Clinical, genetical and microbiological characterization of pediatric patients with Cystic Fibrosis in Ecuador

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Abstract

Summary Objective To characterize the epidemiology and the clinical, pathological, microbiological and genetical profile of children with cystic fibrosis treated in Ecuador. Methods We conducted a cross-sectional analysis of the pediatric population with a confirmed diagnosis of cystic fibrosis (CF) attending to one of the biggest III-level hospitals in Ecuador. All demographic, clinical and genetical variables were obtained from the electronic medical records (EMR) from 2017-2018. Results 47 patients with CF were observed and followed for more than a year. Gender distribution was similar between male (48.9%, n = 23) and female patients (51.1%, n = 24). The Tiffeneau-Pinelli index (FEV1/FVC) changed significantly after 9 months post-diagnosis (85.55 ± 13.26; p <0.05). The most common pathogenic genetical mutation was F508del, found in 52.78% of the cohort (n = 19); H609R, found in 36.11% (n = 13) and the G85E and the N1303K with 11.11% respectively (n = 3). Finally, there were 14.1% (n = 7) of patients with a mutation g.204099A> C, which has only been reported among Ecuadorians. Conclusions This is the first study carried out in Ecuador exploring the clinical, genetical and bacteriological analysis of patients with CF. Children with CF are often colonized by four species of gram-positive bacteria (S. aureus, Coagulase-Negative Staphylococcus, Streptococcus pneumoniae and M. catarrhalis) were the most predominant this condition atients were hospitalized for complications related to cystic fibrosis, with an average of 19 days of stay.

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by a mutation in a gene located on the long arm of chromosome 7. This gene is called cystic fibrosis transmembrane regulator (*CFTR),,* which produces an abnormal production of the cystic fibrosis transmembrane regulator protein (1,480 amino acids) (1–3). The *CFTR* gene encodes the transport of chloride and thiocyanate ions across epithelial cell membranes; resulting in an increase in the thickness of almost every glandular secretion (4, 5). This condition affects the epithelia in the respiratory tract, the exocrine pancreas and hepatobiliary glandular system, the intestine, the genital tract and the exocrine sweat glands (6–9). The resulting pathological process includes a progressive obstructive lung disease with bronchiectasis, mild to severe pancreatic insufficiency, malnutrition, recurrent sinusitis, bronchitis and in more than 98% of men, infertility (9, 10).

Since the description of the disease in 1938, CF has become a severe public health challenge for patients, family, physicians and the entire health system (11–14). CF is one of the most common and lethal genetic diseases worldwide, affecting an estimated 70,000 patients from all ethnic groups around the globe (10). The incidence ranges from 1:2,000 to 1:35,000 newborns worldwide, representing more than 1,000 new cases of CF that are diagnosed each year before the age of two (9, 10, 14, 15). In average, life expectancy is about 37 years, being those patients with earlier diagnosis whom live longer (16). Universal newborn screening and early treatment have markedly improved median survival rates in high-income countries (HIC) in comparison to low- and middle-income countries (LMIC) (14, 17–19). In countries like the United States, Canada or some European countries; average survival curves have improved markedly in the last 30 years, having some patients reaching 50 years of age (20–23). On the other hand, patients from LMICs have poorer outcomes, living in average 10–15 years less than the ones from HIC (18, 23, 24). In Latin-America, the majority of children are misdiagnosed or not diagnosed at all, usually leading to early deaths before reaching the first five years of age (19).

Information from Latin-American countries was first collected through the registry of patients with Cystic Fibrosis (CF) through the 'Registro Latinoamericano de Fibrosis Quistica' (REGLAFQ) in the late nineteen sixties (26). This multicenter collaborative study allowed to determine the epidemiological pattern and some clinical features of these type of patients; nevertheless, further evidence has been gathered independently from country to country (27, 28).

In the region, CF's incidence ranges from 1: 6,000 to 1:12,000. For instance, Mexico, has an incidence of 1: 9,000, Uruguay 1: 9,600, Argentina 1: 6,573 and in Chile an estimated incidence between 1: 8,000 - 9,000 per every live birth(29). In Ecuador, there have been few studies on CF. An analysis from Valle et al. in 2007 determined for the first time, the incidence of the disease in the country. They reported that the estimated incidence is around 1 case per every 11,252 live births(30). Although there are a few reports of CF in Ecuador, there are no detailed reports including diagnostic, microbiologic, genotype, phenotype and pulmonary function data as recommended by international guidelines such as the North American Cystic Fibrosis Foundation (31–33).

The objective of this study was to carry out a complete clinical, pathological, genetical and microbiological characterization of one of the biggest pediatric population with CF in the country.

Methodology

Study design:

A cross-sectional study in a cohort of children with confirmed cystic fibrosis in the Carlos Andrade Marín (HCAM) Specialty Hospital (III-level), was conducted from June 2017 to July 2018.

Setting:

This study was conducted in the biggest hospital of the Social Security Institute (IESS) in the zone. The HCAM hospital received patients from the nearby provinces and cities. The Hospital is located in Quito, the capital of Ecuador, a city located at 2,800 m above sea level, capturing children from all the ethnic groups in the country.

Participants:

All children with confirmed cystic fibrosis diagnosis by a sweat test and pathological mutation report were included. Children whose parents or guardians did not consent their participation were excluded from the analysis.

Ethical considerations:

The considerations for this study were to keep the identities of the patients anonymized through the entire project. Informed consent was gained from every patient, and no data was used from those who did not give consent.

The Institutional Review Board at Carlos Andrade Marin Hospital (HCAM) approved this study. Forty-seven cases met the inclusion criteria and were included in the study.

