BACTERIOLOGICAL STUDY OF DIFFERENT METHODS OF SPUTUM SAMPLING IN EGYPTIAN CHILDREN WITH CYSTIC FIBROSIS

By

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ABSTRACT

Background: *Different respiratory sampling methods exist to identify lower airway pathogens in patients with cystic fibrosis (CF). Bronchoalveolar lavage (BAL) is considered the "gold standard.". Because BAL is invasive and cannot be repeated frequently, identification of lower respiratory tract pathogens in CF patients is challenging. Other sampling techniques are sputum, cough, and nasal swabs.*

The aim of the work: *To differentiate between the microbiological results of bronchoalveolar lavage (BAL), sputum and cough swabs in CF children and to evaluate their accuracy in detecting lower respiratory tract organisms.*

Patients and Methods: *Microbiological results detected by these different sampling methods were compared in all enrolled CF patients (n=31). Specificity, sensitivity, positive (PPV), and negative (NPV) predictive values were calculated.*

Results: *Regarding microbiological results, Concordance was found between BAL and sputum as regards Pseudomonas spp., Klebsiella spp. and Enterobacter spp., except for Staph aureus (P 0.039). overall low sensitivity (7.1%) and low accuracy (16.1%) of cough swabs to detect lower airway pathogens [Pseudomonas spp., Staphylococcus aureus spp., Klebsiella spp. and Enterobacter spp.] in children with CF followed by sputum analysis as it showed sensitivity (85.7%) and accuracy (80.6%) while BAL showed highest sensitivity (86.2%) and highest accuracy (82.9%).*

Conclusion: *Our findings suggested that sputum samples showed a good bacteriologic concordance with BAL in detecting lower airway pathogens, While Cough swabs showed the lowest accuracy in detecting the presence of CF pathogens in clinically stable CF children. However, BAL should be performed in symptomatic patients who could not expectorate and had a negative cough swab and sputum sample.*

Keywords: *bronchoalveolar lavage (BAL); cough swab; cystic fibrosis; Bacteriological study.*

INTRODUCTION

Cystic fibrosis (CF) is a multisystem disorder transmitted in the autosomal recessive mode caused by a mutation in the gene for the CF transmembrane conductance protein (CFTR). CF is primarily a progressive lung disease, characterized by chronic pulmonary infections with opportunistic pathogens **(Dorsey et al., 2017)**.

A number of defective inflammatory responses have been connected to cystic fibrosis transmembrane conductance regulator (CFTR) deficiency including innate and acquired immunity dysregulation, cell membrane lipid abnormalities, various transcription factor signalling defects, as well as altered kinase and toll-like receptor responses **(Cantin et al., 2015)**.

Bronchoalveolar lavage (BAL) is the gold standard for diagnosing lower airway infection and inflammation in young children. However, BAL is an invasive and limited technique that may underestimate infection or inflammation because of regional variability **(Costabel et al., 2019)**. In addition, the effect of repeated general anaesthesia on child development and school performance suggests these procedures should be kept to a minimum **(Schneuer et al., 2018)**.

Escalation to bronchoscopy and BAL has generally been kept for children with CF who have not responded to directed or empirical antibiotic treatment and where oropharyngeal cultures do not explain the persistence of symptoms. There is currently little consensus on how extensive BAL sampling should be, but specific guidelines generated for CF recommend two-lobe BAL, with three-aliquot BAL from the right middle lobe and a single-aliquot BAL from the lingula or the most affected lobe **(Costabel et al., 2019)**.

Because BAL cannot be repeated limitless, diagnosing lower respiratory tract infections in non-expectorating patients is challenging. Other sampling techniques are nasal, cough swabs, and induced sputum which are considered a simple, cost-effective, well tolerated and frequently repeatable approach to sampling the lower airway **(Jain et al., 2018)**.

Ethical Considerations:

1. An approval by ethical guidelines of the Faculty of Medicine's Research Ethics Committee at Ain Shams University was done before the study (NO. FWA 000017585).

2. written informed consent was obtained from patients or their legal guardians.
3. All the data of the patients and results of the study are confidential and the patients have the right to keep it or withdraw from the study at any time.
4. The researcher explains the aim of the study to the patient.

Funding:

This research has not received any funds regarding the study or publication.

