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Qualitative and Quantitative Microbiological Analysis of Sputa of 102 Patients with Cystic Fibrosis

Summary: A microbiological analysis of 102 patients suffering from cystic fibrosis was conducted over a 22 month period. 20 microbial species with the following incidence were identified: *Pseudomonas aeruginosa:* 83.4%; *Candida albicans:* 29.4%; *Staphylococcus aureus:* 24.5%; *Staphylococcus epidermidis:* 11.8%; *Haemophilus influenzae:* 11.8%; *Streptococcus pneumoniae:* 6.9%; *Pseudomonas maltophilia:* 6.8%; *Aspergillus fumigatus:* 5.9%. Other species were present in less than 5% of the patients. In the majority of specimens with *P. aeruginosa,* more than one type (up to six) was detectable. These strains were identical in colony appearance, O-serotype and pyocin-type. Quantitative analysis revealed concentrations of colony-forming units of 10^7 to 10^9 for *P. aeruginosa,* 10^6 to 10^8 for *P. maltophilia,* 10^4 to 10^7 for *S. aureus,* 10^4 to 10^6 for *S. epidermidis* and 10^4 to 10^7 for *C. albicans* in the majority of specimens. Significant differences were observed in the time periods during which the pathogens persisted in the patients. Maximum persistence was observed for *P. aeruginosa. P. maltophilia* and *A. fumigatus* had about similar persistence rates, which were lower than those for *P. aeruginosa* but above those for *S. aureus* and *H. influenzae. S. epidermidis* was eliminated within shorter periods than *S. aureus. C. albicans,* although the second most frequent microorganism identified, showed a very low persistence rate. The microbiological analysis confirms results from other research centers (high incidence of *P. aeruginosa),* but reveals significant regional differences as well (*Pseudomonas cepacia* not detectable, higher incidence of *P. maltophilia* and *C. albicans*). This underlines the necessity for detailed qualitative and quantitative microbiological analysis of sputa from cystic fibrosis patients as a prerequisite for rational analysis of etiological, epidemiological and therapeutical aspects of cystic fibrosis.

Zusammenfassung: *Quantitative und qualitative Analyse von Sputa von 102 Patienten mit zystischer Fibrose.* In Sputumproben von 102 Mukoviszidosepatienten konnten innerhalb von 22 Monaten insgesamt 20 verschiedene Mikroorganismenarten nachgewiesen werden. Ihr Auftreten verteilt sich wie folgt (in % der Patienten): *Pseudomonas aeruginosa* 83,4%, *Candida albicans* 29,4%, *Staphylococcus aureus* 24,5%, *Staphylococcus epidermidis* 11.8%, *Haemophilus influenzae* 11,8%, *Streptococcus pneumoniae* 6,9%, *Pseudomonas maltophilia* 6,8%, *Aspergillus fumigatus* 5,9%. Alle übrigen Spezies kamen in weniger als 5% der Patienten vor. *Ps. aeruginosa* trat überwiegend in mehreren Typen pro Patient auf (bis zu 6). Quantitative Unterschiede zwischen den Spezies wurden erkennbar: pro Gramm Sputum lagen die Koloniebildnerzahlen zwischen folgenden Grenzwerten: *Ps. aeruginosa* 10^7–10^9, *Ps. maltophilia* 10^6–10^8, *S. aureus* 10^4–10^7, *S. epidermidis* 10^4–10^6, *Candida albicans* 10^4–10^7. Der kontinuierliche Nachweis für einzelne Spezies und Typen im individuellen Patienten nahm in folgender Reihenfolge ab: *Ps. aeruginosa, Ps. maltophilia, Aspergillus fumigatus, S. aureus, H. influenzae, S. epidermidis, Candida albicans.* Diese Resultate deuten auf bemerkenswerte Unterschiede in der mikrobiologischen Konstellation verschiedener Zentren und Regionen bei Mukoviszidosepatienten hin und unterstreichen die Notwendigkeit für die Erhebung lokaler und individueller Daten als Grundlage für die Analytik von Ätiologie und Epidemiologie sowie rationale Chemotherapie bei Mukoviszidose.

Introduction

Microorganisms are present in the majority of sputa of patients suffering from cystic fibrosis (1). Respiratory infections play a major role in the reduction of pulmonary function. For this reason, various efforts have been made to reduce or eliminate the microorganisms in the lungs of cystic fibrosis patients. The major therapeutic step has proven to be antimicrobial chemotherapy. In order to achieve optimal efficacy, antimicrobial substances must be selected according to their activity against the microorganisms identified in sputum specimens of the patients for whom antimicrobial chemotherapy has been initiated. Although *Pseudomonas aeruginosa* is identified in the sputa of the majority of cystic fibrosis patients, other microbial species with possible pathogenetic relevance may be present as well (2). Antibiotic susceptibility for most of them cannot be predicted with adequate reliability to en-

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Infection 15 (1987) Nr. 4

sure the selection of an optimal antibiotic. Therefore, susceptibility tests must be performed at least for the gram-negative rods and the staphylococci cultured from the sputum specimens. Elimination of the causative organism from the site of infection (particularly *P. aeruginosa*) cannot be applied as a microbiological criterion of successful treatment in cystic fibrosis as with other infections, despite the fact that an antibiotic concentration high enough to be bactericidal may be reached (3). For this reason, follow-ups must include: determination of the number of organisms of each type, strain identification by appropriate epidemiologic markers (4) and the corresponding minimal inhibitory concentrations against the antibiotic(s) used for therapy.