Demographic, clinical and analytic variables were obtained by reviewing electronic medical records (EMR) at HCAM.

Data:

Demographic variables comprised of age at diagnosis, gender, ethnicity and residence. Clinical parameters included family history, past medical history, respiratory/gastrointestinal persistent symptoms and Shwachman - Kulczycki (SK) score which was considered either excellent (86–100), good (71–85), mild (56–70), moderate (41–55), or severe (≤ 40). Follow-up was conducted through ongoing scheduled clinician appointments. Analytic tests were performed to evaluate pulmonary function using a Spirometer (CareFusion Germany 234 GmbH software) following the ATS/ERS standard criteria. FVC (forced vital capacity), FEV_1 (forced expiratory volume in 1 second) and FEV_1/FVC measurements were used for statistical analysis. In addition, microbiological cultures and sensitivity tests were conducted in all patients; children <5 years of age had deep throat cultures taken, and for children >5 years of age, sputum samples were used. Finally, all patients were analyzed for *CFTR* gene variants by polymerase chain reaction (PCR) and Sanger sequencing by cycling temperature capillary electrophoresis of a panel of 10 specific *CFTR* common mutations for the Ecuadorian population. Also, thirteen patients had *CFTR* full-length sequencing, which were processed in the United States. Since this test was not covered by the IESS, the funds were obtained privately, and some patients could not afford it.

Bias:

Selection bias was reduced by including all cases who gave informed consent. Every case of CF was approached; however, only those who agreed to participate were involved in the study. Information bias was reduced by using medical records from the hospital, which were then reviewed by two researchers to reduce any errors in reporting.

Statistical analysis:

Data were analyzed using the software SAS (Version 9.3; SAS Institute Inc., Cary, NC, USA). For this study, patients were categorized into four subgroups: <5 years, 5 to 9 years, 10 to 14 years old and ≥15 years old. Descriptive statistics, such as simple frequencies and means, were used to calculate demographic distribution, clinical findings, cultures and sensitivity of germs, and genetic profile. For pulmonary function, statistics calculated were a parametric test with Shapiro-Wilk formula and a paired t-test for related samples to evaluate the deterioration of pulmonary function, $p \leq 0.05$ was used for statistical significance.

Results

Demographics:

Out of 48 pediatric patients with CF in the hospital, 47 patients in the database met the inclusion criteria, of which 24 were female, and 23 were male. The one patient was excluded as parental consent was not given, so none of the data was used in this study. At the time of diagnosis, 27.7% (n = 13) were under 5 years, 23.4% (n = 11) were between 5 and 9 years, 31.9% (n = 15) were between 10 and 14 years and 17.02% (n = 8) of patients were 15 years or older, with a median age at diagnosis of 9.2 years (SE +/- 4.98 years). Overall, 89.4% (n = 42), 6.4% (n = 3) and 4.3% (n = 2) of patients were auto-identified as mixed race, white and indigenous, respectively.

Clinical Variables:

Most of the children were referred due to persistent respiratory symptoms (cough, recurrent pneumonia, dyspnea on exertion and chest pain) in 87.2% (n = 41), followed by persistent gastrointestinal symptoms (abdominal distention, increased frequency of stools, flatulence and steatorrhea) in 12.8% (n = 6) and by family history 8.5% (n = 4) of CF cases. Concerning nutritional evaluation using body mass index percentile tables, malnutrition was found in 25.5% (n = 12), the median body mass index (BMI) for females was 17.4 kg/m^2, for males was 15.8 kg/m^2 and the all population median BMI was at the 38^{th} percentile. In the analysis of Shwachman—Kulczycki score at diagnosis, almost half of patients (44.68%; n = 21) obtained an excellent score, followed by good, mild and moderate score in 27.66% (n = 13), 25.5% (n = 12) and 2.1% (n = 1), respectively. Furthermore, there were no severe scores found in the cohort. Presentation of concomitant disorders showed asthma in 25.5% (n = 12) cases, followed by cystic fibrosis-related diabetes in 8.5% (n = 4), meanwhile allergic bronchopulmonary aspergillosis represents 6.4% (n = 3). Age group analysis of clinical data is described in Table 1.

Pulmonary function:

Pulmonary function tests were performed in 28 patients (59.6%). At diagnosis, spirometry showed values of FEV_1 with a mean of 92.67% pred. (SD = ± 16.95), FVC with a mean of 100.81% pred. (SD = ± 15.37) and FEV_1/FVC with a mean of 89.81 (SD = ± 7.69). In concern to follow-up mean difference comparison of FEV_1, values relative to 3 months (Mean = 88.87 ± 22.33; p >0.05), to 6 months (Mean = 89.04 ± 27.66; p >0.05) and to 9 months (Mean = 86.40 ± 24.80; p <0.05). In addition, to follow-up mean difference comparison of FVC, values relative to 3 months (Mean = 98.65 ± 18.75; p >0.05), to 6 months (Mean = 97.04 ± 22.91; p >0.05) and to 9 months (Mean = 95.77 ± 18.94; p <0.05) (Figure 1).

