First report on the prevalence of bacteria in cystic fibrosis patients (CF) in a tertiary care center in Saudi Arabia

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ARTICLE INFO

ABSTRACT

Introduction: Bacterial infections in CF patients are common and start early in life. The prognosis of the disease is substantially dependent on chronic respiratory infection and inflammation. Pseudomonas aeruginosa (PA) infection or chronic colonization have been established to cause a chronic decline in pulmonary function (PFT), and/or increase CF mortality.
Objectives: To obtain the prevalence of all bacterial pathogens in our CF patients and assess their evolution over time.
Method: A retrospective review of 327 patients with confirmed CF of all age groups, who had respiratory culture samples at the first visit and on a regular follow-up between January 1, 1990 and December 2018, was conducted.
Results: A total of 327 patients had a respiratory culture obtained at presentation. Two hundred and sixteen (66%) of 327 patients are alive, while 111 (34%) have died. Respiratory cultures were taken from nasopharyngeal aspiration (NPA) in 199 patients (61%), tracheal aspirate in 9 (3%), bronchoalveolar lavage (BAL)in one (0.29%), and in 124 patients (38%), sputum was induced. The eastern province contributed to the highest number of patients (122, 37.7%). There is a persistent increase in the prevalence of the common bacteria over the follow-up period of 7 years, namely Hemophilus influenzae (H. influenzae), Staphylococcus aureus (S. aureus), and all Pseudomonas (P. aeruginosa) culture types.
Comparing cultures from the first and last follow-up visits, there was an increase in the prevalence of all (P. aeruginosa) cultures from 120 (34%) to 137 (53%), and a decrease in the prevalence of (S. aureus) and (H. influenzae) during the same follow-up period.
Conclusion: There is a progressive increase in the number of patients with the most pathogenic types of bacteria because of the advanced age at presentation. As more adult patients are enrolled, there is a need for improved awareness regarding the early eradication of pathogenic bacteria to prevent progressive pulmonary damage.

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1. Introduction

Cystic fibrosis (CF) is the most common autosomal recessive lethal hereditary disorder in Caucasians [1]. The prognosis of the disease substantially depends on chronic respiratory infection and inflammation, a hallmark of CF[2]. Pseudomonas aeruginosa (P.

Abbreviations

CF	Cystic fibrosis
PFT	Pulmonary function test
P. aeruginosa	Pseudomonas aeruginosa
S. aureus	Staphylococcus aureus
B. cepacia complex	Burkholderia cepacian complex
H. influenzae	Haemophilus influenza
MRSA	Methicillin-resistant Staphylococcus aureus
MSSA	Methicillin-sensitive Staphylococcus aureus
Strep	Streptococcus
RSV	Respiratory syncytial virus
BAL	Bronchoalveolar lavage
CT	Computed tomography
CFFPR	Cystic Fibrosis Foundation Patient Registry
NPA	Nasopharyngeal aspirate
CFTR	Cystic fibrosis Transmembrane conductance Regulator
GT	Gastrointestinal
OR	Odd ratio
CI	Confidence interval
n	number

aeruginosa) is the most dominant pathogen in patients with CF [2]. During the last decades, a variety of treatment strategies have been developed, including improved antibiotic therapies, which had a significant positive impact on prognosis. The median survival age of individuals with CF in industrialized countries increased from 14 years in 1969 to more than 30 years in 2001, and approximately 37% of patients are 18 years of age or older [2]. European registries report similar median survival ages [3,4].

However, during the last 5 years, reported survival rates appear to have reached a plateau in some industrialized countries [2]. Strategies to substantially increase life expectancy in CF include, neonatal screening of the general population to identify CF, early initiation of antimicrobial and anti-inflammatory therapy in identified patients, implementation of effective hygienic measures inside and outside of CF centers, and the establishment of patient registries [5].

The latest developments include strategies against pathogens other than P. aeruginosa. In addition, improved anti-inflammatory therapy, mucolytic therapy, and airway physiotherapy as adjuncts to antibiotic therapy have become more important [6]. Our growing knowledge regarding the transmission of bacterial pathogens from infected patients, contaminated healthy individuals, or the inanimate environment, to patients with CF has resulted in the implementation of better infection control policies within CF centers to minimize the transmission of infection between patients [6,7].