The data presented here were obtained from extensive microbiological analysis of the sputa from 102 cystic fibrosis patients over a 22 month period. The aim of this study is to contribute to a better understanding of the microbiological aspects of the nature of cystic fibrosis.

Materials and Methods

Patients: Sputum specimens of 102 patients were analysed. 49 patients were male and 53 female. The mean age of the male patients was 15.9 ± 5.5 years (range 4 to 31 years), of the female patients 16.2 ± 5.8 years (range 4 to 30 years). Cystic fibrosis had been diagnosed by clinical, as well as laboratory signs and symptoms.

Specimen processing: A fraction of the sputum specimens (about 1 g) was liquefied according to the procedure described by *Hammerschlag* (5) and *Wong* (6).

Colony counts, selective media, identification procedures: Liquefied sputum preparations were diluted in saline in consecutive steps at a rate of 1:10 up to $1:10^6$. 50 µl of each dilution were streaked onto Tryptic Soy Agar (Difco, Michigan, USA) and Mac Conkey Agar (Oxoid, Basingstoke, England). 100 µl were streaked onto Mannitol Salt Agar (Oxoid, Basingstoke, England) and Mueller-Hinton-Agar (Difco, Michigan, USA) with polymyxin (both 16 µg/ml and 128 µg/ml). In order to facilitate the identification of non-*P. aeruginosa* species in the presence of large quantities of *P. aeruginosa* (see *Bauernfeind, Rotter* this issue [7]), a 25 µl spot of pyocins was pipetted onto the plates for the selective inhibition of *P. aeruginosa*. Unliquefied sputa were streaked onto Chocolate Agar (Pepton-Agar, Difco) containing 8% human erythrocytes and 2% horse serum.

Plates were incubated at 35° C and 27° C and inspected on three consecutive days. Colonies dissimilar in their macroscopic appearance were counted separately and subcultured.

API 20E (enterobacteriaceae; API, Nürtingen, FRG) or API 2ONE (non-enteric) were used in the identification of gram-negatives. When necessary, additional markers for identification were tested (8, 9).

Gram-positive cocci were identified by standard procedures (10).

Candida were speciated according to API 20C AUX and Aspergillus by macroscopic and microscopic criteria (11).

Serotyping: Antisera for the *P. aeruginosa* O-serotypes were obtained from rabbits immunized with a suspension of the O-serotype strains exposed to 100° C for 2 h. Slide-agglutination was done with strains pregrown on Tryptic Soy Agar (Difco, Michigan, USA). The density of the suspensions was adjusted to 10^9 cfus/ml (Corning Colorimeter 252, Corning Medical, Halstead, England).

Pyocintyping: The procedure described by *Fyfe* (12) was applied.

Results

Microorganisms in sputa from patients with cystic fibrosis

20 microbial species were identified in the 472 sputa specimens from 102 patients (Table 1), the majority being bacteria. Eleven gram-negative and seven gram-positive species were found. Two fungal species, namely *Candida albicans* and *Aspergillus fumigatus,* were detected as well. As expected, one species – *P. aeruginosa* – was most frequent both in the sputum specimens (88.3%) and amongst the patients (84.3%). Only 7 other of the 20 species were found at an incidence above 1% in sputa or above 5% in patients, namely *S. aureus* (in 11.7% of the sputa, and 24.5% of the patients), *C. albicans* (9.8%, 29.4%), *P. maltophilia* (7.3%, 6.8%), *S. epidermidis* (4.0%, 11.8%), *H. influenzae* (3.2%, 11.8%), *S. pneumoniae* (1.9%, 6.9%) and *A. fumigatus* (2.0%, 5.9%).

Microorganisms in individual sputum specimens

In the majority of the specimens, *P. aeruginosa* was present either as the only organism (61,2%) or associated with one (21,7%) or two (5,5%) other species (Table 2). *P. aeruginosa* was detected with equal frequency in conjunction with either *S. aureus* or *C. albicans* (Table 2). In ad-

Table 1: Microbial species in 472 sputum specimens of 102 patients with cystic fibrosis.

Bacteria	Distribution of species In sputa n	In sputa %	In patients n	In patients %
Gram-negative				
Pseudomonas aeruginosa	418	88.3	86	84.3
Pseudomonas maltophilia	34	7.3	7	6.8
Haemophilus influenzae	15	3.2	12	11.8
Escherichia coli	4	0.9	4	3.9
Proteus mirabilis	4	0.9	3	2.9
Citrobacter freundii	3	0.6	3	2.9
Klebsiella pneumoniae	2	0.4	2	2.0
Salmonella typhimurium	2	0.4	2	2.0
Enterobacter cloacae	1	0.2	1	0.9
Providentia rettgeri	1	0.2	1	0.9
Morganella morganii	1	0.2	1	0.9
Gram-positive				
Staphylococcus aureus	55	11.7	25	24.5
Staphylococcus epidermidis	19	4.0	12	11.8
Streptococcus pneumoniae	9	1.9	7	6.9
Enterococcus faecalis	2	0.4	2	2.0
Streptococcus warneri	2	0.4	1	0.9
Streptococcus saprophyticus	1	0.2	1	0.9
Aerococcus	1	0.2	1	0.9
Fungi				
Candida albicans	46	9.8	30	29.4
Aspergillus fumigatus	9	1.9	6	5.9

Table 2: Pattern of combinations of microorganisms of 472 sputum specimens from patients with cystic fibrosis.

