ISOLATION OF PSEUDOMONAS CEPACIA IN CYSTIC FIBROSIS PATIENT

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Pulmonary infection on cystic fibrosis (CF) patients are associated with a limited qualitative number of microorganisms. During the colonization process, Staphylococcus aureus usually precedes Pseudomonas aeruginosa. This latter is at first non-mucoid, being replaced or associated to a mucoid morphtotype which is rare in other diseases. In 1980, Pseudomonas cepacia appeared as an important agent in CF pulmonary infections with a mean frequency of about 6.1% isolations in different parts of the world. The primus colonization mainly occurs in the presence of pre-existing tissue lesions and the clinical progress of the disease is variable. In some patients it can be fulminant; in others it can cause a gradual and slow decrease in their pulmonary functions. The concern with this germ isolation is justified by its antibiotic multiple resistance and the possibility of direct transmission from a colonized patient to a non-colonized one. We reported the first case of P. cepacia infection in a CF patient in our area. The microbiological attendance to this patient had been made from 1986 to 1991 and the first positive culture appeared in 1988. The sensitivity profile showed that the primus colonization strain was sensitive to 9 of 17 tested antibiotics, however in the last culture the strain was resistant to all antibiotics. These data corroborate the need for monitoring the bacterial flora on CF patients respiratory system.

Key words: Pseudomonas cepacia – cystic fibrosis – pulmonary infection

Pulmonary diseases are responsible for about 90% of deaths occurring in cystic fibrosis (CF) patients, mostly as a consequence of chronic airways infections. The chronic stage comes along with exacerbations of pulmonary infections, progressive losses of pulmonary function and finally death (Gilligan, 1991).

The microbiological profile of respiratory infections is characteristic in those patients referred above, being restricted to a few species of microorganisms. They occur in a sequential order of colonization, the more usual being a Staphylococcus aureus initial colonization, followed by Pseudomonas aeruginosa. These bacteria can be met separately or in association with each other. Other microorganisms less frequently found are Haemophilus influenzae and Pseudomonas cepacia, besides virus and fungi (Wood et al., 1976; Thomassen et al., 1986; Gilligan, 1991).

Staphylococcus aureus was the first characteristic agent described in CF patients with pulmonary infections at the pre-antimicrobial era. It is considered an important agent until today, specially in patients under the age of 10 (Gilligan, 1991). In spite of many virulence factors produced by S. aureus, the mechanisms that may cause pulmonary lesions are poorly known. Patients infected with S. aureus usually respond well to antimicrobial therapy, and it is not difficult to eradicate such microorganism from pulmonary environment. This may be so due to the sensitivity of those strains, whose resistance is usually limited to Penicillin G (Gilligan, 1991). However, the eradication of the agent is difficult and in some cases impossible, concerning P. aeruginosa infections. With the use of anti-staphylococcal drugs, this microorganism became gradually
more frequent in CF patients with pulmonary infections. It may appear as a mucoid variant in a frequency up to 80% of cases (Hoiby, 1982) and can be easily recognized in culture by its large exopolysaccharide production.

So far, the factors that give rise to mucoid *P. aeruginosa* chronic infections are unknown. Initial colonization occurs by the classic non-mucoid variant of *P. aeruginosa,* that becomes mucoid through environmental factors pressure. This second variant persists and is associated with a bad prognosis in chronic infected patients (Schwachman & Kuleczycki, 1968; Hoiby, 1982; Penketh et al., 1983; Marques et al., 1985; Ramphal & Vishwanath, 1987). More recently, *P. cepacia* has been isolated from respiratory secretions in CF patients, with variable isolating rates in different parts of the world (Isle et al., 1986; Thomassen et al., 1986). This microorganism is not as common as those already mentioned but, in many patients, the infection is devastating, compelling some centers of CF patients to monitor its isolation (Gilligan, 1991).

The variability in *P. cepacia* isolation rates may be due to difficulties in its recovery from secretions, specially in those patients simultaneously colonized by mucoid *P. aeruginosa,* the use of selective media being indicated in these cases (Tablan et al., 1987).

The way of transmission of *P. cepacia* in CF patients has not yet been completely defined. Both direct and indirect ways are said to play a role in the transmission between colonized and non-colonized patients (Thomassen et al., 1986; Tablan et al., 1987; Lewin et al., 1990).

Also it has not yet been defined if *P. cepacia* is a true pathogen or merely an opportunist that can be used as an indicator of the degree of the pulmonary involvement, as the initial colonization usually occurs in patients with a worse pulmonary function (Tablan et al., 1987; Taylor et al., 1991).

After the occurrence of infection, the clinical course is variable. It can be devastating, inducing rapidly to death; it can also slowly evolve causing a progressive loss of pulmonary function, or it can remain stable with no important changes in the general physical condition of patients (Gilligan & Schidolow, 1984; Isle et al., 1986; Tomashefski et al., 1988).