The objective of early diagnosis of bacterial lung colonization/infection is to implement antibiotic therapy more rapidly, or even introduce it prophylactically, to influence the outcome for the patient with CF. The spectrum of microbial pathogens in CF lung infections differs considerably from that of other patients with chronic lung diseases. Many environmental bacteria are found in CF airway infections, including S. aureus, P. aeruginosa, Stenotrophomonas maltophilia, B. cepacia complex fungi, atypical mycobacteria, whereas Streptococcus pneumoniae, Haemophilus influenzae, or Moraxella catarrhalis, and bacteria of the endogenous flora, which are often present in other lung diseases, are found less frequently. Furthermore, because of the chronic course of lung disease, bacterial pathogens such as S. aureus or P. aeruginosa change their phenotype, and mucoid or small colony variants [8]

are often observed which are not easily recognized by laboratories not specialized in CF microbiology. In addition, the stress of the local lung environment leads to the occurrence of hypermutable bacterial strains, which show a large variety of genotypic and phenotypic traits including resistance to antimicrobial drugs. Thus, sensitive and highly resistant colonies of a given strain may be present simultaneously in one sputum specimen. Since infection in patients with CF is often polybacterial, selective agars have to be used, particularly for S. aureus, H. influenzae, P. aeruginosa, B. cepacia complex, and atypical mycobacteria [6–9]. Finally, the early diagnosis of lung infections in patients with CF is difficult, since lung infections are often present in small children and infants, who do not expectorate sputum [10–13]. Consequently, other methods such as nasopharyngeal aspirate, cough swabs, sputum induction [14], bronchoalveolar lavage (BAL) [15], and even serological tests [16–18] have a role in diagnosis.

Every microorganism whether regarded as a pathogen or not, should be treated as it causes inflammation which could damage airways. To determine if a microorganism is truly pathogenic in patients with CF, an association of the organism with acute pulmonary exacerbations, increasing chest radiographic signs of infection or altered high-resolution chest CT images, development of an antibody response [19], a chronic decline in pulmonary function, and/or increased mortality has to be established [20]. The epidemiology of microbial pathogens in CF airways has changed over the decades. Factors that may contribute to this change involve [1] antibiotic treatment, [2] increasing age of patients, [3] increased use of inhalation therapy combined with insufficient hygiene, and [4] evolution of the bacterial pathogens themselves.

Most initial P. aeruginosa is nonmucoid, and in general, completely susceptible to pseudomonal-specific antibiotics when they are contracted from the environment. In addition, plug formation, and hence, sputum production is often minimal when P. aeruginosa only colonizes the airways. Therefore, early treatment of P. aeruginosa (shortly after assessment of P. aeruginosa lung colonization) may preserve lung function [21,22], and lead to the eradication of the pathogen [23,24]. However, without treatment, this pathogen often persists in the CF airway.

Microbiological data have been reported before in Saudi Arabia in a small sample of patients [25]. The most common organisms were Staphylococcus aureus, H.influenzae, and P.aeruginosa. Patients became colonized with P.aeruginosa at an early age of 3 years compared to that of 7 years in other reports [25].

In our study, we would like to obtain the prevalence of all bacterial pathogens in our CF patients and assess their evolution over time.

2. Methodology

A retrospective chart review of 327 patients with confirmed CF of all age groups, who had respiratory culture samples at the first visit and on a regular follow-up between January 1, 1990 and December 31, 2018, was conducted.

The patient's papulation was divided into 3 equal periods according to the first culture obtained, period (1) = 1990–2000, similarly period (2) = 2001–2010, and period (3) = 2011–2018.

The most common bacteria that were obtained from the first culture's result were classified as a single culture of Hemophilus influenzae (H. influenzae) (1A), Staphylococcus aureus (S. aureus) (1B), and all types of Pseudomonas (1C) or combined cultures of 2 types: 2 A ((H. influenzae + S. aureus), 2 B (S. aureus + P. aeruginosa), and 2 C (((H. influenzae + P. aeruginosa).

The most common bacteria in the first culture were selected and compared to a similar culture from the last follow-up visit.

2.1. Definition

Patients with CF are defined as those who have typical pulmonary manifestations and/or typical gastrointestinal manifestations (GI) and/or a history of CF in the immediate family, in addition to a sweat chloride concentration of 60 mmol/L or if they have the pathologic CFTR mutations on both chromosomes [26].