Finally, follow-up mean difference comparison of FEV_1/FVC, values relative to 3 months (Mean = 87.86 ± 9.36; p >0.05), to 6 months (Mean = 87.89 ± 11.63; p >0.05) and to 9 months (Mean = 85.55 ± 13.26; p <0.05), as shown in (Figure 1). Age group description values are shown in (Table 2)

Microbiology:

Bacteriological cultures and antibiograms were obtained every three months after diagnosis. The most common isolated organism was *Staphylococcus aureus (*oxacillin sensitive) in 25.89% of the samples, followed by *Haemophilus influenzae* in 17.86% and *Pseudomonas aeruginosa* in 12.50%. Age group analysis showed *Haemophilus influenza* as the most common pathogen in patients younger than 10 years of while *Moraxella Catarrhalis* tends to decrease in frequency in older patients. On the other hand, *Staphylococcus aureus (*oxacillin sensitive and resistant)and *Pseudomonas aeruginosa* become more common as patients get older (Figure 2).

A total of 120 susceptibility tests were performed; four species of gram-positive bacteria (*S. aureus, Coagulase-Negative Staphylococcus, Streptococcus pneumoniae* and *M. catarrhalis)* were the most predominant, with *S. aureus* showing the largest frequency (n = 95). This bacterium was highly susceptible to treatment with sulfas, vancomycin, linezolid and ciprofloxacin. Highest resistance reported corresponded to erythromycin. Others, gram-positive organisms only were present in 10% of isolates, as shown in Table 3. We also found ten gram-negative organisms, of which six are described in Table 3; the other four pathogens are *Acinetobacter spp, Achromobacter spp, Serratia spp and Raultella planticola.* These four were isolated only once, and susceptibility tests were not performed. The most frequent gram-negative pathogen was *Pseudomonas spp,* foundin 29.17% of cultures. *Pseudomonas spp* were susceptible to Cefepime, Ceftazidime, Meropenem, Imipenem and Piperacillin/Tazobactam, but showed resistance in almost 20% of cases against Amikacin and Gentamicin, as well as nearly 10% of isolates against Ciprofloxacin. *Haemophilus spp* was found in 16.67% of tests, demonstrated high sensitivity to Ampicillin/Sulbactam, Azithromycin, Ceftriaxone and Cefuroxime, however it was resistant against Co-trimoxazole in less than half of cases and only in 15% of cases against Ampicillin alone. *Enterobacter spp* was isolated in 10.8% tests, and it was highly sensitive to Ceftazidime and Gentamicin followed by Ceftriaxone and Piperacillin/Tazobactam.

Genetic Analysis:

All mutations found were detailed in correlation with the ClinVar database and the Single Nucleotide Polymorphism Database (dbSNP). The results of the Ecuadorian patients are shown in Table 4.

Only 36 of the 47 patients underwent *CFTR* sequence analysis due to the cost of testing and lack of available funding. Of those tested, 23 had a targeted mutation panel, and 13 a full-length gene sequencing.

The most common pathogenic mutation was F508del, found in 52.78% (n = 19) of tested patients, which were expressed in homozygous (n = 4) and heterozygous (n = 15) state. The second most common mutation was H609R, found in 36.11% (n = 13) of tested patients divided in homozygous (n = 2) and heterozygous (n = 11). The rest of the patients were compound heterozygous for other reported mutations. It is important to note that the patients in homozygous state are indigenous, as the 7% of Ecuadorian population, what may be related to a founder effect (36). The third most common pathogenic mutations were G85E, and N1303K found in 11.11% of the patients equally. Less common variants (W1098X, G542X, R170H) are displayed in table 5.

There was some polymorphism reported and also some not reported mutations. The most important were g.204099A>C in 19.44% (n = 7), followed by M470V in 16.67% (n = 6), c.869+11C>T in 11.11% (n = 4), and g.206359C>A in 11.11% (n = 4). Finally, the never reported mutations were g.19395G>A, c.164+12T>C, Q1463* and others as shown in table 5.

Discussion

Cystic fibrosis is an autosomal recessive disorder seen worldwide, with typically a higher prevalence in Caucasians (13). A significant problem in developing countries is that CF patients have a shorter life expectancy with many patients diagnosed at a later time once symptoms of the disease, in particular, respiratory problems, have manifested (34). This study shows the average age of diagnosis of 9.2 years, with only 27% of patients diagnosed before the age of five. This later stage of diagnosis varies significantly across the world. In high-income countries, more than 95% of the patients are diagnosed during the first year of life (13,35).

For clinical evaluation, respiratory symptoms (cough, recurrent pneumonia, dyspnea on exertion and chest pain), followed by gastrointestinal symptoms (abdominal distention, increased frequency of stools, flatulence and steatorrhea) were typical expressions of the disease, as seen in similar reports from Latin America (29,36). As previously reported, BMI values are often low. In our cohort, young children have an average BMI of 16.6 kg/m^2, being this number higher in girls (17.4 kg/m^2) than in boys (15.8 kg/m^2). Culhane et al. reported higher BMI values for girls being slightly lower (21 kg/m^2) than boys (22 kg/m^2(37).

Despite the lateness of diagnosis, malnutrition was presented in only a quarter of patients. The Shwachman-Kulczycki scores arouse a 'good' or an 'excellent' score in more than 70% of patients, grade system used to track progress in this type of patients (38,39).

In terms of comorbidities, the diagnosis of asthma in CF patients is difficult due to the age of the patient and the respiratory parameters. Nevertheless, around 20 to 30% of the patients with CF can have a concomitant diagnosis of Asthma (40). Our results show that 1:3 patients had a concomitant and previously diagnosed with asthma, as reported elsewhere (41)

Pulmonary function test (FEV_1 and FVC) were within the normal range in the majority of patients. It was found a mild-lower airway obstruction in all age groups, which is an interesting finding since lung function would have been expected to be lower in patients with a delayed CF diagnosis, a situation previously reported (39). Lung function at follow up showed a significant drop only at nine months. Besides this finding, follow-up guarantees an adequate assessment of the disease (42).