Conflict of Interest:

The authors declared that there were no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

Calculation of Sample Size: Estimation was done using the Epi info7 program for sample size calculation. According to **Eyns et al. (2018)**, there was a large effect size comparing the different sputum samples' microbiological yield. A sample size of at least 31 cases in each of the three patients' groups achieves a power of 80% to detect an effect size of at least 0.6 using Chi-squared test with 3 degrees of freedom and at a level of significance of 0.05.

Inclusion criteria:

1. Both genders were included in the study.

2. All CF patients who presented to the hospital aged less than 18 years.

3. Patients with documented diagnosis of CF who were diagnosed according to CF Foundation guidelines based upon positive sweat tests and/or the presence of a mutation in the homozygous state or two heterozygous mutations on the gene encoding the CFTR protein and clinical evaluation **(Margaroli et al., 2019)**.

Exclusion Criteria were any patients with:

Underlying chronic lung disease (Childhood interstitial lung disease and TB).

Study Design: This was a cross-sectional study. It was conducted on 31 patients attending the Cystic Fibrosis Clinic of Children's Hospital, Ain Shams University for their follow-up in the period of January 2023 to August 2023. The enrolled CF patients were selected by a simple random method.

Patients attending the CF Clinic and eligible for bronchoscopy were divided into two groups expectorating and non-expectorating depending on their

ability to expectorate on demand. A cough swab, sputum and BAL were obtained from patients. A cough swab and sputum were drawn by the CF physiotherapist, and BAL was obtained by the pulmonologist.

All enrolled CF patients were subjected to:

1. **Complete history-taking including:**
    - age, sex and consanguinity, birth order, residence.
    - Age of onset of the CF disease (neonatal, infants, childhood).
    - Perinatal history of meconium ileus, prolonged neonatal jaundice or previous NICU admission.
    - Family history of CF and degree of consanguinity.
    - Duration of symptoms and comorbidities.
    - Special emphasis on the history of respiratory symptoms such as (cough, expectoration, dyspnea, hemoptysis, and wheezes), gastrointestinal symptoms such as (steatorrhea, failure to gain weight, meconium ileus, abdominal distension and symptoms of pancreatic insufficiency), and symptoms of pseudo-barter syndrome (vomiting, sweating, dehydration, or loss of weight).
    - Medication history (antibiotics oral or injections, bronchodilators, mucolytics, vitamins, minerals and pancreatic enzyme replacement).
2. **Thorough clinical examination including:**
    - Anthropometric measurements including body weight and height according to CDC growth centiles.
    - Complete chest examination (including chest inspection for shape and symmetry, auscultation for intensity of breath sounds, type of breathing and adventitious sounds).
    - Complete abdominal examination.
3. **Laboratory investigations including:**

Sweat chloride test:

Sweat chloride in healthy children should be less than 30 mmol/L. Values greater than 60 mmol/L are diagnostic of cystic fibrosis. Intermediate values between 30 to 60 mmol/L are considered to be an elevated but not diagnostic level. Cystic fibrosis is possible in these

patients, but repeat or alternative testing is necessary **(Davis, 2006)**.

Cystic fibrosis DNA analysis:

Cystic fibrosis transmembrane conductance regulator gene (CFTR) mutation analysis was done by Multiplex polymerase chain reaction (PCR). A panel of 32 CFTR mutations was tested.

Sputum specimen Collection and processing:

The patients were advised not to ingest food for 1 to 2 hours before expectoration and to rinse their mouths with saline or water just before expectoration. Sputum specimens were collected in a sterile container and transferred to the Main Microbiology Lab within 1 hour. In younger children who could not expectorate sputum, induced sputum was collected by postural drainage and thoracic percussion assisted by a respiratory therapist. Before specimen collection, patients were advised to brush the buccal mucosa, tongue, and gums with a wet toothbrush. Direct Gram stain examination and then culture were done **(Tille, 2022)**.

Cough swab specimen Collection and processing:

A cough swab was collected using a sterile, nylon-tipped swab. The swab was held towards the back of the oral pharyngeal cavity avoiding contact with the mucosa as patients were asked to cough. The swab was then removed from the oropharynx and placed into the accompanying tube and transferred to the Main Microbiology Lab within 1 hour **(Eyns et al, 2018)**.

