CURRENT MICROBIOLOGICAL DATA ON LOWER RESPIRATORY TRACT INFECTION IN CYSTIC FIBROSIS
PART II: RECOMMENDATIONS FOR MICROBIOLOGICAL DIAGNOSIS IN CYSTIC FIBROSIS

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CURRENT MICROBIOLOGICAL DATA ON LOWER RESPIRATORY TRACT INFECTION IN CYSTIC FIBROSIS. PART II: RECOMMENDATIONS FOR MICROBIOLOGICAL DIAGNOSIS IN CYSTIC FIBROSIS (Abstract): Current standards of sputum examination in respiratory infections with opportunistic bacteria cannot be applied to patients with cystic fibrosis due to their particularities. In 2010, a working group from Great Britain established standards for microbiological processing of samples from patients with cystic fibrosis. Recommendations on sample collection, transportation, storage, and processing, identification of bacterial isolates and in vitro antibiotic susceptibility are made. Also recommended is that the multidisciplinary team monitoring patients with cystic fibrosis to include a microbiologist. **Key words**: CYSTIC FIBROSIS, LOWER RESPIRATORY TRACT INFECTION, OPTIMIZATION OF MICROBIOLOGICAL DIAGNOSIS.

Current standards of sputum examination in respiratory infections with opportunistic bacteria (1, 2, 3) cannot be applied to patients with cystic fibrosis (CF) due to the previously detailed particularities (4): onset of clinical manifestations in the first year of life, diverse pathogen population with different degrees of implication in the worsening of respiratory insufficiency, difficulties in identifying some of them to species level by conventional techniques.

In these circumstances, expert groups from North America and Europe became concerned with the optimization of microbiological evaluation of samples from lower respiratory tract (LRT) in CF patients as the therapeutic decision and infection control depend on it (5, 6, 7). The main recommendations in the latest guidelines drafted by a working group from United Kingdom in September 2010 (8) are presented, being of interest for all microbiologists dealing with such cases. Quality of care for these patients depends on the introduction of these rules in current practice.
Sample collection, transport, and storage

Sputum is the recommended specimen for routine investigation in patients, both at each hospital visit and during exacerbations. Sputum is of equal value as specimens taken during surgery or by bronchoscopic bronchoalveolar lavage (the golden standard for the investigation of LRT infections). In infants and small children hypopharyngeal aspirate is recommended as it is a simple and non-invasive method. Pharyngeal exudate is not indicated because of false-positive results.

For best results, the samples have to be processed as soon as possible after collection. Any delay in processing with storage at room temperature can cause bacterial overgrowth which could mask the true pathogens. On the other hand, refrigeration at 4°C for 2-3 hours does not significant negative consequences. Thus, if processing is delayed, refrigeration is preferable to storage at ambient temperature.

Sample processing

Homogenization. Numerous studies recommend sputum homogenization of by mechanic disruption with glass beads or by mucolytic agents. However, the British expert group believes that there is insufficient evidence to support the requirement of using homogenization in CF.

Sputum Gram stains. Generally, this is a very important method which allows rejection of unrepresentative samples, and for the representative ones it gives quick information about morphology and staining characteristics of bacteria associated with inflammatory cells, and guides etiology and empirical antibiotic therapy. In CF there is insufficient evidence to recommend it for routine use as a method for assessing specimen quality and to predict culture results.

Conventional culture. Unlike other respiratory infection, in CF the use of selective medium in addition to usual culture medium (blood agar, chocolate agar) is mandatory; prolonged incubation time leads to higher detection rates of three major pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex (BCC). Their presence should be reported irrespective of quantity.

For *S. aureus*, Chapman medium incubated at 35-37°C in air and examined next day and after at least other 24 hours is recommended. *P. aeruginosa* can be isolated in good conditions on MacConkey agar incubated at 35-37°C in air and examined next day and after at least other 24 hours. This medium did not give satisfactory results in the isolation of BCC. BCSA medium (*Burkholderia Cepacia Selective Agar*) with polymyxin, gentamicin and vancomycin has a high sensitivity and specificity for BCC as compared to other selective media (9, 10), being thus recommended. To maximize sensitivity, it should be incubated at 35-37°C in air for 5 days, with daily culture examination.

*Haemophilus influenzae* is a frequent commensal in the upper airways that may be a sputum contaminant, reason why qualitative evaluations are recommended. Growth on cefsulodin chocolate agar favors the isolation by inhibitory effect against *P. aeruginosa*. Incubation 35-37°C in CO₂ at 35-37°C for 24-48 hours.

According to the guidelines, the use of selective medium for *Stenotrophomonas*
maltoophilia and other Gram-negative bacilli should be considered by laboratories according to their particular clinical needs.

Culture and identification of atypical mycobacteria is a matter for specialized laboratories.

For the isolation of filamentous fungi Sabouraud medium with additional antibiotic (ciprofloxacin, amikacin, ceftazidime or polymyxin) to inhibit *P. aeruginosa* should be used. Plate should be incubated at 35-37°C for 24-48 hours, then prolonging cultures up to 7 days at room temperature (22°C).

There is not sufficient evidence to support the use of molecular assays for the detection of microbial agents in CF in addition to routine culture.

**Identification of bacterial isolates**

Phenotypic identification to species level of isolates from LRT in CF is particular challenging for non-fermentative Gram-negative bacilli. *P. aeruginosa* strains can be accurately identified by the presence of three phenotypic characters: colonies with blue-green pigmentation (pyocyanin), positive oxidase test and growth at 42°C. Growing strains displaying unusual characters can be misidentified as other Gram-negative non-fermentative bacilli: *Achromobacter* spp, BCC, *Burkholderia gladioli*, *Pandorea* spp, *Ralstonia* spp, *S. maltophilia*, with important clinical and therapeutic consequences. An “excellent” identification from API 20NE kit (BioMerieux) in conjunction with colonial appearance, oxidase test, and colistin susceptibility can be used to confirm *P. aeruginosa*, *S. maltophilia*, and *Achromobacter* spp. This does not apply to a “very good”, “good”, or “acceptable” result due to a significant percentage of false-positive results; in these cases molecular techniques should be used by referring laboratory. However, BCC strains suspected by phenotypic methods should be immediately reported to the clinic, and immediate measures for preventing contact with other non-infected patients should be taken.

Additional molecular typing is recommended to identify strains with epidemiogenic potential.

**Susceptibility testing guidelines**

Antibiotic susceptibility of strains with clinical significance should be assessed by a standard method (CLSI, EUCAST); disc diffusion provides results comparable with broth dilution method (reference method). Automated testing is not recommended for Gram-negative non-fermentative bacilli due to possible major errors.

A good correlation between susceptibility findings and clinical response was found in patients intermittently infected with *P. aeruginosa*. In patients with initial infection, early treatment with appropriate antibiotics resulted in a much slower progression to chronic infection. In chronic infections, the presence of glycocalyx containing slow-multiplication bacteria makes that antibiotic therapy guidance by *in vitro* tests to be unsatisfactory. Currently there are no specific breakpoints for this condition.

**Multidisciplinary team**

The microbiologist, as laboratory service provider and infection prevention adviser, should participate in the meetings of the multidisciplinary team monitoring CF patients whenever particular situations are identified.
Current microbiological data on lower respiratory tract infection in cystic fibrosis

REFERENCES