Brief communication

Microbiology of the middle meatus compared to sputum in young patients with cystic fibrosis from Bahia – Brazil

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ABSTRACT

Lower airway infection is a major cause of morbidity and mortality in patients with cystic fibrosis. It is currently unknown if the infection of the upper airway can cause exacerbation of lower respiratory tract infection. This study aimed to determine the microbiological profile of the anterior paranasal sinuses outflow tract (middle meatus) of cystic fibrosis outpatients. The microbiological profile was defined using endoscopically directed middle meatal cultures. Paired middle meatal and sputum specimens were collected from 56 outpatients for aerobic cultures. A semi-quantitative leukocyte count of the middle meatal samples was performed. The median age of patients was nine years (3–20 years). Staphylococcus aureus (37%), Staphylococcus coagulase-negative (25%), Neisseria (14%), Pseudomonas aeruginosa (11%), and Streptococcus pneumoniae (7%) were the most prevalent microorganisms in the middle meatal cultures. Using the middle meatal leukocyte count, 16 out of 54 patients (29.6%) presented sinus infection. The most frequently identified pathogens in patients with sinus infections were Staphylococcus aureus (10 patients), Pseudomonas aeruginosa (4 patients), and Streptococcus pneumoniae (3 patients). Agreement of paired middle meatal and sputum cultures was significantly higher among patients with infection in middle meatal (69%). The most common middle meatal pathogens were the typical cystic fibrosis spectrum. This suggests the potential for participating in post-nasal lower airway seeding.

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Cystic fibrosis (CF) is the most frequent autosomal recessive disorder of European descent populations, with an incidence of 1:2000–1:8000 live newborns.1,2 Mutations of the CF transmembrane conductance regulator (CFTR) gene lead to a defective secretion of chloride and a hyperabsorption of sodium. This results in depletion of airways surface liquid, which creates abnormally thick and viscous mucus. This mucus is responsible for the reduction in the mucociliary
clearance and susceptibility to Airways infection and inflam-
mation seen in patients with CF.  

CF is a multisystemic disease. Patients commonly present
with chronic upper and lower Airways infection. Lower respira-
tory tract infection is a major cause of morbidity and mortality
in patients with CF. Pseudomonas aeruginosa is the most preva-
lent bacteria in death-related pulmonary infection. It is
also a common pathogen in chronic rhinosinusitis (CRS). In
patients with CF, radiographic evidence of sinusitis is almost
universally detected. Due to persistent symptoms, CRS affects
the quality of life of CF patients. Additionally, it may be impli-
cated in increased pulmonary exacerbations, and probably
acts as a bacterial reservoir for chronic drainage to lower
Airways. Colonization of both the upper and inferior respira-
tory tract with identical Staphylococcus aureus and Pseudomonas
aeruginosa has been investigated in previous studies and there
is potential for cross-infection between these two sites.

Optimal treatment strategies for bacterial infection in CF
depend upon reliable detection of pathogens. In contrast to
pulmonary infection, antimicrobial therapy for CRS in CF
patients is usually determined empirically. This is especially
common in pediatric populations, because there is limited
application of the gold standard maxillary sinus puncture.
Endoscopically directed middle meatal (MM) culture is an
alternative in acute and CRS. It represents not only the
maxillary, but also all anterior sinuses outflow.

At this time, few studies have investigated the MM micro-
biological flora in CF. There is no consensus in the field as
to whether the bacteria from the upper Airways are implic-
cated in lower respiratory tract infections of these patients.
This study aims to determine the microbiological profile of
the anterior paranasal sinuses outflow tract (MM) of CF out-
patients younger than 21 years of age. A better understanding
of microbial flora, an important component of CF disease, can
lead to improved diagnostic and treatment strategies.

A prospective study evaluating consecutive CF outpatients
receiving care at the Pediatric Pulmonary Clinic of Bahia Ref-
ence Center for CF, in Octávio Mangabeira Hospital, was
conducted from December 2007 to Jun 2008. Paired MM and
sputum samples were collected from study participants, who
were also submitted to paranasal sinus computed tomo-
graphic (CT) scan. All patients or parents completed a standard
questionnaire, which provided information on demographics,
signs and symptoms of rhinosinusitis, associated pulmonary
disease and history of hospital admission.