Bronchoalveolar lavage specimen Collection and processing were carried out using a pediatric flexible fiberoptic bronchoscope, samples were collected in a sterile container and transferred to the Main Microbiology Lab within 1 hour, where, 5 ml of each sample was centrifuged at 3000 rpm for 20 minutes, the supernatant was discarded, and the sediment was tested through Direct Gram stain examination then the culture was done **(Tille, 2022)**.

Bacterial culture of specimens:

Each specimen was cultured on Blood agar, Chocolate agar and MacConkey agar plates (Oxoid, UK) by semi-quantitative technique, plates were incubated aerobically for 24-48 hours at 37°C. Positive bacterial cultures were identified through manual identification using biochemical reactions and Vitek 2 Compact (BioMérieux, France) **(Tille, 2022)**.

Statistical analysis:

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 23. Quantitative variables will be first subject to the normality test (Kolmogorov v Simonov). The significance of the obtained results was judged at the (0.05) level. Continuous variables were present in mean ±SD, and their differences were assessed by the independent T-test. Categorical variables were described as numbers (percentages) and were compared by chi-squared test. Operating Characteristic (ROC) curve analysis was conducted.

RESULTS

All results will be demonstrated in the following tables and figures:

Table (1): Demographic data of the studied CF patients

Variables		**No. = 31**
Age (year)	Median (IQR)	5 (3 - 9)
	Range	2 – 15
Sex	Female	13 (41.9%)
	Male	18 (58.1%)
Consanguinity	Negative	13 (41.9%)
	Positive	18 (58.1%)
Family history of similar condition.	Negative	24 (77.4%)
	Positive	7 (22.6%)
Residence	Urban	19 (61.3%)
	Rural	12 (38.7%)
Onset of disease (months)	Median (IQR)	4 (0.03 - 5)
	Range	0.03 – 12

Baseline demographic data **Table (1)** including age, gender, Consanguinity, onset of CF disease, family history of similar condition and consanguinity were obtained prior to sampling.

Table (2): Clinical findings of the enrolled CF patients

Variables		Total no. = 31
Respiratory symptoms		
Cough (dry or productive)	Negative	11 (35.5%)
	Productive	16 (51.6%)
	Dry	4 (12.9%)
Dyspnea	Negative	8 (25.8%)
	Positive	23 (74.2%)
Finger Clubbing	Negative	9 (29.0%)
	Positive	22 (71.0%)
GIT symptoms		
Steatorrhea	Negative	4 (12.9%)
	Positive	27 (87.1%)
Abdominal distension	No	13 (41.9%)
	Positive	18 (58.1%)
Failure to gain weight	Negative	7 (22.6%)
	Positive	24 (77.4%)
Hepatomegaly	Negative	19 (61.3%)
	Positive	12 (38.7%)
Meconium ileus	Negative	26 (83.9%)
	Positive	5 (16.1%)
Clinical severity scoring according to the Shwachman-kulczycki score	Intermediate	7 (22.6%)
	Moderate	12(38.7%)
	Good	12(38.7%)

Table (2) Respiratory symptoms were prevalent, with 64.5% experiencing cough (51.6% productive, 12.9% dry), 74.2% having dyspnea, and 71.0 % exhibiting finger clubbing. Gastrointestinal symptoms included steatorrhea in 87.1%, abdominal distension in 58.1%, and 77.4% with failure to gain weight. Mild hepatomegaly was observed in 38.7%, and 16.1% had meconium ileus. Assessment of clinical severity using the Shwachman-Kulczycki score showed that 38.7% were categorized as "Moderate" and 38.7% as "Good," while 22.6% fell into the "Intermediate" category.