Prevention of complications is essential in managing this disease; periodical cultures had proven to be an important clinical step to perform an early treatment. Most common gram-positive germs were oxacillin susceptible and resistant *S. Aureus*. Age group analysis showed a decrease of positive *Moraxella Catharralis, Haemophilus Influenzae* cultures in older patients and an increase of positive *S. Aureus* and *P. Aeruginosa* cultures in elderly patients, homogenous finding as those reported (25). It has been suggested that finding *Pseudomonas aeruginosa* in patients with CF is a marker of the severity of the disease(43,44). This concomitant infection causes quicker deterioration in lung function and increases the cost of treatments(45,46). In our results, *P. aeruginosa* was detected in 12.5% of airway cultures and those patients were treated immediately with a double intravenous (IV) antibiotic therapy targeted to *P. aeruginosa;* the most common combination of antibiotics was tobramycin and ceftazidime.

Regarding molecular findings, as expected, F508del mutation was the most commonly reported (52.78% of tested patients). Around the world, this mutation accounts for an estimated 30%–80% of pathogenic variants, depending on the ethnic group (47). In addition, 78% of patients who had this mutation were heterozygous, which was also expected.

An important fact to note is the analysis of H609R mutation (caused by the transition of adenosine to guanine at nucleotide 1958 - exon 13), to our best knowledge, it has been carried out and documented only in Hispanic offspring (33,48–50). However, in this analysis, 13 patients (38.23%) had this mutation, two in a homozygous state, and six were compound heterozygous with F508del, which enhances the importance of this specific mutation in Ecuadorian population.

The other mutations had been previously described in Ecuadorian patients with CF (33,50). Nefzi et al. analyzed Latin American CF patients and found four common mutations: F508del (31.37%), G542X (1.96%), G85E (1.96%) and N1303K (1.96%), with 63.7% of Ecuadorian CF mutations remaining unidentified (42). In the second report, in order of frequency, the mutations reported were F508del (37.1%), G85E (8.9%), G542X (2.4%), N1303K (2.4%), with a detection rate of 53.22% of the total of CF patients studied. All four of these mutations were found in our tested patients in the following percentage: 52.77%, 5.56%, 11.11%, and 11.11%, respectively.

CFTR exhibits an important allelic heterogeneity, a situation in which different mutations in the same gene produce variations in clinical manifestations, with this heterogeneity well known to be related to ethnic origin. As we can clearly exemplify with H609R mutation, only reported in Hispanic offspring (33,48–50). As well as for the other common mutations: G542X, G85E and N1303K, all of them consistently found in Ecuadorian patients in different publications, and frequencies more prevalent than in Caucasian mutation panels.

In 7 of our patients, the polymorphism g.204099A>C was reported, all in homozygous state, three of them related to M470V homozygous polymorphism as described above and the others with the following features:

- M470V(Heterozygous)/g.204099A>C
- F508del/W1098X/g.204099A>C
- R170H/G330*/g.204099A>C
- F508del/ Gln685Thrfs/c.869+11C>T/g.204099A>C
- H609R/M470V/g.204099A>C.
- G85E/H609R/M470V/g.204099A>C
- G970S /M470V/ g.204099 A>C.M470V

The polymorphism g.204099A> C has been reported only in Ecuadorian populations, establishing its role as a predisposing genetic factor in positive cases.

This study was carried out to understand better the demographics of those diagnosed with cystic fibrosis, as well as investigate the bacterial colonization of the airways and analyze the genetic mutations related to the disease found in the patients. The bacterial analysis showed microbial susceptibility to an array of available antibiotics, and this data could be useful in managing the therapies given to patients as well as monitoring the emergence of antibiotic resistance in the bacteria.

Limitations:

A limitation of this study is that the research was only conducted one hospital in Ecuador, therefore may not be generalizable for the whole population. To have a better understanding of the clinical, genetic and microbiological of CF in Ecuador, a larger sample size, over multiple hospitals around the country

would be needed. Another limitation was the access to funding and equipment. Due to the lack of availability of equipment and finances, only 36 of the 47 patients underwent genetic analysis, which was conducted on a first-come, first-served basis, as the patients presented to the hospital. Therefore 11 relevant results were missed, which would have been important for the treatment of the patients.

The findings from this study may have broader implications for managing CF around the globe. Knowing the bacterial colonization within the respiratory tract of the patient can inform the best treatment for the patient and can help prevent the rise of unnecessary antibiotic usage. The results published could help physicians in giving the best care for the individual affected, and the genetic characteristics could be useful in mapping the epidemiology of the disease.

Conclusions

This is the first study carried out in Ecuador exploring the clinical, genetical and bacteriological analysis of patients with CF. Cystic fibrosis in Ecuador is a relatively uncommon health problem in children and young people from different background and geographical location. Due to the lack of universal screening, children are being diagnosed later in their childhood, a situation that might affect their prognosis.

It is relevant to establish that the g.204099A> C genetic variant has been only reported in Ecuadorian populations. Complete genetical screening is not available among the public health system network, jeopardizing the diagnosis for those children with more inferior socioeconomic status.

It is important to note that performing this type of full analysis, where an extensive clinical follow-up, complete bacterial colonization analysis and genetic testing should be the standard of care among the health system in Ecuador, South America and the majority of developing countries with limited resources.