Inclusion criteria were diagnosis of CF confirmed by sweat
test or genotype and age ranging from 3 to 20 years. Patients
were excluded if they had a history of nasoenteral feeding, antibiotic, oral or nasal corticosteroid therapy in the
last 30 days. Those patients who did not tolerate nasal endo-
scopic examination were also excluded. Informed consent
was obtained from all patients prior to examination. The study
was approved by the Institutional Review Boards of the Oswaldo
Cruz Foundation, Brazilian Ministry of Health.

Two MM samples per patient were collected unilaterally in
all cases under endoscopic guidance: one for culture and the
other for cytological analysis. Routinely, the turbinates, MM,
sphenoethmoidal recess and nasopharynx were assessed for
the presence of polyps, purulent secretion, and adenoid hyper-
trophy. The method was preformed, as follows: nasal mucosa
were sprayed with 0.25–0.5% oximetazoline and 2% xylo-
caine solutions using an atomizer. Endoscopy was performed
by retracting nasal ala using a 2.7-mm 30° telescope. Ster-
ile cotton-tipped wire swabs (sterile nasopharyngeal calcium
alginate tipped applicators; Puritan Products Co., Guilford, ME)
were carefully placed into the MM near the natural maxillary
ostium, and turned until the fibers got wet. In order to decrease
sample contamination with flora from vestibular, septum or
turbinate areas, decongestion of nasal mucosa was performed
and the passage of swab was guided by endoscopy. The first
swab was placed into the Stuart transport medium and sent
to the clinical microbiology laboratory. The other was used to
perform a smear on two macroscopic slides, which were fixed
with 70% alcohol and sent to the pathology laboratory. Sputum
samples were collected by the physiotherapist, during routine
respiratory physical therapy, or immediately after the conclu-
sion of otolaryngologic procedures. Sputum and MM samples
were immediately sent to microbiological analysis.

Chocolate agar, blood agar, MacConkey agar, Sabouraud
Dextrose agar, and Burkholderia cepacia agar base (oxoid) were
inoculated with the samples and incubated for 18–48 h at 36 °C.
Mycological analysis was performed by direct examination
and culture in Sabouraud medium with chloramphenicol
and cycloheximide (BBL). The cultures were incubated at room
temperature for at least three weeks.

The number of polymorphonuclear leukocytes (PMNs) in
the MM samples was determined by a semiquantita-
tive method, using hematoxylin and eosin (H&E) staining
procedure. Smears were examined under oil immersion objec-
tive at 1000× magnification independent of the cultures
results. PMNs were counted in 20 oil immersion fields, and
the average number of PMNs per field was calculated. The
results were classified as follows: many (>10 PMNs/field), few
(6–10 PMNs/field), rare (1–5 PMNs/field), none (0 PMNs/field).

In addition to the paired MM and sputum samples micro-
biological evaluation, all patients were submitted to sinus CT
scan. Three constant anatomic-functional structures, in cor-
respondence to MM microbiological evaluation during this
study, were chosen for description: maxillary, anterior eth-
moid sinuses and ostomeatal unit. The degree of opacification
at these sites was classified according to Lund-Mackay score.
Scores for maxillary and ethmoid sinuses were 0 – no opaci-
fication, 1 – partial opacification and 2 – total opacification.
For the ostomeatal unit was scored as 0 – no opacification
and 2 – total opacification. A Toshiba 64-canals multidectec-
tor unit was used to perform axial sections and multiplanar
reconstructions without intravenous contrast of all patients.
A radiologist and the main investigator analyzed the images.

McNemar’s test was used to assess the significance of the
difference between two paired proportions. The chi-square
test was used for the comparison of categorical variables. Sta-
tistical significance was determined at the 5% (p<0.05) level.
Statistical analysis was performed using SPSS 14.0 (SPSS, Inc.,
Chicago, IL).