Table (3): Bacteriological results of the enrolled CF patients

Bacteriological results		No.	%
I. Bronchoalveolar lavage	Negative	3	9.7%
	Positive	28	90.3%
Normal Flora		2	6.5%
Pseudomonas		17	54.8%
Klebsiella		8	25.8%
Staph aureus		4	12.9%
Enterobacter spp		1	3.2%
II. Sputum	Negative	5	16.1%
	Positive	26	83.9%
Flora		9	29.0%
Pseudomonas		13	41.9%
Klebsiella		4	12.9%
III. Cough swab	Negative	29	93.5%
	Positive	2	6.5%
Pseudomonas		2	6.5%

Table (3): Bronchoalveolar lavage was conducted in 90.3% of patients, revealing various bacteria including Pseudomonas spp. (54.8%) and Klebsiella spp. (25.8%). Sputum analysis, performed in 83.9% of cases, showed Pseudomonas spp. (41.9%), Klebsiella spp. (12.9%), and flora (29.0%). Cough swabs, though less common (6.5%), also detected Pseudomonas spp. (6.5%).

Table (4): Comparison between BAL and sputum as regards bacteriological results

Bacteriological results	Bronchoalveolar lavage		Sputum		Test value	P-value	Sig.
	No.	%	No.	%			
No	3	9.7%	5	16.1%	0.574	0.449	NS
Yes	28	90.3%	26	83.9%			
Flora	2	6.5%	9	29.0%	5.415	0.020	S
Pseudomonas	17	54.8%	13	41.9%	1.033	0.309	NS
Klebsiella	8	25.8%	4	12.9%	1.653	0.199	NS
Staph aureus	4	12.9%	0	0.0%	4.276	0.039	S
Enterobacter spp	1	3.2%	0	0.0%	1.016	0.313	NS

Table (4): The analysis indicates no significant difference in the results between the two methods regarding Pseudomonas, Klebsiella and Enterobacter spp Additionally, "Staph aureus" results showed a significant difference (p = 0.039, S).

Table (5): Comparison between Bronchoalveolar Lavage and cough Swab samples regarding Bacteriological results

Bacteriological results	Bronchoalveolar lavage		Cough swab		Test value	P-value	Sig.
	No.	%	No.	%			
No	3	9.7%	29	93.5%	43.658	<0.001	HS
Yes	28	90.3%	2	6.5%			
Flora	2	6.5%	0	0.0%	2.067	0.151	NS
Pseudomonas	17	54.8%	2	6.5%	17.075	<0.001	HS
Klebsiella	8	25.8%	0	0.0%	9.185	0.002	HS
Staph aureus	4	12.9%	0	0.0%	4.276	0.039	S
Enterobacter spp	1	3.2%	0	0.0%	1.016	0.313	NS

Table (5): Highly significant differences were observed between the two methods (p = 0.000, HS). Especially for "Pseudomonas" (p < 0.001, HS), "Klebsiella" (p = 0.002, HS), and "Staph aureus" (p = 0.039, S), with more detection in bronchoalveolar lavage samples. However, there was no significant difference for "Flora" (p = 0.151, NS) and "Enterobacter spp" (p = 0.313, NS).

Table (6): Investigations of the studied CF patients

Investigations		No. = 31
Diagnostic data		
Sweat chloride test value	Mean ± SD	93.00 ± 20.82
	Range	69 – 153
Cystic Fibrosis Genotyping	**F508del:** I. Homozygous mutation	21 (67.7%)
	II. Hetrozygous mutation	7 (22.6%)
	CFTR gene revealed **none of the 34 common** mutations but the presence of IVS8-7T polymorphism	3 (9.7%)

Table (6): showed the sweat chloride test and cystic fibrosis genotyping results for the studied cystic fibrosis patients.

Table (7): ROC curve for BAL, sputum and cough swabs in the detection of the lower respiratory tract pathogens in CF patients

Item	Accuracy	Sensitivity	Specificity	PPV	NPV	AUC	P-value
Sputum	80.6%	85.7%	66.7%	92.3%	20.0%	0.595	0.593
Cough swab	16.1%	7.1%	100%	100%	10.3%	0.536	0.841
Bronchoalveolar lavage	82.9%	86.2%	74.3%	94.1%	21.9%	0.601	0.744

