Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *88* (1): 125-129, jan./mar. 1993

ISOLATION OF *PSEUDOMONAS CEPACIA* IN CYSTIC FIBROSIS PATIENT

ELIZABETH DE ANDRADE MARQUES; ROSA MARIA CARVALHO PINTO*; LUDMA TROTTA DALLALLANA*; ELSA FUCHSHUBER RODRIGUES DE OLIVEIRA & ITALO SUASSUNA

Serviço de Microbiologia e Imunologia, Departamento de Patologia e Laboratórios, Faculdade de Ciências Médicas, UERJ, Av. 28 de Setembro, 77 – 3º andar-fds, 20551-030 Rio de Janeiro, RJ, Brasil
*Setor de Mucoviscidose, Instituto Fernandes Figueira – FIOCRUZ, Av. Rui Barbosa, 716, 22250-020 Rio de Janeiro, RJ, Brasil

Pulmonary infection on cystic fibrosis (CF) patients are associated with a limited qualitative number of microorganisms. During the colonization process, Staphylococcus aureus *usually preceedes* Pseudomonas aeruginosa. *This latter is at first non-mucoid, being replaced or associated to a mucoid morphotype 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-existent 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 resistence 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 resistent 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 funtion 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 Penicilin 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

This work was supported by CNPq and FINEP.

Received 23 June 1992.
Accepted 18 August 1992.

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 & Kulczycki, 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 monitore 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 involviment, 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 intermitent 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 sputa were examined. Oropharynx secretion had been collected with sterile swabs and immediatelly 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 tampoons 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% CO_2 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 microrganisms 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).

TABLE I

Biochemical characterization of *Pseudomonas cepacia* in cystic fibrosis patient

Test		Results
Metabolism type in OF medium		Oxidative
Growth on MacConkey Agar		Positive
Oxidase		Positive
Pyocian		Negative
Pyoverdin		Negative
Polymyxin sensitivity		Negative
Acid:	fructose	Positive
	galactose	Positive
	xylose	Positive
	lactose	Positive
	saccharose	Positive
	mannitol	Positive
Nitrate reduction		Negative
Arginine dihydrolase		Negative
Ornithine decarboxylase		Negative
Lysine decarboxylase		Positive
Hydrolysis:	urea	Negative
	DNA	Negative
	gelatin	Positive
Growth on:	SS Agar	Negative
	6/5% NaCl	Negative

The mucoid and non-mucoid variants of *P. aeruginosa* were differentiated by its colonical 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 chemotherapics tested 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); Ceftazidime (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); Sulfisoxazole (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

TABLE II

Antibiotics susceptibility profile of isolated *Pseudomonas cepacia* from respiratory secretions of a cystic fibrosis patient in four different occasions

Antibiotics	Antibiotics susceptibility			
	6 Oct. 88	15 May 91	2 Aug. 91	11 Oct. 91
Amoxicillin	R	R	R	R
Ampicillin	R	R	R	R
Carbenicillin	R	R	R	R
Cephalothin	R	R	R	R
Cefoxitin	R	I	R	R
Cefotaxime	S	S	S	R
Ceftzidime	NT	S	NT	R
Cefoperazone	NT	S	NT	R
Ceftriaxone	S	S	S	R
Amicacin	S	S	R	R
Gentamicin	S	S	R	R
Netilmicin	S	S	R	R
Tobramycin	S	R	R	R
Ofloxacin	NT	NT	NT	R
Chloranphenicol	S	S	S	R
Sulfisoxazole	I	S	R	R
Trimethopim-Sulfamethoxazole	I	S	S	R

R = resistent; S = sensitive; I I = intermediate; NT = non-tested.

multiresistent, was sensitive to some of the tested antibiotics, in contrast with the further strains obtained in the final culture which were resistent 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 liquefaction 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 tipical streptococci colonies.

Little is known about the importance of *P. cepacia* on CF pathogenesis. However, the relation between the antimicrobial resistence

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 resistence develops during or after therapy. Some strains may be resistent 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 resistent to all antimicrobials tested.

The isolation and identification of the pathogens involved on 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

BALOWS, A.; HAUSLER Jr, W. J.; HERRMANN, K. L.; ISENBERG, H. A.; SHADOMY, H. J. (eds), 1991. *Mamual of Clinical Microbiology,* 5th ed. American Society for Microbiology. Washington, D. C.

BECK, G.; PUCHELLE, E.; LAROCHE, D.; MOUGEL, D. & SADOUL, P., 1982. Bacteriologie quantitative des expectorations recueillies par une techinique simple limitant la contamination salivaire. *Bull. Europ. Physiopath. Resp., 18:* 885-892.