Fifty-six CF patients were evaluated during the study
period. The median age was nine years, ranging from three to
20 years (mean ± SD, 9 ± 4 years). Fifty three percent of patients
were male, 25% white, 74% mulatto, and 2% black. The body
mass index ranged from 11 to 22 (mean, 16). The median
for Shwachman score was 85 (range, 65–100). At endoscopic
examination, 38% presented purulent MM secretion, 18% had obstructive adenoid hypertrophy and 12% had nasal polyps. Opacification of maxillary sinus was present in 73% of cases. Ethmoid sinus and ostiomeatal unit were opaque in 61% of patients (Table 1). Severe edema of MM was observed in 18% of patients.

Out of 112 cultures (paired specimens – MM and sputum), 108 (96%) were positive with a total of 147 isolates. The most frequent bacteria were S. aureus, CoNS, Neisseria sp., P. aeruginosa, and S. pneumoniae in both MM and sputum samples. The exception was α-hemolytic Streptococcus, which was more commonly isolated in sputum cultures (p = 0.001) (Table 2). Gram-negative bacteria were isolated in 29% of the MM cultures and in 50% of sputum samples. Based on PMN counts in MM samples, 17% of patients presented many, 13% had few, 61% had rare, and 9% did not present any PMNs. Thirty percent of patients (16/54) had more than six PMNs per field. These patients were most frequently colonized by S. aureus (62%), P. aeruginosa (25%) and S. pneumoniae (18%). In patients with rare PMNs, the most frequent bacteria were CoNS (12/38), S. aureus (11/38), Neisseria sp. (6/38) (Table 3). The most frequent bacteria isolated in the MM secretion from patients with severe edema in MM were S. aureus (50%) and S. pneumoniae (30%).

The present study was the first to evaluate the microbiologic flora of the MM in CF outpatients from Brazil using an endoscopically directed MM sample collection. The invasive gold standard maxillary sinus culture puncture is not routinely used in clinical practice. Compared to methods involving direct sinus cavity assessment (endoscopy-guided sinus aspiration, sinus puncture and intraoperative sampling), endoscopic culture may be considered a non-invasive procedure.

S. aureus was the most frequent pathogen, followed by CoNS, Neisseria sp., P. aeruginosa and S. pneumoniae. Gram-negative bacteria accounted for nearly one third of the total samples. Similarly, a high frequency of S. aureus and P. aeruginosa isolated in sputum of CF patients, mainly in patients below 17 years old was described. Methicillin resistant S. aureus was isolated from 6% of these patients, indicating

### Table 1 – Demographic and clinical findings of 56 cystic fibrosis patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>9 ± 4 (3–20)</td>
</tr>
<tr>
<td>Gender, %</td>
<td>53</td>
</tr>
<tr>
<td>Ethnic group, %</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>25</td>
</tr>
<tr>
<td>Mulatto</td>
<td>74</td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>16 ± 2 (11–22)</td>
</tr>
<tr>
<td>Shwachman score</td>
<td>85 (65–100)</td>
</tr>
<tr>
<td>Endoscopic exam, %</td>
<td></td>
</tr>
<tr>
<td>Middle meatus purulent secretion</td>
<td>38</td>
</tr>
<tr>
<td>Adenoid hypertrophy</td>
<td>18</td>
</tr>
<tr>
<td>Polyps</td>
<td>12</td>
</tr>
<tr>
<td>CT scan, %</td>
<td>73</td>
</tr>
<tr>
<td>Maxillary opacification</td>
<td>61</td>
</tr>
<tr>
<td>Ethmoid opacification</td>
<td>61</td>
</tr>
<tr>
<td>Ostitomeatal complex opacification</td>
<td>61</td>
</tr>
</tbody>
</table>

Note: CT, computed tomography.

* Lund–Mackay score 1 or 2.
* Lund–Mackay score 2.
* Mean ± SD (range).
* Median (range).

