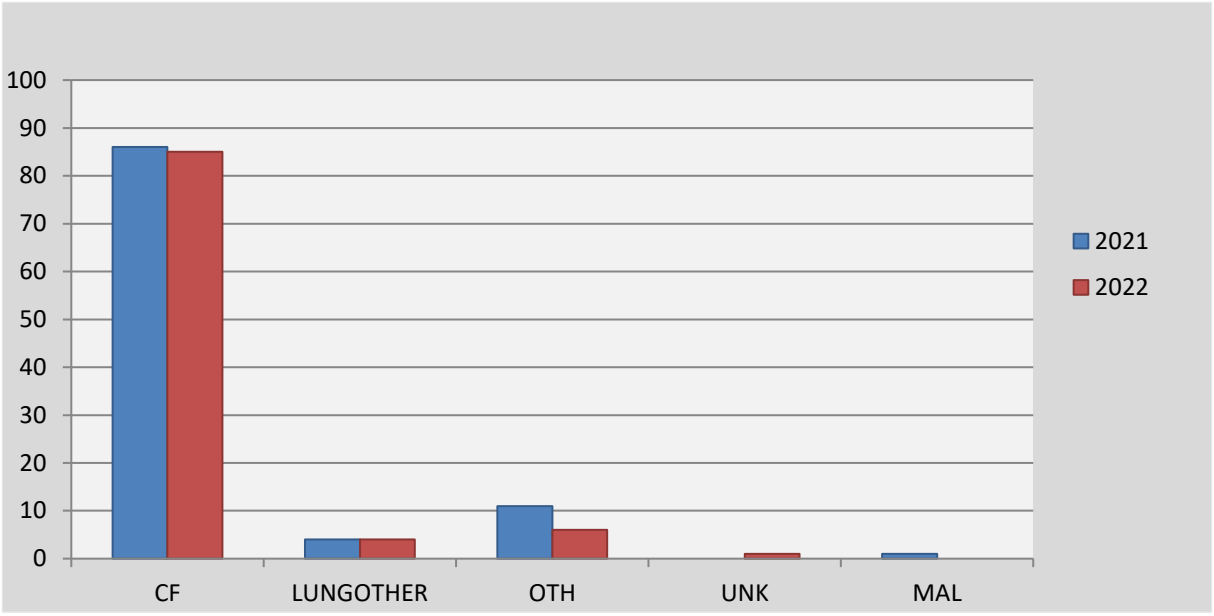

Figure 11. NRC-BCC-GNNF patients' (CF/non CF) distribution according to disease (MAL: malignancy, OTH: other, LUNGOTHER: other lung disease).

IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF BCC AND OTHER GNNF STRAINS FOR THE ROUTINELY TESTED ANTIMICROBIAL AGENTS

In vitro antimicrobial susceptibility testing of BCC-GNNF species to antimicrobial agents was performed routinely for all the referred isolates. Antimicrobial Susceptibility tests were performed according to previously described methods (11,12). Briefly, the antimicrobial susceptibility testing was performed by microdilution, with the Sensititre Antimicrobial Susceptibility Testing System (Thermofischer). No species-specific EUCAST breakpoints available for *Burkholderia* spp. and only breakpoints are available for *Achromobacter* spp. species. Table 2 and Table 3 show the antimicrobial susceptibility testing results for *Burkholderia* spp. and *Achromobacter* spp. isolates of 2021-2022 (to avoid bias, only one isolate was selected by patient).

Table 2. Minimal inhibitory concentrations for *Burkholderia* spp.

	MIC (mg/L)									
Antibiotic	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**
Piperacillin					8	9	7	6	3	12
Piperacillin-tazobactam					11	12	4	5	1	12
Temocillin							14	17	7	7
Cefepime			1		9	8	4	10	13	
Ceftazidime				2	12	13	5	5	8	
Ceftazidime-avibactam				3	15	12	8	3	4	
Ceftolozane-tazobactam			1	11	13	5	3	4	8	
Meropenem				8	13	12	3	4	5	
Aztreonam					4	10	8	5	4	14
Ciprofloxacin			3	12	9	9	4	8		
Amikacin								1	3	41
Tobramycin				0			1	3	41	
Tigecycline		1	3	9	12	5	5	10		
Colistin						1		1	43	
Trimethoprim-suflamethoxazole				28	3		6	8		

Note: includes only one isolate per patient; no EUCAST interpretation available.

Table 3. Minimal inhibitory concentrations for *Achromobacter* spp.

Achromobacter spp.	**MIC (mg/L)**										**EUCAST interpration**		
											Breakpoints		
Antibiotic	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**S ≤**	**R >**	**%S+I**
Piperacillin					37	6	1	2	2	8			
Piperacillin-tazobactam				33	10	2	1	1	9		4	4	80%
Temocillin				1					2	53			
Cefepime						3	4	16	33				
Ceftazidime					7	16	14	8	11				
Ceftazidime-avibactam					4	20	13	7	12				
Ceftolozane-tazobactam						3	5	4	44				
Meropenem				35	2	7	2	3	7		1	4	79%
Aztreonam								1	4	51			
Ciprofloxacin			1	6	8	15	9	17					
Amikacin						5	3	1	6	41			
Tobramycin				3	3	2	2	7	39				
Tigecycline		9	7	18	12	6	2	2					
Colistin				8	16	9	5	3	15				
Trimethoprim-suflamethoxazole				37	3	1	3	5	7		0,125	0,125	66%

Note: includes only one isolate per patient; EUCAST interpretation available only for piperacillin–tazobactam, meropenem and trimethoprim–sulfamethoxazole for *A. xylosoxidans*.

CONCLUSIONS

- In previous years, three Belgian centers were contributing to a large proportion of the referred isolates. One of the three centers has referred only very few isolates in 2021-2022. The reason for this decline should be investigated.

- It is not possible to determine the national prevalence of BCC and GNNF among CF patients based solely on the isolates referred to the NRC, since not all Belgian centers send all their isolates. To address this issue, our NRC plans to organize a new national surveillance study and invite all Belgian CF centers to participate.

- No BCC outbreaks occurred during 2021-2022.

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