GILLIGAN, P. H., 1991. Microbiology of airway disease in patients with cystic fibrosis. *Clin. Microbiol. Rev., 4:* 35-51.

GILLIGAN, P. H. & SCHIDOLOW, D. V., 1984. The role of *Pseudomonas cepacia* in pulmonary disease of cystic fibrosis patients. *Clin. Microbiol. News, 6:* 42-44.

HOIBY, N., 1982. Microbiology of lung infection in CF patients. *Acta Paediatr. Scand.* (Suppl.), *301:* 33-54.

ISLE, A.; MACLUSKY, I.; COREY, M., 1986. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J. Pediatr., 134:* 669-771.

LEWIN, L. O.; BYARD, P. J. & DAVIS, P. B., 1990. Effect of *Pseudomonas cepacia.* Colonization on survival and pulmonary function of cystic fibrosis patients. *J. Clin. Epidemiol., 43:* 125-131.

LONON, M. K.; WOODS, D. E. & STRAUS, D. C., 1988. Production of lipase by clinical isolates of *Pseudomonas cepacia. J. Clin. Microbiol., 26:* 979-984.

MARQUES, E. A.; DALLALANA, L. T.; LIMA, K. M. R.; LOBO, S. T.; PINTO, R. M. & SUASSUNA, I., 1985. Incidência e caracterização e *Pseudomonas aeruginosa* em pacientes com mucoviscidose. *J. Pediatr., 59:* 374-380.

MCKEVITT, A. I.; BAJAKSOUZIAN, S.; KLINGER, J. D. & WOODS, D. E., 1985. Purification and characterization of an extracellular protease from *Pseudomonas cepacia. Infect. Immun., 57:* 771-778.

MCKEVITT, A. I. & WOODS, D. E., 1984. Characterization of *Pseudomonas cepacia* isolates from patients with cystic fibrosis. *J. Clin. Microbiol., 19:* 241-293.

PARRY, M. F. & NEU, C., 1977. Effect of N-Acetyl-Cysteine on antibiotic activity and bacterial growth *in vitro. J. Clin. Microbiol., 5:* 58-61.

PENKETH, A.; PITT, T.; ROBERTS, D.; HODSON, M. E. & BATTEN, J. C., 1983. The relationship of phenotype changes in *Pseudomonas aeruginosa* to the clinical conditions of patients with cystic fibrosis. *Am. Rev. Respir. Dis., 127:* 605-608.

RAMPHAL, R. & VISHWANATH, S., 1987. Why is *Pseudomonas* the colonizer and why does it persist? *Infection, 15:* 281-287.

SHWACHMAN, H. & KULCZYCKI, L. L., 1968. Long-term study of one hundred five patients with cystic fibrosis A. M. A. *J. Dis. Child, 96:* 6-15.

STROOBANT, J., 1986. Viral infection in cystic fibrosis. *J. Royal. Soc. Med.* (Suppl.), *12:* 19-22.

TABLAN, O. C.; CARSON, L. A.; CUSICK, L. B.; BLAND, L. A.; MARTONE, W. J. & JARVIS, W. R., 1987. Laboratory proficiency test results on use of selective media for isolating *Pseudomonas cepacia* from simulated sputum specimens of patients with cystic fibrosis. *J. Clin. Microbiol., 25:* 485-487.

TABLAN, O. C.; MARTONE, W. J.; DOERSHUK, C. F.; STERN, R. C.; THOMASSEN, M. J.; KLINGER, J. D.; WHITE, J. W.; CARSON, L. A. & JADVIS, W. R., 1987. Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis. Risk factors and outcomes. *Chest, 91:* 527-532.

TAYLOR, R. F. H.; GAYA, H. & HUDSON, R. E., 1991. *Pseudomonas cepacia:* pulmonary infection in patients with cystic fibrosis. *Ped. Pulmon.* (Suppl.), 6: 1991. North American Cystic Fibrosis Conference, Dallas, Texas.

THOMASSEN, M. J.; DEMKO, C. A.; DOERSHUK, C. F.; STERN, R. C. & KLINGER, J. D., 1986. *Pseudomonas cepacia:* Decrease in colonization in patients with cystic fibrosis. *Am. Rev. Resp. Dis., 134:* 669-671.

TOMASHEFSKI, J. F.; THOMASSEN, M. J.; BRUCE, M. C.; GOLDBERG, H. I.; KONSTAN, M. W. & STERN, R. C., 1988. *Pseudomonas cepacia*-associated pneumonia in cystic fibrosis. *Arch. Pathol. Lab. Med., 112:* 166-172.

WOOD, R. E.; BOAT, T. F. & DOERSHUK, C. F., 1976. Cystic Fibrosis: state of the art. *Am. Rev. Respir. Dis., 113:* 833-878.