### Table 2 – Middle meatal and sputum microbiological profile of 56 cystic fibrosis patients.

<table>
<thead>
<tr>
<th>Culture results</th>
<th>Middle meatus</th>
<th>Sputum</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 56</td>
<td>n (%)</td>
<td>n = 56</td>
</tr>
<tr>
<td>Total growth rate</td>
<td>53 (95)</td>
<td>55 (98)</td>
<td>0.500</td>
</tr>
<tr>
<td>No growth</td>
<td>3 (5)</td>
<td>1 (2)</td>
<td>0.500</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>21 (37)</td>
<td>18 (32)</td>
<td>0.678</td>
</tr>
<tr>
<td>SCN</td>
<td>14 (25)</td>
<td>9 (16)</td>
<td>0.302</td>
</tr>
<tr>
<td>Neisseria sp.</td>
<td>8 (14)</td>
<td>12 (21)</td>
<td>0.424</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>6 (11)</td>
<td>10 (18)</td>
<td>0.219</td>
</tr>
<tr>
<td>Non-mucoid</td>
<td>1 (2)</td>
<td>3 (5)</td>
<td></td>
</tr>
<tr>
<td>Mucoid</td>
<td>5 (9)</td>
<td>7 (11)</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>4 (7)</td>
<td>2 (4)</td>
<td>0.500</td>
</tr>
<tr>
<td>α-hemolytic Streptococcus</td>
<td>2 (4)</td>
<td>15 (27)</td>
<td>0.001</td>
</tr>
<tr>
<td>Corinebacterium sp.</td>
<td>2 (4)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>1 (2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Tatumella pyseos</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Achromobacter xylosidans</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Sphingobacterium multivoran</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Candida sp.</td>
<td>0</td>
<td>2 (4)</td>
<td></td>
</tr>
</tbody>
</table>

CoNS, coagulate-negative Staphylococci.
* p < 0.05, McNemar’s test.
that colonization of respiratory tract by pathogenic microorganisms of these patients occurs at young age.\textsuperscript{16} The MM microbiological profile found in this study differed from both the maxillary sinus flora of CF pediatric surgical patients and the MM aspirates of a CF general population, in which greater rates of \textit{P. aeruginosa} and \textit{H. influenzae} were found.\textsuperscript{15,17} In fact, surgical patients frequently have more severe chronic sinus disease, with persistent \textit{P. aeruginosa} infection.\textsuperscript{18} Likewise, this organism is more frequently isolated in older patients with purulent MM secretion.\textsuperscript{15} Patients enrolled in the present study were younger than 21 years old, followed in outpatient clinic and had no clinical signs of acute CF exacerbation. Furthermore, such differences in the microbiological profiles are unlikely to be explained by the sampling method, because there is a high concordance rate between maxillary sinus and endoscopically directed MM cultures.\textsuperscript{13,14}

About one third of included patients presented MM purulent secretion and positive culture to \textit{S. aureus, P. aeruginosa} and \textit{S. pneumoniae}. In contrast, ostiomeatal complex opacification at CT scans was found in two thirds of the cases. The CT may overestimate the diagnosis of sinus disease and cannot accurately differentiate between mucosal thickening and infection.\textsuperscript{15} Comparing pathogen profile from MM and sputum cultures, the most frequent isolated bacteria were similar. The exception was seven-fold higher frequency of \textit{a}-hemolytic Streptococci in sputum culture as compared to MM culture. This may represent sample contamination with oropharyngeal microflora, especially because the studied patients were composed of young children and it is difficult to collect spontaneous sputum samples. The agreement rate of bacterial profile was higher in the group of patients with MM infection. This may reflect the post-nasal lower airway colonization by pathogenic bacterial species. The genotypic characterization of isolates from both MM and sputum samples could confirm the traffic of pathogenic bacteria from upper to lower airways. There is a high genotypic concordance of \textit{P. aeruginosa} and \textit{S. aureus} isolates from nasal and sputum samples, suggesting that the flow of pathogenic bacteria from upper to lower airways may be a frequent event.\textsuperscript{12} Moreover, it was demonstrated that there is no significant differences in the bacterial community diversity in lung between baseline and exacerbation samples of patients with CF.\textsuperscript{20}

One of the limitations of this study was its cross-sectional design, limiting the understanding of the complex dynamic characteristic of chronic infections in CF patients. In the future, prospective studies should be conducted to define the impact of sinus infection on pulmonary disease in CF patients. In conclusion, the most common MM pathogens in CF young outpatients were the typical CF spectrum and \textit{S. pneumoniae}. This finding suggests the potential for post-nasal lower airway seeding.

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### Conflicts of interest

The authors declare no conflicts of interest.

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