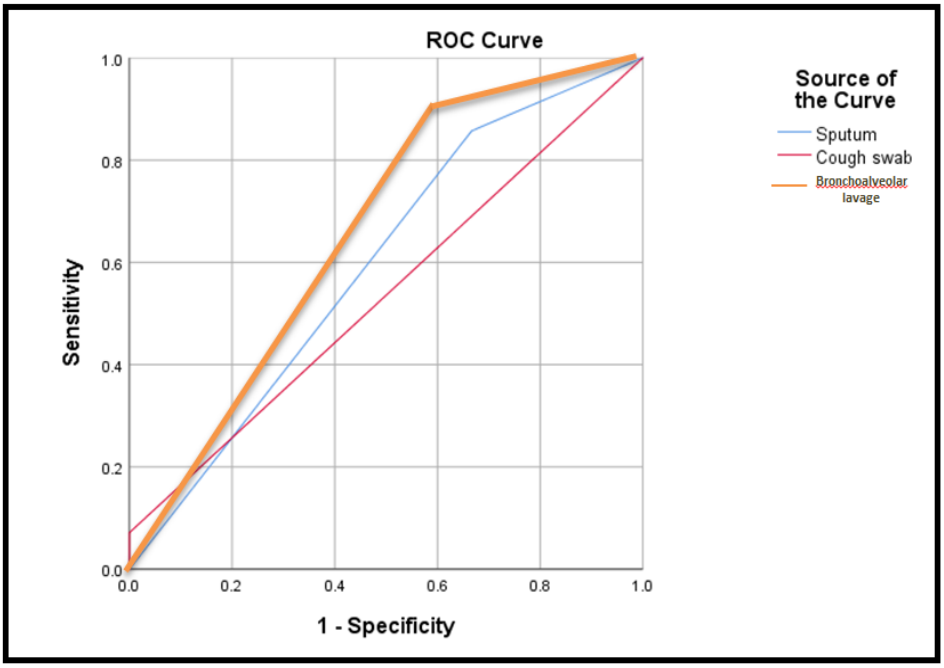

Figure (1): ROC curve for BAL, Sputum and Cough swabs

Receiver operating characteristic (ROC) analysis was performed to determine the value of BAL, Sputum and Cough swabs in the detection of the lower respiratory tract pathogens in CF patients.

DISCUSSION

The data represented a cohort of 31 individuals with a wide age range (2 to 15 years) and a median age of 5 years. Notably, 58.1% of the cohort had a history of consanguinity, possibly indicating

a genetic predisposition to the condition under investigation.

This data also emphasized the relevance of family history, as 22.6% had 1st- degree relatives with a similar condition. Geographically, the majority of the cohort (61.3%) resided in urban areas, highlighting potential disparities in healthcare access and environmental influences between urban and rural settings. The disease onset ranged from a few days to a year, with a median onset of 4 months, suggesting variable disease presentation and progression among individuals.

In line with our research findings, a study carried out in Egypt by **El-Falaki et al. (2014)** reported that the mean age of individuals with CF was 3.91 years, with a standard deviation of 4.18 years. In this cohort, 61% were male, while 39% were female. Furthermore, the study indicated that consanguinity was observed in 50% of the cases. Regarding residence distribution, the majority (61.1%) resided in greater Cairo, followed by 27.8% in the Delta region and 11.1% in Upper Egypt. Notably, a positive family history of CF was identified in 6 individuals, making up 16.7% of the total studied population.

The current study revealed that a significant majority (64.5%) of the individuals in this cohort experienced coughing. Among those with a cough, 51.6% have a productive cough. Furthermore, a substantial portion (74.2%) of the individuals exhibited dyspnea. This could be due to airway obstruction or reduced lung function, both of which are common in cystic fibrosis. Additionally, 71.0% of the individuals had clubbing. These respiratory symptoms collectively reflected the significant burden consistent with cystic fibrosis on the respiratory system.

The study done by **El-Falaki et al. (2014)** highlighted the early onset of CF, with a significant number of patients diagnosed during the first year of life and experiencing symptoms in the neonatal or infantile period. Chronic cough is a consistent feature affecting a high percentage of patients.

The data also provided insights into gastrointestinal symptoms associated with cystic fibrosis. An overwhelming majority (87.1%) of the individuals experience steatorrhea, which is the presence of excessive fat in the stool. This is a classic symptom of cystic fibrosis-related pancreatic insufficiency. Additionally, 58.1%

exhibit abdominal distension, which can be linked to malabsorption. A substantial portion (77.4%) of the individuals had failed to gain weight or had low weight, highlighting the nutritional challenges that often accompany cystic fibrosis. Furthermore, 38.7% showed mild hepatomegaly, which could also be associated with liver involvement in cystic fibrosis. Lastly, 16.1% have a history of meconium ileus, a bowel obstruction that could occur in newborns with cystic fibrosis.