2.2. Inclusion criteria

All confirmed CF patients of all the age groups, who had respiratory culture results (positive or negative) during their follow-up period in the CF clinic between January 1, 1990 and December 2018 were included.

2.3. Types of samples

Nasopharyngeal aspirates (NPA) were collected from patients below the age of 4 years, who were unable to expectorate. Induced sputum samples were obtained from patients above 4 years of age. Bronchoalveolar lavage (BAL) samples were collected from patients with severe CF pulmonary disease. Cultures were repeated every 3−6 months during the follow-up period.

2.4. Method of sample collection

Sputum cultures, NPA samples, and bronchoalveolar lavage were collected for bacterial cultures and processed in accordance with standard methodology [26]. Samples were collected by following standard hospital precautions.

2.5. Ethical considerations

The Declaration of Helsinki and good clinical practice guidelines were followed. Data collection and data entry were supervised by the principal investigator. All data needed were obtained by a retrospective chart review. All data were stored in the pediatrics research unit; accessed only by the principal investigator and the assigned clinical research coordinator. The entire patient information was kept strictly confidential. Each patient was given a study number, and all patients' data were entered into the designated data sheet (EXCEL) without any patient identification. The department of Biostatistics Epidemiology and Scientific Computing (BESC) carried out the statistical analysis of the data.

2.6. Statistical method

Scale variables were summarized by means, standard deviations, or medians and interquartile range (IQR) as appropriate. Categorical data were presented by frequencies and percentages. Paired T-test or Wilcoxon signed test was used to assess the differences between the first and last culture results. A P-value of $< .05$ was considered as the level of significance. Data were analyzed by JMP 15.0 from SAS.

3. Results

A total of 327 patients had a respiratory culture obtained at presentation. Two hundred and sixteen (66%) of 327 patients are alive, while 111 (34%) have died. Respiratory cultures were taken from nasopharyngeal aspiration (NPA) in 199 (61%)patients, tracheal aspirate in 9 (3%), BAL in one (0.29%), and in 124 patients (38%), sputum was induced.

The eastern province contributed to the highest number of patients (122, 37.7%); whereas in other provinces, it was as follows:

central province (83, 25.44%), north province (42, 12.87%), west province (39, 11.97%), and south province (39, 11.97%).

There is a persistent increase in the prevalence of the common CF bacteria from the first culture over the 3 periods (1990−2018) (Table 1), namely Hemophilus influenzae (H. influenzae), Staphylococcus aureus (S. aureus), and pseudomonas culture types such as (pseudomonas aeruginosa (P. aeruginosa), mucoid pseudomonas, and all other pseudomonas) (the same culture may contain >1 bacteria, simultaneously) (Table 1) (P=<.0001).

When the 3 most common bacteria were selected and classified into single or double bacterial types and the first culture at the mean age of 5.6 (6.7) years and the last follow-up culture at the mean age of 12 (7.7) years were compared, there was an increase in the prevalence of all Pseudomonas (P. aeruginosa) culture from 120 (34%) to 137 (53%) (Table 2)and a decrease in the prevalence of S. aureus and H. influenzae (Table 2).

The prevalence of the combination of 2 bacterial cultures remained the same during the first and last follow-up culture periods.

Overall, there is an increase in H.influenzae and Pseudomonas cultures during the 18 years (2000−2018) possibly owing to an increase in culture orders and increased CF diagnosis in adolescent and adult population >18 years. ($P = .0001$).

4. Discussion

In the United States, progressive pulmonary disease, marked by recurrent exacerbations, bacterial infection, and declining lung function, drives morbidity and mortality [27]. Studies of the CF lung reveal diverse microbiology. Methicillin-sensitive Staphylococcus aureus (MSSA) and Pseudomonas aeruginosa are the two organisms most commonly isolated from the airway [27]. Opportunistic organisms, including Burkholderia cepacia complex, Stenotrophomonas maltophilia, Achromobacter xylosoxidans, nontuberculous mycobacteria, and fungal organisms, commonly colonize and infect patients with CF [27].