The variability in the clinical course may be related to the degree of pulmonary involvement at the time of the initial colonization. The more stable evolution occurs more often in patients with a discreet pulmonary involvement while a less favourable advance is related to more severe pulmonary diseases (Gilligan & Schidolow, 1984; Isle et al., 1986). Clinical evolution may also be associated with the virulence of the infecting strain of *P. cepacia,* considering that some strains are highly virulent (McKevitt & Woods, 1984; McKevitt et al., 1985; Lonon et al., 1988).

The synergistic interaction of *P. cepacia* with other less easily characterized microorganisms like virus may also influence the course of the disease. A viral infection may interfere in some way in the natural local host defenses, or expose receptors that favour *P. cepacia* adhesion (Wood et al., 1976; Stroobant, 1986).

Another factor that may influence the clinical course of the disease is the antimicrobial sensitivity of the infecting strain. Patients initially colonized with multiresistant strains usually course with a persistent infection, while those colonized with less resistant strains have a tendency to present intermittent infections (Taylor et al., 1991).

In this study, we describe a five-year-bacteriologic follow-up of the first case of *P. cepacia* infection in a CF patient attended at the mucoviscidose sector of the Instituto Fernandes Figueira – FIOCRUZ (RJ).

**MATERIALS AND METHODS**

The patient studied, N. P. S., female, born in 4 March 1979, was attended at the sector of mucoviscidose of the Instituto Fernandes Figueira (RJ) and was bacteriologically accompanied from 12 April 1986 to 10 November 1991.

During this period, three oropharynx secretions and 18 sputas were examined. Oropharynx secretion had been collected with sterile swabs and immediately processed or introduced into a Stuart transport medium for periods not superior to 4 hr. Sputa were obtained through spontaneous expectoration by the protecting technique with cotton tampons in order to avoid spittle contamination (Beck et al., 1982).

Sputa were liquefied in a 2.5% (v/v) N-acetyl-cystein solution, in 13 x 100 mm tubes
with glass pearls during 30 min with occasional agitation.

Clinical specimens were sown in sheep-blood agar, Eugon agar (Difco) and CLED agar (BBL). They had been incubated at 35 °C, the first two in 5 to 10% CO₂ atmosphere and the last one in aerobiosis. The first observation of the plates was made after 18 hr, to avoid confluent growth of mucoid P. aeruginosa, and again after 48 hr and 72 hr.

The isolated microorganisms were identified by conventional bacteriological methods (Ballows et al., 1991) and in the specific case of non-fermenter gram-negative bacilli, the following tests were used: metabolism type in OF medium, oxidase, growth in MacConkey Agar, and motility. On the P. cepacia characterization was used: pyocianin and pyoverdin production, polymyxin sensitivity, fructose, galactose, xylose, lactose, saccharose and mannitol utilization in OF medium, nitrate reduction, decarboxylases (arginine, ornithine, and lysine) production, urea hydrolysis, DNase and gelatinase production, growth on SS Agar, and NaCl 6.5%. All results were compatible with P. cepacia (Table I).

The mucoid and non-mucoid variants of P. aeruginosa were differentiated by its colonial morphology in the different isolation culture media.

Antimicrobial sensitivity testing of P. cepacia strains had been done following by the diffusion method, according to Barry (Ballows et al., 1991). The chemotherapy tests were: Amoxicillin (10 mcg/ml); Ampicillin (10 mcg/ml); Carbenicillin (100 mcg/ml); Cephalothin (30 mcg/ml); Cefoxitin (30 mcg/ml); Cefotaxime (30 mcg/ml); Cefoperazone (30 mcg/ml); Cefazidime (30 mcg/ml); Ceftriaxone (30 mcg/ml); Amikacin (30 mcg/ml); Gentamicin (10 mcg/ml); Netilmicin (30 mcg/ml); Tobramycin (10 mcg/ml); Ofloxacin (5 mcg/ml); Chloramphenicol (30 mcg/ml); Sulfoisoxazole (300 mcg/ml) and Trimethoprim-sulfamethoxazole (1.25/23.75 mcg/ml).

RESULTS

The first oropharynx secretion cultured showed the growth of S. aureus and Haemophilus sp. which had been present in 18 of 21 cultures realized during all the period of our research.

A second culture of oropharynx secretion obtained after three months indicated the presence of mucoid P. aeruginosa, besides the bacteria already mentioned. Mucoid P. aeruginosa was also isolated in posterior specimens.

Non mucoid P. aeruginosa were isolated from sputum after one year of attendance, on the fourth culture and also in subsequent cultures. Both P. aeruginosa morphotypes had been isolated in an intermittent way.

Group A Beta hemolytic Streptococcus was obtained in various cultures, and Streptococcus pneumoniae in a single one.