Declarations

Availability of data and materials:

The datasets used during the current study are available from the corresponding author on reasonable request. His email is e.ortizprado@gmail.com

Ethics approval and consent to participate:

The study was approved by the Institutional Review Board at Carlos Andrade Marin Hospital (HCAM). The study participants were patients who received medical care at the Hospital, and all of the information was anonymized for this publication. The patients received the standard of care for children with CF and written informed consent was routinely obtained from their parents when performing genetical testing and pulmonary function tests.

Consent to publish:

Not Applicable

Competing interests:

All authors declare not having any conflict of interest.

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Authors' Contributions:

YLV developed the specific study idea, she conducted the clinical interviews, and she was fully in charge of the follow-up. She was fully responsible for retrieving information from the health records. EOP was in charge of the conceptualization of the manuscript, performed the preliminary data analysis and wrote the primary draft of the manuscript. LGB and KSR performed the statistical and data analysis, created the tables and figures and contribute with the final draft of the manuscript. AV and AL were responsible for interpreting some of the information from the medical records as well as to retrieve the information from the bacteriological reports. They contributed with the final draft of the manuscript. EA was responsible for interpreting the genetical results and for writing them down in the manuscript. GFP was responsible for the critical review of the manuscript, the final draft of the text, and he provided critical inputs on the interpretation of the overall results and the elaboration of the manuscript.

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Abbreviations

BMI = Body Mass Index

CF = Cystic Fibrosis

CFTR = cystic fibrosis transmembrane regulator

dbSNP = Single Nucleotide Polymorphism Database

EMR = Electronic Medical Records

FEV_1 = Forced Expiratory Volume in 1 Second

FVC = Forced Vital Capacity

HCAM = Carlos Andrade Marin Hospital

HIC = High Income Countries

IV = Intravenous

IESS = Ecuadorian Institute of Social Security

LMIC = Low- and Middle-Income Countries

PCR = Polymerase Chain Reaction

REGLAFQ = Registro Latinoamericano de Fibrosis Quistica

SK = Shwachman—Kulczycki

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Tables

Table 1 Clinical findings reported among patients with CF in Ecuador

Clinical findings reported at CF Diagnosis				
	< 5 (%)	6 to 10 (%)	11 to 15 (%)	≥ 16 (%)
Number of individuals (n)	13	11	15	8
Familiar history with asymptomatic patient	15.4	9.1	6.7	0.0
Symptoms				
Persistent respiratory symptoms	69.2	90.9	93.3	100.0
Persistent gastrointestinal symptoms	38.5	0.0	6.7	0.0
Signs				
Digital clubbing	0.0	27.3	13.3	25.0
Abnormal liver function test	7.7	0.0	0.0	0.0
Sinus disease	0.0	9.1	6.7	25.0
Malnutrition	23.1	18.2	33.3	25.0
Body mass index percentile (average)	*44.8	*37.6	*37.8	*28.3
Score Shwachman – Kulczycki				
Excellent	53.9	63.6	33.3	25.0
Good	46.1	27.3	20.0	12.5
Mild	0.0	9.1	46.7	50.0
Moderate	0.0	0.0	0.0	12.5
Severe	0.0	0.0	0.0	0.0
Comorbidities				
Cyrstic fibrosis related Diabetes	0.0	0.0	6.7	37.5
Asthma	0.0	36.4	40.0	25.0
Pulmonary Hypertension	0.0	0.0	6.7	12.5
Celiac disease	0.0	0.0	6.7	0.0
Cholelithiasis	0.0	0.0	6.7	0.0
Allergic bronchopulmonary aspergillosis	0.0	9.1	13.3	0.0
Pancreatitis	0.0	18.2	0.0	0.0
Meconium ileus/other intestinal obstruction	0.0	9.1	6.7	0.0

* Values expressed in average

Table 2 Pulmonary function per age group

Age group	Follow-up	FEV1 %pred.	FVC %pred.	FEV1/FVC
5 to 9 years (n =11)	At diagnosis	105.3 (13.1)	106,3 (15.9)	98,5 (8.2)
	3-month	95,8 (27.2)	100,8 (22.3)	92,5 (10.9)
	6-month	104,7 (30.7)	109,4 (25.3)	93,7 (11.9)
	9-month	105,3 (29.5)	107,0 (23.5)	95,3 (10.9)
10 to 14 years (n =15)	At diagnosis	87,7 (12.8)	97,5 (11.5)	87,9 (4.3)
	3-month	87,1 (20.9)	97,0 (18.5)	88,0 (6.6)
	6-month	86,6 (22.7)	94,1 (20.1)	88,0 (8.3)
	9-month	80,4 (18.3)	92,3 (16.3)	81,7 (11.9)
≥ 15 years (n =8)	At diagnosis	90,4 (19.9)	99,6 (18.3)	87,6 (8.0)
	3-month	85,8 (22.3)	100,0 (18.2)	83,0 (11.6)
	6-month	76,9 (31.9)	90,1 (25.3)	72,8 (29.4)
	9-month	79,3 (25.8)	91,3 (17.2)	83,8 (15.1)

*FEV1 (Forced expiratory ventilation at first second) and FVC (Forced vital capacity) are expressed as a percentage of the predicted values (% pred.), while FEV1/FVC is expressed in percentage.