Our study reveals that there is a persistent increase in the prevalence of the common CF bacteria from the first culture over the 3 periods (1990−2018) (Table 1), namely Hemophilus influenzae (H. influenzae), Staphylococcus aureus (S. aureus), and Pseudomonas culture types such as Pseudomonas aeruginosa (P. aeruginosa), mucoid Pseudomonas, and all other Pseudomonades. This might be because of improved awareness about obtaining respiratory culture and an increase in the adolescent and adult diagnosed patients with CF.

Published cross-sectional data from the Cystic Fibrosis Foundation Patient Registry (CFFPR) showed that dominant airway infections differ with age [27]. MSSA most commonly infects pediatric patients, while P. aeruginosa infection increases in frequency with age and commonly dominate the bacterial community in adult patients [27]. Simultaneously, our study showed that when the first and last follow-up of 7 years were compared, there was an increase in the prevalence of all Pseudomonas (P. aeruginosa) cultures from 120 (34%) to 137 (53%) (Table 2), and a decrease in the prevalence of S. aureus and H. influenza (Table 2).

Our explanation is that there is a progressive increase in all Pseudomonas cultures which is caused by persistent colonization and the need for early eradication is advised. Without a clear understanding of the underlying microbial interactions, efforts to prevent, treat, or eradicate specific organisms, such as P. aeruginosa, may produce unexpected and undesirable outcomes.

In a French CF patient registry, 2013−2014, to identify CF patients aged ≥20 years, the clinical outcomes, and CF transmembrane conductance regulator (CFTR) genotypes, microbiological data of patients who reported positive at least once

Table 1
Prevalence of common types of bacteria through 3 periods of follow-up (1990—2018).

Variable cultures	Period 1 (1990—2000) (%)	Period 2 (2001—2010) (%)	Period 3 (2011—2018) (%)	Total (%)	P-value
Number of cultures	63 (7.3)	462 (53.2)	343 (39.5)	868 (100)	.19
Mean age (SD) yrs.	3.29 (3.7)	4.8 (5.6)	5.6 (7)		
Minimum	<1 month	<1 month	<1 month		
Maximum	11	25	29		
Staphylococcus aureus	8 (0.9)	78 (9.0)	55 (6.3)	141 (16.2)	.69
Hemophilus influenzae	14 (1.6)	61 (7.0)	20 (2.3)	95 (10.9)	.0001
Pseudomonas aeruginosa	10 (1.2)	94 (10.8)	50 (5.8)	154 (17.17)	.09
Mucoid pseudomonas	3 (0.3)	100 (11.5)	103 (11.9)	206 (23.7)	.0001
Other pseudomonas	0 (0.0)	6 (0.7)	0 (0.0)	6 (0.7)	.07
Burkhderia cepacia	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.1)	.47
Streptococcus	7 (0.8)	38 (4.4)	18 (2.1)	63 (7.3)	.12
Klebsiella	0 (0.0)	5 (0.6)	2 (0.2)	7 (0.8)	.56
E.coli	0 (0.0)	6 (0.7)	13 (1.5)	19 (2.2)	.02
Other gram negative	7 (0.8)	16 (1.8)	3 (0.3)	26 (3)	.0001
Achromobacter	0 (0.0)	1 (0.1)	1 (0.1)	2 (0.2)	.90
Other	6 (0.7)	22 (2.5)	22 (2.5)	50 (5.8)	.25
Normal flora	16 (1.8)	99 (11.4)	86 (9.9)	201 (23.2)	.44
No growth	63 (7.3)	462 (53.2)	343 (39.5)		

(Total 327 patients, 868 cultures).
N·B (The same culture may contain 2-3 types of bacteria, simultaneously).

Table 2
Comparison of 3 common types of bacteria in CF patients with single or double bacterial combination.

Cultures	First C (Total 305)(%)	Last F/U C (Total 259) (%)
1A (*H. influenzae*)	7 (2.3)	3 (1.1)
1B (*S. aureus*)	62 (20)	44 (17)
1C (*P. aeruginosa*)	120 (34.9)	137 (53.1)
2 A (*H. influenzae + S. aureus*)		1 (0.3)
2 B (*S. aureus + P. aeruginosa*)	10 (3.2)	10 (3.8)
2 C (*H. influenzae + P. aeruginosa*)	3 (0.98)	1 (0.3)
Others	102 (33.5)	62 (24)
Total	304 (100)	258 (100)
Mean age	5.6 (6.2)	12 (7.7)