Specimens *with Pseudomonas aeruginosa*			
Number of species per specimen		Number of specimens	% of specimens
1	*Pseudomonas aeruginosa*	289	61.2
Total		289	61.2
2	*Pseudomonas aeruginosa* + *Staphylococcus aureus*	27	5.7
	Pseudomonas aeruginosa + *Candida albicans*	27	5.7
	Pseudomonas aeruginosa + *Staphylococcus epidermidis*	14	3.0
	Pseudomonas aeruginosa + *Pseudomonas maltophilia*	13	2.8
	Pseudomonas aeruginosa + *Haemophilus influenzae*	9	1.9
	Pseudomonas aeruginosa + *Proteus mirabilis*	3	0.6
	Pseudomonas aeruginosa + *Salmonella typhimurium*	2	0.4
	Pseudomonas aeruginosa + *S. warneri*	2	0.4
	Pseudomonas aeruginosa + other species	6	1.3
Total		103	21.7
3	*Pseudomonadaceae* + *Staphylococcus aureus* + *Pseudomonas maltophilia*	6	1.3
	Pseudomonadaceae + *Candida albicans* + *Staphylococcus aureus*	2	0.4
	Pseudomonadaceae + *Candida albicans* + *Staphylococcus epidermidis*	2	0.4
	Pseudomonadaceae + *Candida albicans* + *Streptococcus pneumoniae*	2	0.4
	Pseudomonadaceae + two other species	14	2.9
	triple combinations each appearing only once		
Total		26	5.5

Specimens *without Pseudomonas aeruginosa*			
Number of species per specimen		Number of specimens	% of specimens
1	*Pseudomonas maltophilia*	10	2.1
	Staphylococcus aureus	8	1.7
	Aspergillus fumigatus	3	0.6
	Candida albicans	2	0.4
	Escherichia coli	1	0.2
	Morganella morganii	1	0.2
Total		25	5.3
2	*Staphylococcus aureus* + *Aspergillus fumigatus*	3	0.6
	Staphylococcus aureus + *Candida albicans*	2	0.4
	Pseudomonas maltophilia + *Candida albicans*	2	0.4
	other double combinations each appearing only once, including *Staphylococcus aureus, Pseudomonas maltophilia Haemophilus influenzae, Streptococcus pneumoniae, Citrobacter freundii, Escherichia coli, Candida albicans, Aspergillus fumigatus*	8	1.7
Total		15	3.3
3	*Pseudomonas maltophilia* + *S. saprophyticus* + *Candida albicans*	1	0.2
Total		1	0.2
	sterile or physiological flora	11	2.3

dition, combinations of *P. aeruginosa* with *S. epidermidis, P. maltophilia* and *H. influenzae* were found at a low rate of incidence, while other species in the same sample were associated only very rarely with *P. aeruginosa.*

Types of Pseudomonas aeruginosa in sputum specimens

Strain identity was established by several criteria: namely the appearance of the colonies on TSA-Agar, O-serotyping and pyocin-typing. Heterogeneity of *P. aeruginosa* according to this typing pattern was observed in many of the specimens (Table 3).

Quantitative microbiological analysis of sputum specimens

The laboratory analysis of sputum specimens included the enumeration of colony-forming microorganisms. The range of cfus was between ≤ 40 and more than 10^9 per ml of sputum (Figure 1). Microbial concentrations were highest for *P. aeruginosa* and lowest for *C. albicans.* The concentrations of the staphylococci were lower than those for both *Pseudomonas* species. However, concentrations up to 10^9 cfu per ml of *S. aureus* were detected. Enterobacteriaceae were detected in the sputa predominantly at concentrations below those for *P. aeruginosa* or *P. maltophilia* in numbers comparable to *C. albicans.* Concentrations below 10^5/ml may be due to contamination of the sputum specimens by gram-negative rods colonizing the oral cavity (Table 4).