Table 3 Bacteriological culture results

	Microorganisms	Isolations (n)	Antibiotics	Susceptibility (n)	Susceptibility (%)	Resistance (n)	Resistance (%)	Non-described (n)	Non-descrit (%)
Gram-positive	Staphylococcus aureus	95	Ciprofloxacin	55	57.89	4	4.21	36	37.89
			Clindamycin	46	48.42	38	40.00	11	11.58
			Erythromycin	19	20.00	61	64.21	15	15.79
			Gentamicin	64	67.37	15	15.79	16	16.84
			Linezolid	55	57.89	0	0.00	40	42.11
			Oxacillin	40	42.11	39	41.05	16	16.84
			Co-trimoxazole	74	77.89	1	1.05	20	21.05
			Vancomycin	51	53.68	0	0.00	44	46.32
	Coagulase-Negative Staphylococcus	3	Clindamycin	0	0.00	2	66.67	1	33.33
			Gentamicin	2	66.67	0	0.00	1	33.33
			Erythromycin	0	0.00	2	66.67	1	33.33
			Oxacillin	1	33.33	1	33.33	1	33.33
			Penicillin	0	0.00	2	66.67	1	33.33
			Co-trimoxazole	2	66.67	0	0.00	1	33.33
			Vancomycin	2	66.67	0	0.00	1	33.33
	Streptococcus pneumoniae	7	Ceftriaxone	4	57.14	0	0.00	3	42.86
			Clindamycin	2	28.57	1	14.29	4	57.14
			Penicillin	4	57.14	2	28.57	1	14.29
			Co-trimoxazole	2	28.57	3	42.86	2	28.57
	Corynebacterium spp	2	Doxycycline	1	50.00	0	0.00	1	50.00
			Levofloxacin	1	50.00	0	0.00	1	50.00
	Escherichia coli	11	Amikacin	6	54.55	3	27.27	2	18.18
Gram negatives			Ampicillin/sulbactam	0	0.00	8	72.73	3	27.27
			Cefepime	1	9.09	9	81.82	1	9.09
			Ceftazidime	1	9.09	9	81.82	1	9.09
			Ciprofloxacin	1	9.09	7	63.64	3	27.27
			Gentamicin	4	36.36	4	36.36	3	27.27
			Imipenem	10	90.91	0	0.00	1	9.09
			Piperacillin/tazobactam	4	36.36	3	27.27	4	36.36
	Enterobacter spp	13	Ceftazidime	10	76.92	0	0.00	3	23.08
			Ceftriaxone	8	61.54	0	0.00	5	38.46
			Gentamicin	10	76.92	0	0.00	3	23.08
			Piperacillin/tazobactam	8	61.54	0	0.00	5	38.46
	Haemophilus spp	20	Ampicillin	13	65.00	3	15.00	4	20.00
			Ampicillin/sulbactam	14	70.00	1	5.00	5	25.00
			Azithromycin	15	75.00	0	0.00	5	25.00
			Ceftriaxone	13	65.00	0	0.00	7	35.00
			Cefuroxime	13	65.00	1	5.00	6	30.00

			Co-trimoxazole	6	30.00	9	45.00	5	25.00
	Klebsiella spp	5	Amikacin	3	60.00	0	0.00	2	40.00
			Ampicillin/sulbactam	3	60.00	0	0.00	2	40.00
			Cefepime	3	60.00	0	0.00	2	40.00
			Ceftazidime	4	80.00	0	0.00	1	20.00
			Ciprofloxacin	3	60.00	0	0.00	2	40.00
			Imipenem	3	60.00	0	0.00	2	40.00
	Moraxella spp	7	Amoxicillin/clavulanate	4	57.14	0	0.00	3	42.86
			Ampicillin	0	0.00	5	71.43	2	28.57
			Ampicillin/sulbactam	5	71.43	1	14.29	1	14.29
			Azithromycin	6	85.71	0	0.00	1	14.29
			Cefuroxime	6	85.71	0	0.00	1	14.29
	Pseudomonas spp	35	Amikacin	11	31.43	6	17.14	18	51.43
			Cefepime	27	77.14	3	8.57	5	14.29
			Ceftazidime	31	88.57	1	2.86	3	8.57
			Ciprofloxacin	21	60.00	4	11.43	10	28.57
			Gentamicin	16	45.71	8	22.86	11	31.43
			Imipenem	20	57.14	1	2.86	14	40.00
			Meropenem	26	74.29	1	2.86	8	22.86
			Piperacillin/tazobactam	25	71.43	2	5.71	8	22.86

Table 4 Classification of mutations

Exon	Genetic identification	Protein identification	dbSNP ID	Clinical Significance	Molecular consequence	Class of allele mutation	Clinical classification	CFTR 2 database patient reports
1	**g.19395G>A**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
2	**g.43555G>C**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
2	**g.43575G>C**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
2	**g.43580G>T**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
2	**g.43583A>G**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
2	**g.43592T>C**	**c.164+12T>C**	rs121908790	Uncertain	Intron variant	Non classified	Non classified	Not reported
2	**g.43594A>G**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
3	**g.48340G>A**	**p.G85E**	rs75961395	Pathogenic	Missense variant	Class II	A group	584
5	**g.73512G>A**	**R170H**	rs1800079	Pathogenic	Missense variant	Non classified	Non classified	11
6a	**g.70332G>T**	**621+1G>T**	rs78756941	Pathogenic	Splice donor variant	Class I	A group	1,293
6a	**g.74534G>C**	**G542X**	rs113993959	Pathogenic	Nonsense variant	Class I	A group	3,489
6b	**g.206154C>T**	**c.869**+11C>T	rs1800503	Benign	Intron variant	Non classified	Non classified	Not reported
7	**g.79435G>T**	**G330***	rs79031340	Pathogenic	Nonsense variant	Non classified	Non classified	23
10	**g.98696A>G**	**M470V**	rs213950	Benign	Missense variant	Non classified	C group	209
10	**g.98808_98811delTCT**	**F508del**	rs113993960	Pathogenic	Inframe variant	Class II	A group	65,046
13	**g.131210A>G**	**H609R**	rs397508310	Pathogenic	Missense variant	Non classified	Non classified	9
13	**c.2052dupA**	**Gln685Thrfs**	rs121908746	Pathogenic	frameshift variant	Non classified	Non classified	324
13b	**Not reported**	**2347delG**	rs397508353	Pathogenic	Frameshift variant	Non classified	Non classified	38
14a	**g.134218T>G**	**T854T**	rs1042077	Benign	Synonymous variant	Non classified	Non classified	36
15	**g.142999 G>A**	**G970S**	rs397508453	Uncertain	Missense variant	Non classified	Non classified	10
15	**g.143018G>T**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
17a	**g.149918T>A**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
17b	**g.74629T>C**	**W1098X**	rs397508533	Pathogenic	Nonsense variant	Non classified	Non classified	9
20	**g.181807A>G**	**P1290P**	rs1800130	Benign	Synonymous variant	Non classified	Non classified	Not reported
21	**g.192094C>G**	**N1303K**	rs80034486	Pathogenic	Missense variant	Class II	A group	2,147
22	**g.204099A>C**	**-**	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported
24	**g.129569G>A**	**1812-1G>A**	rs121908794	Pathogenic	Splice acceptor variant	Non classified	Non classified	31
24	**g.206271 G>A**	**Q1463***	rs886044425	Uncertain	Nonsense variant	Non classified	Non classified	Not reported