1A: *Hemophilus influenzae*.
1B: *Staphylococcus aureus*.
1C: *Pseudomonas aeruginosa*.
2A: *Hemophilus influenzae + Staphylococcus aureus*.
2B: *Staphylococcus aureus + Pseudomonas aeruginosa*.
2C: *Pseudomonas aeruginosa + Hemophilus influenzae*.
#: Number.
C: Culture.
P: < 0.0001.

for *P. aeruginosa* ("Pyo" group, $n = 1827$) were compared to those of patients with no history of *P. aeruginosa* isolation ("Never" group, $n = 303$). Predictive factors of non colonization by *P. aeruginosa* were identified by multivariate logistic regression model with backward selection. Absence of *aspergillosis* (odds ratio (OR) [95% CI] = 1.64 [1.01—2.66]) and diabetes (2.25 [1.21—4.18]), pancreatic sufficiency (1.81 [1.30—2.52]), forced expiratory volume 1 (FEV1) $\geq$ 80% (3.03 [2.28—4.03]), older age at CF diagnosis (1.03 [1.02—1.04]), and absence of F508del/F508del genotype (2.17 [1.48—3.19]) were predictive clinical factors associated with the absence of infection ("Never" group). Microbiologically, this same group was associated with more frequent detection of *H.influenzae* and lower rates of *Stenotrophomonas maltophilia, Achromobacter xylosoxidans,* and *Aspergillus* spp. (all $P < .01$) in sputum [28].

This study strongly suggests that the absence of pulmonary colonization by *P. aeruginosa* in a minority of CF adults (14.2%) is associated with a milder form of the disease. Our study did not correlate bacterial culture and CFTR genotype or PFT data. Further study is required to assess such a correlation. Recent progress in the development of drugs to correct CFTR deficiency thus may be decisive in the control of *P. aeruginosa* lung infection [28].

5. Conclusion

There is a progressive increase in the number of patients with the most pathogenic types of bacteria because of the advanced age at presentation. As more adult patients are enrolled, there is a need for improved awareness regarding the early eradication of pathogenic bacteria to prevent progressive pulmonary damage.

Ethical statement

I testify on behalf of all co-authors that our article is submitted to the *International Journal of Pediatrics and Adolescent Medicine*.

Limitations

Our CF patients reflected approximately 80% of the CF population in KSA.

Declaration of competing interest

No conflict of interest between authors.

Visual abstract

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpam.2021.07.001.

References

[1] Ratjen F, Döring G. Cystic fibrosis. Lancet 2003;361:681–9.

[2] Cystic fibrosis foundation patient registry 2001 annual data report. Bethesda (MD), USA: Cystic Fibrosis Foundation; 2002.

[3] Stern M, Sens B, Wiedemann B, Busse O. Qualitätssicherung Mukoviszidose-Ü berblick über den Gesundheitszustand der Patienten in Deutschland 2001. Zentrum fü r Qualitätsmanagement im Gesundheitswesen. Germany: Ä rzte-kammer Hannover; 2002.

[4] Koch C, McKensey SG, Kaplowitz H, Hodson ME, Horms HK, Navarro J, Mastella G. International practice patterns by age and severity of lung disease in cystic fibrosis: data of the Epidemiologic Registry of Cystic Fibrosis (ERCF). Pediatr Pulmonol 1997;24:147–54.

[5] The road for survival improvement of cysticfibrosis patients in Arab countries (Hanaa Banjar a,*, Gerhild Angyalosi.

[6] Döring G, et al. For the Consensus Committee. Antibiotic therapy against Pseudomonas aeruginosa in cystic fibrosis: a European consensus. Eur Respir J 2000;16:749–67.

[7] Antibiotic treatment for Cystic Fibrosis. Report of the UK cystic fibrosis trust antibiotic group. second ed. London, UK: Cystic Fibrosis Trust; 2002.

[8] Kahl B, Herrmann M, Everding AS, Koch HG, Becker K, Harms E, et al. Persistent infection with small colony variant strains of Staphylococcus aureus in patients with cystic fibrosis. J Infect Dis 1998;177:1023–9.

[9] Burns JL, Emerson J, Stapp JR, Yim DL, Krzewinski J, Louden L, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clin Infect Dis 1998;27:158–63.