Pseudomonas cepacia was isolated for the first time after two years. In the same specimen we also found mucoid and non mucoid P. aeruginosa, S. aureus and Haemophilus sp. The following eight cultures carried on for 18 months didn't give rise to P. cepacia. This one appeared again in three cultures we did between 1990 and 1991.

The antibiotic sensitivity profile of the P. cepacia isolated on four occasions showed us that the initial strain, in spite of being

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**TABLE I**

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism type in OF medium</td>
<td>Oxidative</td>
</tr>
<tr>
<td>Growth on MacConkey Agar</td>
<td>Positive</td>
</tr>
<tr>
<td>Oxidase</td>
<td>Positive</td>
</tr>
<tr>
<td>Pyocian</td>
<td>Negative</td>
</tr>
<tr>
<td>Pyoverdin</td>
<td>Negative</td>
</tr>
<tr>
<td>Polymyxin sensitivity</td>
<td>Negative</td>
</tr>
<tr>
<td>Acid: fructose</td>
<td>Positive</td>
</tr>
<tr>
<td>galactose</td>
<td>Positive</td>
</tr>
<tr>
<td>xylose</td>
<td>Positive</td>
</tr>
<tr>
<td>lactose</td>
<td>Positive</td>
</tr>
<tr>
<td>saccharose</td>
<td>Positive</td>
</tr>
<tr>
<td>mannitol</td>
<td>Positive</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>Negative</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>Negative</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>Negative</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>Positive</td>
</tr>
<tr>
<td>Hydrolysis: urca</td>
<td>Negative</td>
</tr>
<tr>
<td>DNA</td>
<td>Negative</td>
</tr>
<tr>
<td>gelatin</td>
<td>Positive</td>
</tr>
<tr>
<td>Growth on: SS Agar</td>
<td>Negative</td>
</tr>
<tr>
<td>6/5% NaCl</td>
<td>Negative</td>
</tr>
</tbody>
</table>
multiresistant, was sensitive to some of the tested antibiotics, in contrast with the further strains obtained in the final culture which were resistant to all the antibiotics tested (Table II).

**DISCUSSION**

All the microorganisms described as usual in respiratory infections of CF patients were found in our patient. Curiously, the primary isolation of the mucoid variant of *P. aeruginosa* preceded the isolation of the non-mucoid variant. This way have occurred due to overgrowth of the mucoid strain.

*Pseudomonas cepacia* was isolated after two years, along with *Haemophilus* sp., *S. aureus* and non-mucoid *P. aeruginosa*. Subsequent cultures were negative for *P. cepacia* for one year, then it reappeared persistently until the last bacteriologic examination of our study. Similar situations have been described before. Although most patients colonized with *P. cepacia* show a persistent colonization, in some of them a transitory infection may occur, and in such cases the clinical evolution tends to be more benign (Taylor et al., 1991).

Mucoid *P. aeruginosa* disappearance coincided with the first *P. cepacia* infection. The reduction of the number of *P. aeruginosa* and its disappearance during *P. cepacia* infection has been documented in some studies employing quantitative sputum cultures (Gilligan, 1991; Taylor et al., 1991). Another possibility to be considered here is the influence of mucolytic agents like N-acetyl-cysteine, employed on sputum liquefication before culture on the growth of *P. aeruginosa* (Parry & Neu, 1984).

*Staphylococcus aureus*, usually a frequent but easily eradicated agent in CF patients, and *Haemophilus* sp., also not characterized by persistent pulmonary infections, were obtained in almost all cultures apparently without influence of the presence of *P. cepacia* and *P. aeruginosa*.

Moreover, group A beta hemolytic *Streptococcus*, rare in pulmonary infection exacerbations in CF patients, was isolated in seven cultures. We are not sure if it participates in the infective process, or if the patient was a carrier and contaminated the sputum on the superior airways during collection. Negative cultures for this microorganism coincided with the growth of mucoid *P. aeruginosa* which may have hidden the presence of the typical streptococci colonics.

Little is known about the importance of *P. cepacia* on CF pathogenesis. However, the relation between the antimicrobial resistance
and the persistence of infection, as well as the disadvantageous clinical evolution of *P. cepacia* infected patients are well known. Due to the dangers carried on by this infection, special attention must be given to the compromised patients.

The first infecting strains are usually sensitive to some antimicrobials, but resistance develops during or after therapy. Some strains may be resistant to all antibiotics and *P. cepacia* eradication will be hardly obtained (Gilligan, 1991).

A similar situation was spotted in the patient studied. The *P. cepacia* strain initially isolated was sensitive to several of the antibiotics. On subsequent isolate strains, drug resistance increased gradually and the last cultured strain was resistant to all antimicrobials tested.

The isolation and identification of the pathogens involved in CF pulmonary infections may contribute to the survival of these patients in an important way, because those microorganisms present different relationships with the host and with the disease evolution. The bacteriologic monitoring of these patients is also relevant to make therapeutic measures possible so that their life quality and life expectancy can be improved and lengthened.

REFERENCES