Pseudomonas aeruginosa types in sputa of selected patients

In the majority of the sputa from cystic fibrosis patients colonized by *P. aeruginosa,* more than one type thereof was identified (90.2%). Since 72 of the patients (70.6%) were infected by more than one type, heterogeneity was not restricted to some of the patients. Within a two year

Infection 15 (1987) Nr. 4

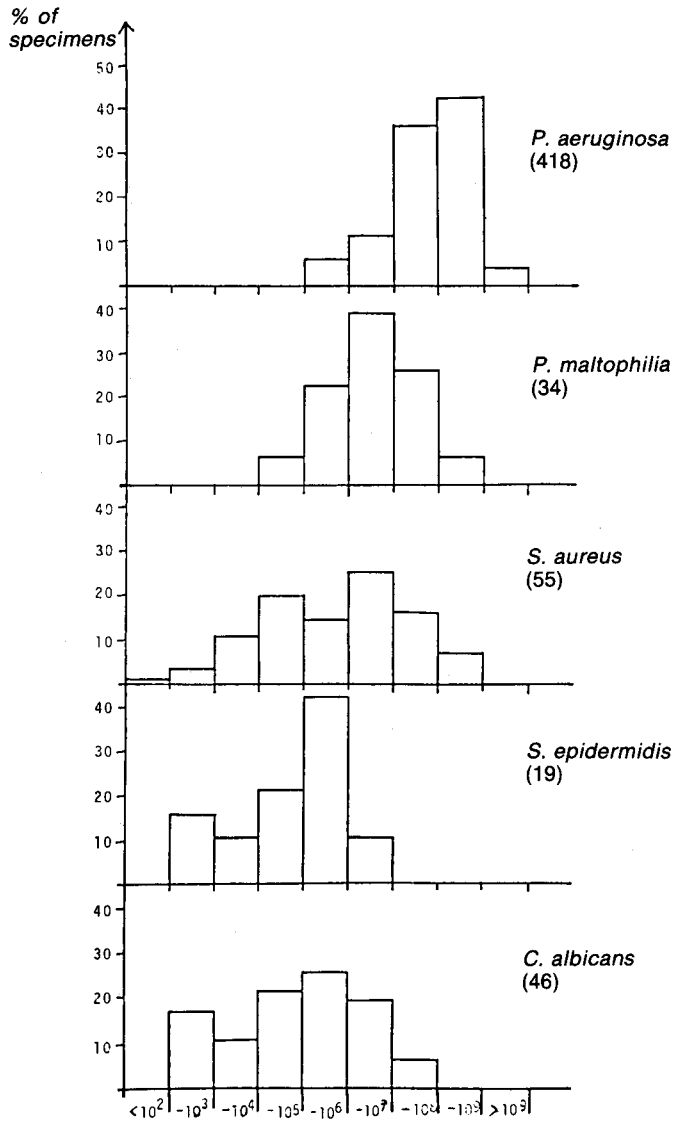


Figure 1: Concentration of microorganisms in sputa of patients with cystic fibrosis (number of specimens).

investigational period, as many as 19 types were identified in the sputum specimens of individual patients. The time periods over which specific types of *P. aeruginosa* persisted are depicted for several patients in Figures 2–5. These patients were selected in preference to others, because bacteriological data was collected and analysed for them for the longest time period (up to 22 months). Although various types were identified in most of these patients, one strain clearly prevailed in each patient throughout extensive parts of the observation period, i.e. additional types persisted for the most part over shorter periods only. Mucoid and non-mucoid strains identical both in sero- and pyocin-type were, for the most part, present only alternatively. Both types were found to persist in patients for extended time-periods.

Persistence of various microbial species in cystic fibrosis patients

The overall frequency of species in patients or specimens (Table 1) does not indicate how long specific types of bacteria persisted in individual patients. Table 5 therefore, compares various species or types, as to the frequency in which they were found in consecutive specimens of the same patient. The sum of isolates found in series, divided by the total number of isolates, is taken as a measure for the persistence of microorganisms in the sputa of the patients (persistence rate).

Table 3: Types* of *Pseudomonas aeruginosa* in sputa of cystic fibrosis patients.

Number of types per sputum	1	2	3	4	5	6
Percentage of specimens	24.7	55.7	17.8	1.6	0	0.2

* defined by: pyocin-, sero- and colony-type.