24	g.206359C>A	-	Not reported	Not reported	Not reported	Non classified	Non classified	Not reported

Table 5 Molecular findings in CF Patients

Exon	Genetic identification	Protein identification	Number of reports	Heterozygosis	Homozygosis
1	g.19395G>A	-	2	-	2
2	g.43555G>C	-	1	1	-
2	g.43575G>C	-	1	1	-
2	g.43580G>T	-	1	1	-
2	g.43583A>G	-	1	1	-
2	g.43592T>C	c.164+12T>C	2	2	-
2	g.43594A>G	-	1	1	-
3	g.48340G>A	p.G85E	4	4	-
5	g.73512G>A	R170H	1	1	-
6a	g.70332G>T	621+1G>T	1	1	-
6a	g.74534G>C	G542X	2	2	-
6b	g.206154C>T	c.869+11C>T	4	3	1
7	g.79435G>T	G330*	1	-	1
10	g.98696A>G	M470V	6	3	3
10	g.98808_98811delTCT	F508del	19	15	4
13	g.131210A>G	H609R	13	11	2
13	c.2052dupA	Gln685Thrfs	1	1	-
13b	Not reported	2347delG	1	1	-
14a	g.134218T>G	T854T	1	1	-
15	g.142999 G>A	G970S	1	1	-
15	g.143018G>T	-	1	1	-
17a	g.149918T>A	-	1	1	-
17b	g.74629T>C	W1098X	3	3	-
20	g.181807A>G	P1290P	1	1	-
21	g.192094C>G	N1303K	4	4	-
22	g.204099A>C	-	7	-	7
24	g.129569G>A	1812-1G>A	1	1	-
24	g.206271 G>A	Q1463*	2	2	-
24	g.206359C>A	-	4	-	4