[10] Konstan MW, Hilliard KA, Norvell TM, Berger M. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. Am J Respir Crit Care Med 1994;150:448–54.

[11] Rosenfeld M, Gibson RL, McNamara S, Emerson J, Burns JL, Castile R, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. Pediatr Pulmonol 2001;32:356–66.

[12] Burns JL, Gibson RL, McNamara S, Yim D, Emerson J, Rosenfeld M, et al. Longitudinal assessment of Pseudomonas aeruginosa in young children with cystic fibrosis. J Infect Dis 2001;183:444–52.

[13] Dakin CJ, Numa AH, Wang H, Morton JR, Vertzyas CC, Henry RL. Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. Am J Respir Crit Care Med 2002;165:904–10.

[14] De Boeck K, Alifier M, Vandeputte S. Sputum induction in young cystic fibrosis patients. Eur Respir J 2000;16:91–4.

[15] Armstrong DS, Grimwood K, Carlin JB, Carzino R, Gutierrez JP, Hull J, et al. Lower airway inflammation in infants and young children with cystic fibrosis. Am J Respir Crit Care Med 1997;156:1197–204.

[16] Dö ring G, Høiby N. Longitudinal study of immune response to Pseudomonas aeruginosa antigens in cystic fibrosis. Infect Immun 1983;42:197–201.

[17] Brett MM, Simmonds EJ, Ghoneim ATM, Littlewood JM. The value of serum IgG titres against Pseudomonas aeruginosa in the management of early pseudo-monal infection in cystic fibrosis. Arch Dis Child 1992;67:1086–8.

[18] West SE, Zeng L, Lee BL, Kosorok MR, Laxova A, Rock MJ, et al. Respiratory infections with Pseudomonas aeruginosa in children with cystic fibrosis: early detection by serology and assessment of risk factors. J Am Med Assoc 2002;287:2958–67.

[19] Høiby N, Frederiksen B. Microbiology. In: Hodson ME, Geddes D, editors. Cystic fibrosis. London: Arnold; 2000. p. 83–108.

[20] Saiman L, Siegel J. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. Infect Control Hosp Epidemiol 2003;24. S6–S52 [Suppl.].

[21] Petersen NT, Høiby N, Mordhorst CH, Lind K, Flensborg EW, Bruun B. Respiratory infections in cystic fibrosis patients caused by virus, chlamydia and mycoplasma-possible.

[22] Ratjen F, Comes G, Paul K, Posselt HG, Wagner TO, Harms K. Effect of continuous anti-staphylococcal therapy on the rate of P.aeruginosa acquisition in patients with cystic fibrosis. Pediatr Pulmonol 2001;31:13–6.

[23] Ferson MJ, Morton JR, Robertson PW. Impact of influenza on morbidity in children with cystic fibrosis. J Paediatr Child Health 1991;27:308–11.

[24] Johansen HK, Høiby N. Seasonal onset of initial colonisation and chronic infection with Pseudomonas aeruginosa in patients with cystic fibrosis in Denmark. Thorax 1992;47:109–11.

[25] Banjar H. Microbiological data of cystic fibrosis patients in a tertiary care center in Saudi Arabia. Kuwait Medical Journal, Sept. 2004;36(3):179–81.

[26] Banjar H, *, Al-Qahtani H, Yasin W, Al-wgait W, Al-Amer H, Raja R, Al-Nakhli A, Karkour K. The first report of Methicillin-resistant Staphylococcus aureus (MRSA) in cystic fibrosis (CF) patients in Saudi Arabia. IJPAM 2020;7: 186–90. https://doi.org/10.1016/j.ijpam.2019.10.005.

[27] Granchelli,a AM,Adler FR,Keogh R , Kartsonaki, C , Cox,D ,Lioua, T, Microbial interactions in the cystic fibrosis airway. J Clin Microbiol;56(8):1-13.

[28] Vongthilath Réchana, Richaud Thiriez Bénédicte, Dehillotte Clémence, Lemonnier Lydie, Guillien Alicia, Bruno Degano, Dalphin Marie-Laure, Dalphin Jean-Charles, Plésia Patrick. Clinical and microbiological characteristics of cystic fibrosis adults never colonized by *Pseudomonas aeruginosa*: analysis of the French CF registry. https://doi.org/10.1371/journal.pone.0210201.