A study done by **Hamed et al. (2022)** provided gastrointestinal manifestations, including vomiting (40%), steatorrhea (40%), failure to thrive (FTT) (50%), recurrent abdominal pain (86.7%), and delayed passage of meconium (26.7%), were prevalent, affecting a substantial proportion of CF patients, with recurrent abdominal pain being particularly common. Hepatic complications were also notable, with a significant portion of patients exhibiting pathologies such as hepatomegaly (10%), coarseness of the hepatic parenchyma (23.3%), periportal fibrosis (13.3%), liver steatosis (16.7%), and cholecystolithiasis (6.7%). Moreover, pancreatic abnormalities like pancreatic cytosis (16.7%) and lipomatosis (26.7%) were observed in a considerable number of patients.

The current study revealed that the mean sweat chloride test value of 93.00 ± 20.82 suggests that these individuals have an elevated level of chloride in their sweat. This is a significant finding, as elevated sweat chloride is a hallmark of cystic fibrosis (CF), a genetic disorder that primarily affects the respiratory and digestive systems. The range of values from 69 to 153 indicated some variability in the severity of chloride ion imbalance among the individuals. This test is crucial for the initial screening of CF and can be a strong indicator of the disease.

The genotyping results provide further insights into the genetic basis of the condition. A majority (67.7%) of the individuals in this cohort were found to have a F508del homozygous mutation. Another significant portion (22.6%) had a F508del heterozygous mutation. The remaining 9.7% did not have any of the 34 common CFTR gene mutations but had the presence of the IVS8-7T polymorphism. This polymorphism is known to be associated with CF and could contribute to the clinical presentation of the disease in these individuals.

A study done by **Gonska et al. (2021)** demonstrated differences in sweat chloride concentrations among groups, reflecting varying degrees of disease severity and providing valuable information for the diagnosis and clinical management of cystic fibrosis and related disorders. **De Boeck (2020)** revealed that homozygous for F508del, which accounts for 45%-50% of study patients.

A study done by **Raffey et al. (2020)** revealed that Δ F508 genotype (the most common genotype), especially homozygous and compound heterozygous genotypes was significantly prevalent from other genotypes.

The study's data pertained to bacteriological results and the types of bacteria detected in different sample groups, namely bronchoalveolar lavage (BAL), sputum, and cough swab. Among those who underwent BAL, a substantial 90.3% yielded positive bacteriological results, indicating the presence of respiratory bacteria. Notably, prevalent isolates included Pseudomonas (54.8%), Klebsiella (25.8%), and Staphylococcus aureus (12.9%), suggesting a high likelihood of respiratory infections within this group. Pseudomonas, a common pathogen in lung conditions in CF patients, underscored the importance of prompt infection identification and treatment to prevent complications.

Sputum samples were obtained, 83.9% were positive, with Pseudomonas (41.9%) and Klebsiella (12.9%) being prominent. These findings aligned with chronic lung disease pathogens, indicating ongoing respiratory infections. Lastly, the cough swabs exhibited a lower 6.5% positivity rate, primarily with Pseudomonas isolates. Importantly, this group's challenge lay in sputum production difficulties, potentially affecting the sample's adequacy for analysis.

In a study done by **Williams & Davies (2012)**, CF patients were predisposed to Pseudomonas infections, and the bacteria could become a permanently established component of the chronically infected lung in more than 80% of patients.

A study done by **Blau et al. (2014)** revealed that induced sputum samples showed good bacteriologic correlation with BAL with no statistically significant difference.

CONCLUSIONS

This study has provided a comprehensive assessment of the bacteriological yield for different

respiratory sampling techniques of Egyptian children with cystic fibrosis. The findings revealed that bronchoalveolar lavage and sputum both yield high rates of positive bacteriological results, highlighting their efficacy in detecting respiratory infections in this CF patient population.

RECOMMENDATION

Based on the data presented, several recommendations can be made, highlighting the importance of regular respiratory system sampling by BAL or sputum in CF patients for early detection and eradication of infection for prevention of further lung damage.

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