Discussion

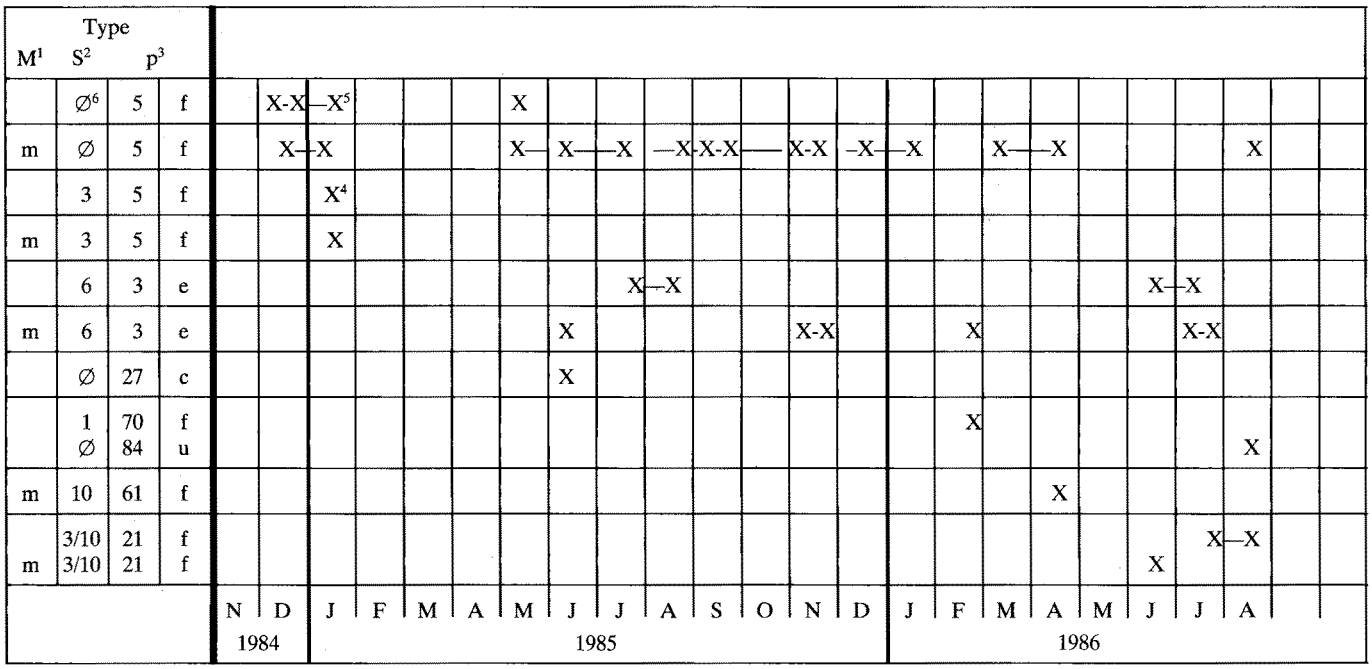
The overall spectrum of microorganisms from 472 sputa of patients with cystic fibrosis (Table 1) confirms the dominant role of *P. aeruginosa,* which was found in 88.3% of all the specimens (81% according to *Thomasson* [4]). Although 19 other microbial species were identified as well, only seven of them were present in more than 1% of the sputa, namely *S. aureus, C. albicans, P. maltophilia, S. epidermidis, H. influenzae, S. pneumoniae, A. fumigatus* (sequence in decreasing incidence).

Of the non-aeruginosa *Pseudomonas* species, *P. maltophilia* was the most frequent (7/102 = 6.8%, age 8–24 years) in our patients. Six of the seven patients in whom *P. maltophilia* was identified were male children. This species has been identified only rarely in other studies (13). The majority of our *P. maltophilia* strains were iso-

Table 4: Number of cfus per ml of sputum for Enterobacteriaceae.

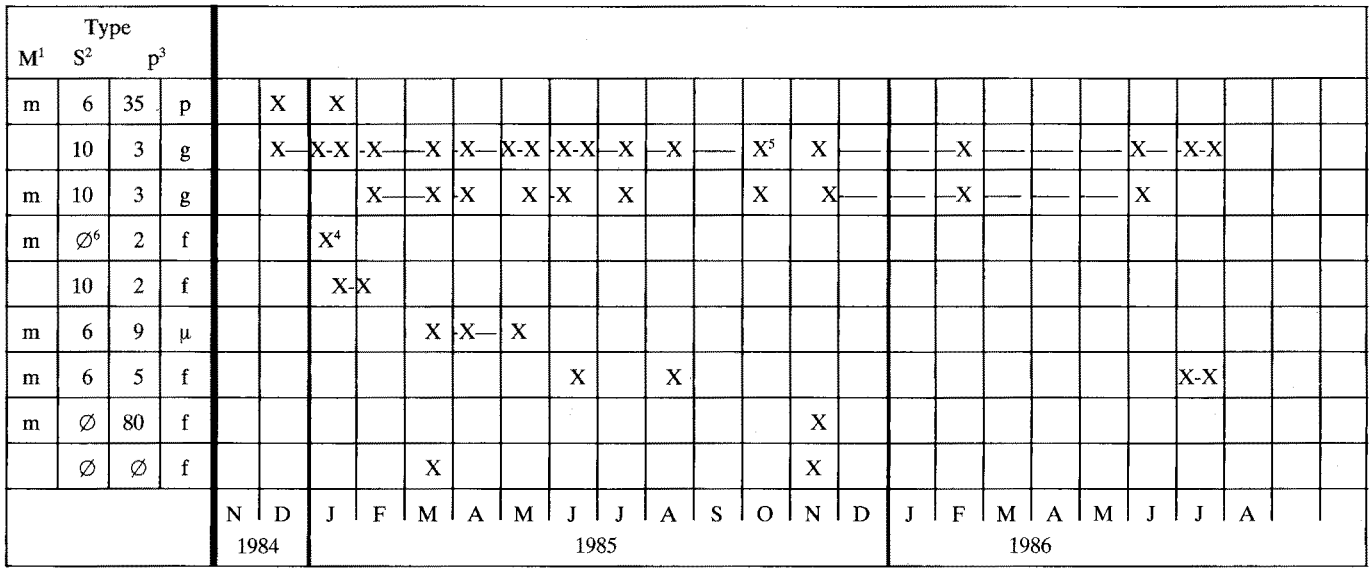
Species (n)	cfus/ml
Escherichia coli (4)	3×10^4 1×10^6 3×10^6 3×10^6
Klebsiella pneumoniae (2)	3×10^4 2×10^5
Enterobacter cloacae (1)	6×10^7
Citrobacter freundii (3)	4×10^3 8×10^5 1×10^7
Proteus mirabilis (4)	3×10^4 1×10^5 4×10^6 6×10^6
Proteus rettgeri (1)	3×10^4
Morganella morganii (1)	1×10^5
Salmonella typhimurium (2)	1×10^5 1×10^6

Figure 2: Pseudomonas aeruginosa-types in cystic fibrosis (patient number 4).