Figures

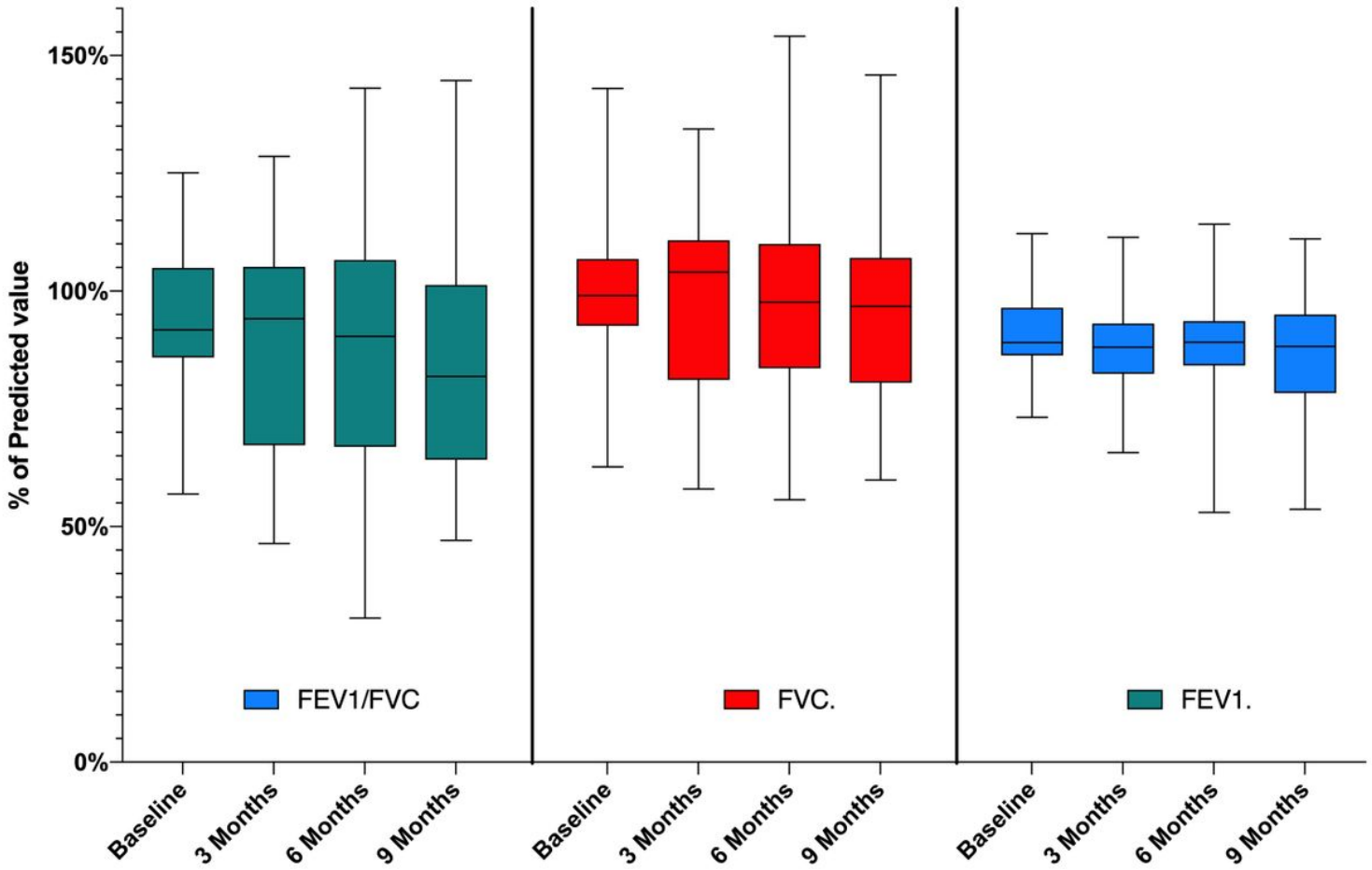

Figure 1

Spirometry results from baseline up to 9 months follow-up in patients from Ecuador

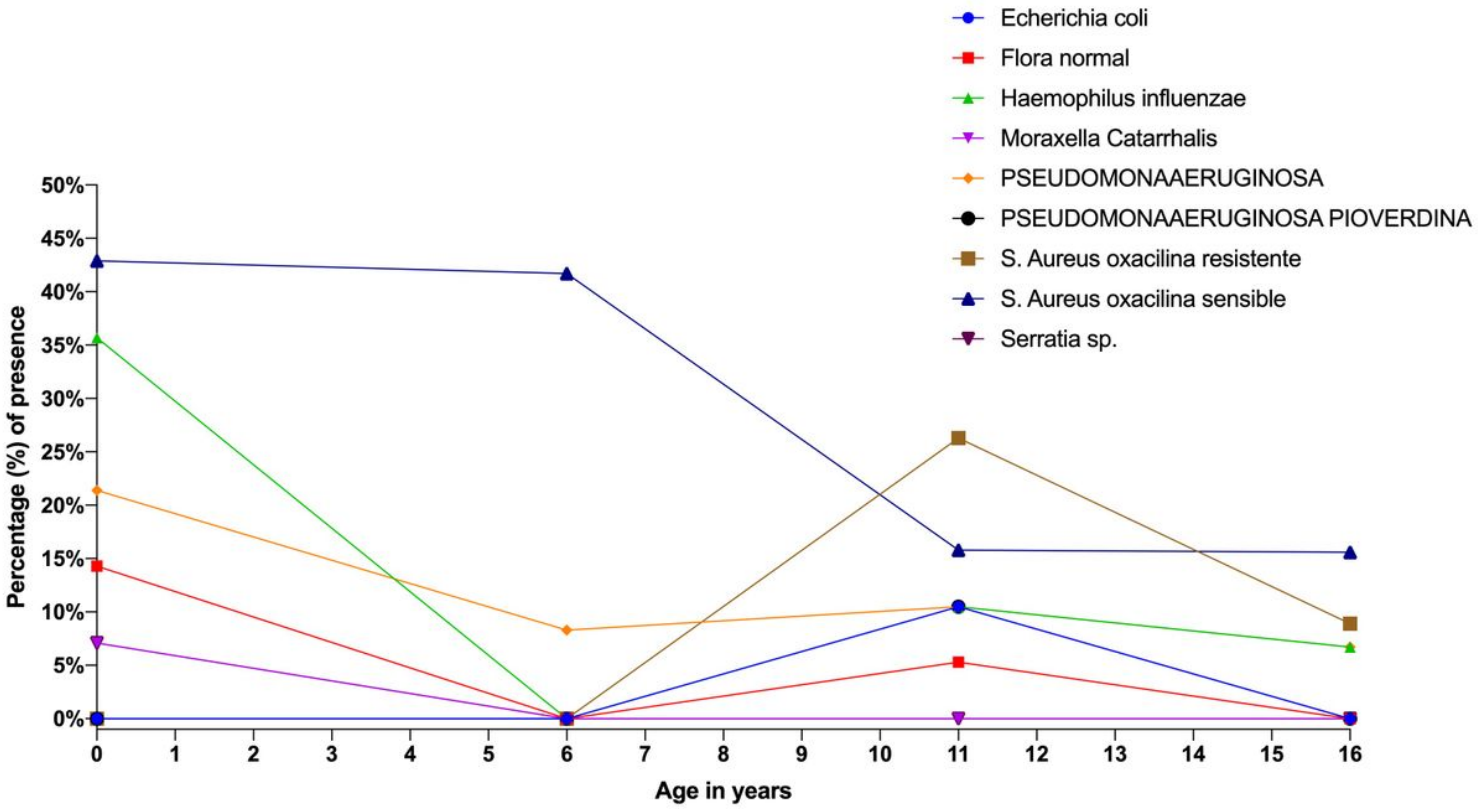

Figure 2

Presence of different microorganism per age group according to the bacteriological culture results