[1] = Mucoid; [2] = O-Serotype; [3] = Pyocin-type (main-, subtype); [4] = type identified only once; [5] = type identified in consecutive samples; [6] = non-typeable.

Figure 3: Pseudomonas aeruginosa-types in cystic fibrosis (patient number 7).



[1] = Mucoid; [2] = O-Serotype; [3] = Pyocin-type (main-, subtype); [4] = type identified only once; [5] = type identified in consecutive samples; [6] = non-typeable.

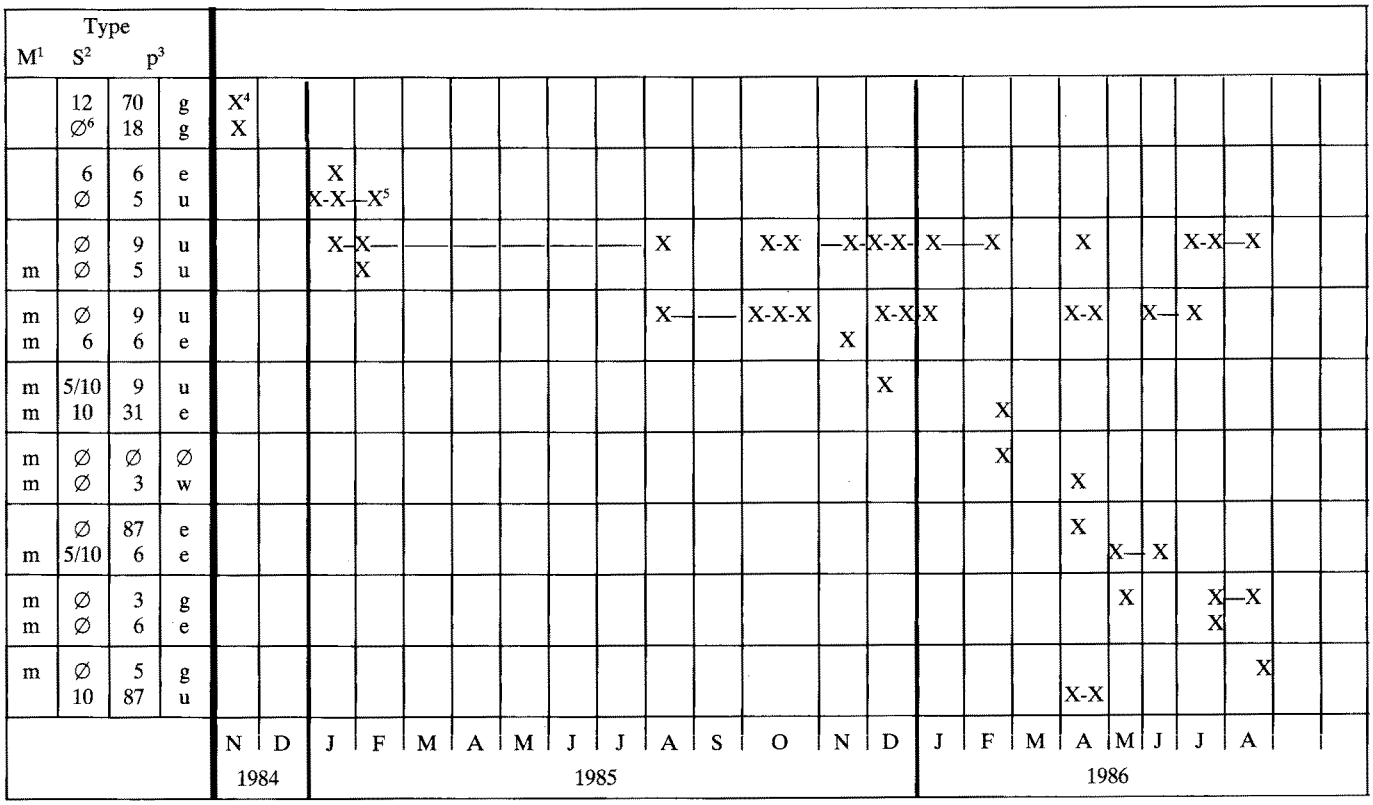
lated from two patients. *P. aeruginosa* was also present concurrently in one of these patients, whereas the other had *P. maltophilia* only. *P. cepacia* was not present in our patients at all. Major differences between CF-centers in incidence of *P. cepacia* were reported (14–17) indicating cross-infection routes of spread, which until recently could not be analysed, as appropriate epidemiological markers were not accessible. Bacteriocin-typing elaborated by *Govan* (18) should facilitate future analysis.

H. influenzae was approximately as frequent in our patients (11.8%) as in Canadian studies (5 to 15% [19]). Colonies of *H. influenzae* may be masked on unselective media in the presence of high concentrations of *P. aeruginosa*. Identification is significantly improved when media with selective inhibition of the growth of *P. aeruginosa* are used (see *Bauernfeind, Rotter,* this issue [7]).

To date, little attention has been paid to *C. albicans,* the second most frequent microorganism in our patients

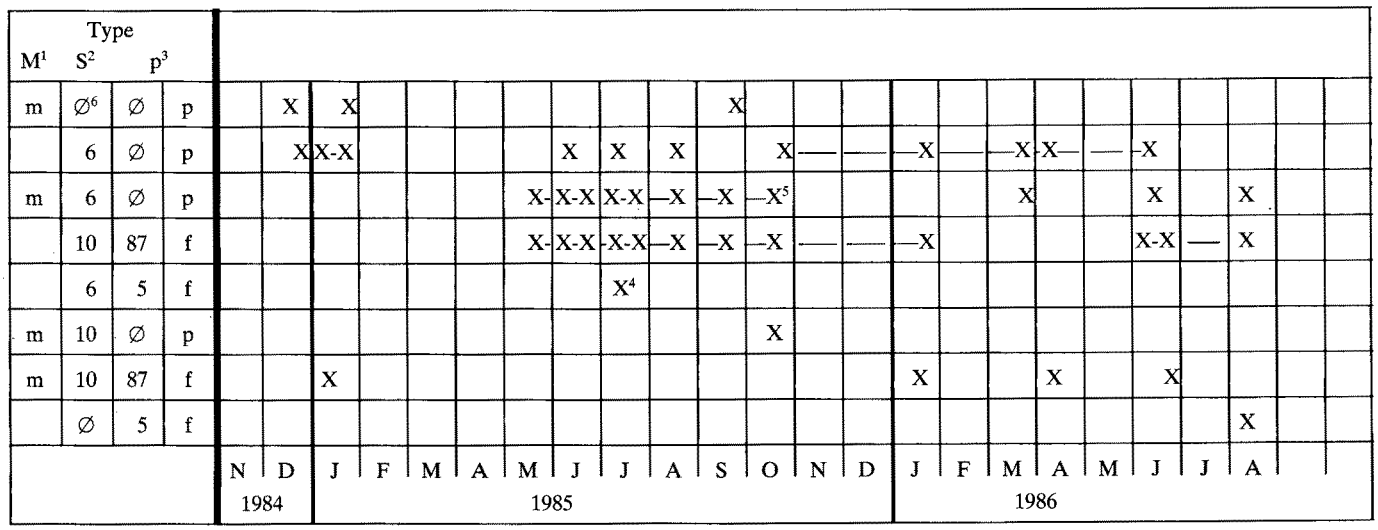
Infection 15 (1987) Nr. 4

Figure 4: Pseudomonas aeruginosa-types in cystic fibrosis (patient number 1).



[1] = Mucoid; [2] = O-Serotype; [3] = pyocin-type (main-, subtype); [4] = type identified only once; [5] = type identified in consecutive samples; [6] = non-typeable.

Figure 5: Pseudomonas aeruginosa-types in cystic fibrosis (patient number 8).



[1] = Mucoid; [2] = O-Serotype; [3] = Pyocin-type (main-, subtype); [4] = type identified only once; [5] = type identified in consecutive samples; [6] = non-typeable.

(29.4%). This high incidence may be due to the extensive prophylactic use of antistaphylococcal agents, i.e. oral cephalosporins or co-trimoxazole. *C. albicans* persisted in most of our patients only for short periods of time (Table 5). Even large numbers of this microorganism (up to 10^8 cfus/ml) disappear from the sputa within short time periods without antifungal therapy.

A. fumigatus was detected in six (aged 9–24 years) of our 102 patients (5.9%), two male and four female children. *Schonheyder* observed 156 patients over a period of 22 months at monthly intervals in 1982 and 1983 in Denmark. He reported a prevalence rate for *A. fumigatus* of

Table 5: Persistence of various species in sputa of cystic fibrosis patients.

Species	Detectable in consecutive specimens in series of																			No of serial isolates total no of isolates
	2	3	4	5	6	7	8	9	10	11	12	15	17	19	21	22	25	26		
Pseudomonas aeruginosa	17	9	6	5	5	2	2	3	1	1	1	2	1	1	1	1	1	1		390/418 = 93.3%
Aspergillus fumigatus	1		1																	6/ 9 = 66.7%
Pseudomonas maltophilia	3	1	1				1													21/ 34 = 61.8%
Staphylococcus aureus	6	2	2																	26/ 55 = 47.3%
Haemophilus influenzae	3																			6/ 15 = 40.0%
Staphylococcus epidermidis	3																			6/ 19 = 31.6%
Enterobacteriaceae	1	1																		5/ 19 = 26.3%
Candida albicans	3																			6/ 48 = 12.5%
Streptococcus pneumoniae																				0/ 8 = 0 %

40.4% (20). An even higher incidence (57%) was reported by *Nelson* in 1979 (21), while in other studies the incidence of *A. fumigatus* was much lower (22). These data indicate regional differences which may be partly due to cross-colonizations in specific centers. It appears that *A. fumigatus* may contribute to pulmonary damage in patients with chronic infection due to *P. aeruginosa* (20–25).
S. aureus was prevalent in about 90% (26) of CF patients prior to the antistaphylococcal therapy (1). In the 1970s, between 14 and 39% of CF patients were reported to have *S. aureus* in their sputa (for reviews, see *Govan, Høiby,* 27, 28). These numbers approximate our findings (29.4%).
Most of the reports do not contain data about *S. epidermidis,* detected in 11.8% of our patients. The speciation of coagulase-negative staphylococci is obligatory to define their clinical role and the contradictory aspects of antistaphylococcal therapy and prophylaxis (27). It was evident that *S. epidermidis* occurred in our patients, yet only for shorter periods of time than *S. aureus* (Table 4).
In two thirds of the sputa, only *P. aeruginosa* was identified (Table 2), while monoinfections with other species were observed in 5.4% of the sputa altogether (*P. maltophilia* in 2.2% and *S. aureus* in 1.7%, others 1.5%). Thus, an evaluation of the pathogenetic significance of the majority of non-*P. aeruginosa*-species in chronic cystic fibrosis is complicated for the most part by the presence of *P. aeruginosa,* whose clinical significance in cystic fibrosis is established in 89% of the mixed infections. In mixed infections, 19 of the 20 species identified were involved mainly in combinations including *P. aeruginosa* (*M. morganii* was found only once and in a pure culture). There was, however, one exception: *A. fumigatus* was associated significantly more often with species other than *P. aeruginosa.*
The mechanisms for mutual exclusion still have to be explored. Antimicrobially-active products may suppress the colonization of the respiratory tract in cystic fibrosis patients by other strains susceptible to these compounds. Monoinfection by *P. aeruginosa* does not imply infection by a single strain. In the majority of specimens and patients, more than one strain was identified according to the three epidemiological markers (colony-, sero-, pyocin type) selected for this study. These three particular markers were selected over others (e.g. lysotype) because of easy accessibility (colony appearance), epidemiological and possible prophylactic relevance (O-serotype) and a suspected role of antibiosis due to pyocin production in mixed infections, e.g. of various pyocin-types of *P. aeruginosa.* Epidemiological typing of *P. aeruginosa* allows for the analysis of persistence of change in specific types. Although more than one type was identified in most of our patients, one type prevailed throughout extended periods. The major strains were detectable in their mucoid type (Figure 2) or both mucoid and non-mucoid types were present simultaneously (Figure 3) or alternately (Figure 4). In rare cases, two different types coexisted (Figure 5).
When sputum specimens were classified according to the concentration of colony-forming organisms, the largest classes were those with 10^8 cfus/g for *P. aeruginosa,* 10^6 cfus/g for *P. maltophilia* and *S. aureus,* 10^5 cfus/g for *S. epidermidis* and *C. albicans.* Sputa with more than 10^9 cfus/g were found for *P. aeruginosa* only (Figure 1).
The eradication of *P. aeruginosa* from the lungs of cystic fibrosis patients is, therefore, severely impaired by its high concentration (for other reasons, see *Ramphal, Govan,* this issue [29, 30]). Killing rates by antibiotics are lower for high inocula, especially when degradative enzymes such as β-lactamases are produced. Furthermore, the risk that mutants resistant to chemotherapeutics (e.g. 4-quinolones) are selected, increases with the number of organisms present. Microorganisms other than *P. aeruginosa* were found in sputa usually at concentrations 100 times (*P. maltophilia, S. aureus*) or 1000 times (*S. epidermidis, C. albicans*) lower (see Figure 1) than for *P. aeruginosa.* Thus, complete elimination of these microorganisms from the sputa is observed more often and also accomplished within a shorter period of time (Table 4). Evaluation of the clinical relevance of microorganisms in cystic fibrosis has to be characterized not only by their frequency in sputa or patients (Table 1) but by their persistence in individual patients as well (Table 4). The comparison of these microbiological parameters reveals that *C. albicans,* although being the second most prevalent microorganism in our patients, has a very low persistence rate of 12.5%. In spite of concentrations of *C. albicans* up to 10^8 cfus/ml, these organisms appear to be eliminated

Infection 15 (1987) Nr. 4

from the sputa without chemotherapy within short periods of time. *S. pneumoniae* was never found in two consecutive specimens from the same patient, while the persistence rate of *H. influenzae* was 40%. *S. epidermidis* clearly was less persistent than *S. aureus,* which was detectable in as many as five specimens taken in series.
Both *P. maltophilia* and *A. fumigatus* were found to be the most persistent species next to *P. aeruginosa.*
Assuming adequate antibacterial chemotherapy in all the patients, eradication of the majority of the pathogens colonizing cystic fibrosis patients is not a major problem (see Table 5). On the other hand, some *(S. aureus)* are more difficult to eliminate and in the case of *P. aeruginosa* and *P. maltophilia* can be eliminated rarely and for short periods of time only. In these cases, only extensive bacteriological analysis, including colony-counts, epidemiological typing and determination of MICs of single antibiotics or *in vitro* evaluation of combinations (checkerboard titrations, killing curves) can generate etiological, epidemiological and therapeutically meaningful information. Purely qualitative diagnostic procedures are useless and misleading. The clinical significance of less frequent species must be elucidated by additional data on antibodies of IgG or IgA class both in sera and in sputum (secretory IgA) of the patients.

Acknowledgement

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