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SPUTUM BACTERIOLOGY AND ITS ANTIBIOTIC SUSCEPTIBILITIES IN TURKISH CYSTIC FIBROSIS PATIENTS*

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To identify lower respiratory tract pathogens and their in-vitro antibiotic susceptibilities in Turkish cystic fibrosis (CF) patients, a total of 383 sputum cultures were evaluated from 45 CF children in 168 symptomatic and 215 control periods over 25 months. Microorganisms were isolated in 252 of the cultures. The isolation rate was 82 percent for symptomatic periods and 53 percent for control periods. The most common microorganism was P. aeruginosa in the symptomatic period and S. aureus in the control period. Other microbiological species included E. coli, H. influenzae, K. pneumoniae, S. epidermidis, β-hemolytic streptococcus, H. parainfluenzae, K. oxytoca, E. aerogenes and E. aglomerans. P. cepacia was not found. In 20 cultures more than one microorganism was isolated at the same time. In in-vitro conditions, high susceptibility rates were detected to amikacin, ciprofloxacin and ceftazidim for P. aeruginosa; cefuroxime, ceftriaxone, amikacin, cephalothin, chloramphenicol and erythromycin for S. aureus; amikacin and ceftriaxone for E. coli; ampicillin-sulbactam, amoxicillin-clavulanate, cefuroxime, ceftazidime, ceftriaxone and aztreonam for H. influeanzae; and aztreonam and amikacin for K. pneumoniae. Lower respiratory tract pathogens and their antibiotic susceptibilities in Turkish CF children were not significantly different from those indicated previously in the literature. *Key words: cystic fibrosis, sputum bacteriology, antibiotic susceptibility.*

Chronic pulmonary disease and its complications of recurrent pulmonary infections determine the quality of life and survival of cystic fibrosis (CF) patients. Since the early description of CF, various efforts have been made to identify and eliminate the microorganisms in the lungs of CF patients. The aim of this study was to detect microbiological flora in the respiratory system and antibiotic susceptibility in CF patients in our country.

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Material and Methods

The study group comprised 45 CF children, 22 female and 23 male, who were being followed at Hacettepe Children's Hospital between January 1991 and February 1993. Their ages ranged from two months to 16 years. They were diagnosed with CF by obtaining at least two sweat chloride values higher than 60 mEq/L with the method of pilocarpin iontopheresis[1], and typical clinical and laboratory findings. Sputum cultures were taken from the patients at routine monthly check-ups and during lower respiratory system infections. Sputum brought to the oropharynx by coughing in children, and by stimulating the coughing reflex in infants, was removed with cotton-tipped swabs. Samples were immediately sent to the laboratory in Stuarts transport medium and inoculated onto appropriate media by the same microbiologist who was working with the subject. All the samples were streaked directly onto two five-percent sheep blood agar (Oxoid); one eosin-methylene blue agar (EMB) (Oxoid); one chocolate agar with 10 percent horse blood containing 300 µg/ml bacitracin. 5 µg/ml vancomycine, 1 µg/ml clindamycine (CHOC-BVC) which was selective for Haemophilus species[2]; one cetrimide agar for Pseudomonas aeruginosa; and one OFPBL (oxidation fermentation medium supplemented with 300,000 U/L polymyxcin B sulfate, 200 U/L bacitrain and lactose) for Pseudomonas cepacia[3]. One sheep blood agar and CHOC-BVC were incubated in five to 10 percent CO_2 at 35 °C for 18-24 hours. One sheep blood agar, EMB agar, cetrimide agar and OFPBL agar were incubated in normal atmosphere at 35 °C for 18-24 hours. If there was no bacterial growth on CHOC-BVC agar at the end of 24 hours, the plates were reincubated for an additional 24 hours. Standard microbiological techniques were used to identify the microorganisms[4]. Antibiotic susceptibilities of detected microorganisms were evaluated by the Bauer-Kirby disk diffusion method according to NCCLS standards[5].

Antibiotic disks used for gram-negative microorganisms included ampicillin-sulbactam (10/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), amikacin (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), aztreonam (30 µg) and ciprofloxacin (5 µg). Antibiotics disks used for gram-positive microorganisms included ampicillin (10 µg), ampicillin-sulbactam (10/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), amikacin (30 µg), erythromycin (15 µg), cephalothin (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg) and chloramphenicol (30 µg). Antibiotic disks used for Haemophilus species were ampicillin (10 µg), ampicillin-sulbactam (10/10 µg), amoxicillin-clavulanate (20-10 µg), aztreonam (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ceftazidime (30 µg), cefuroxime (30 µg) and chloramphenicol (30 µg).

Results

During the study period a total of 383 cultures were taken from CF children (215 in control and 168 was in symptomatic periods), and microorganisms were isolated in 252 of them (114 in control, 138 in symptomatic periods). The isolation rate was 82 percent during symptomatic periods and 53 percent for control periods.

The most frequently isolated microorganism was Pseudomonas aeruginosa (23%) in symptomatic periods and Staphylococcus aureus (19%) in control periods. All cultivated microbiological species are shown in Table I. In 20 sputum cultures, more than one microorganism was isolated at the same time-frequently in the symptomatic period (Table II). The in-vitro antibiotic susceptibilities of the main isolated microorganisms are shown in Table III. In in-vitro conditions, high susceptibility rates were detected to amikacin, ciprofloxacin and ceftazidime for P. aeruginosa; cefuroxime, ceftriaxone, amikacin, cephalothin, chloramphenicol and erythromycin for S. aureus; amikacin and ceftriaxone for E. coli; ampicillin-sulbactam, amoxicillin-clavulanate, cefuroxime, ceftazidime, ceftriaxone and aztreonam for H. influeanzae; and aztreonam and amikacin for K. pneumoniae.

Table I: Microbial Species in 252 Sputum Specimens of 46 CF Children and Distribution According to Age as well as Symptomatic and Control Periods

P. aeruginosa	S	6	6	9	7	4	32 (23)
	C	–	3	3	8	5	19 (17)
S. aureus	S	2	2	7	2	4	17 (12)
	C	–	2	7	2	11	22 (19)
E. coli	S	3	1	11	1	–	16 (12)
	C	4	3	6	1	2	16 (14)
H. influenzae	S	4	2	11	3	2	22 (16)
	K	1	3	–	2	–	6 (5)
K. pnemoniae	S	7	1	2	–	1	11 (8)
	C	7	6	2	1	–	16 (14)
S. pneumoniae	S	3	0	1	4	2	10 (7)
	C	2	2	6	4	–	14 (12)
β Hemolytic streptococcus	S	1	–	8	3	2	14 (10)
	C	–	–	5	2	–	7 (6)
H. parainfluenzae	S	1	1	8	1	2	13 (9)
	C	–	2	2	–	1	5 (4)
S. epidermidis	S	–	–	2	–	–	2 (1.4)
	C	–	1	–	–	–	1 (0.9)
K. oxytoca	S	–	–	–	–	–	–
	C	–	1	–	–	–	1 (0.9)
E. aerogenes	S	–	–	–	1	–	1 (0.7)
	C	–	–	–	–	–	–
E. agglomerans	S	–	–	–	–	–	–
	C	1	–	–	–	–	1 (0.9)
Total	S						138 (100)
	C						144 (100)

S: Symplomatic period C: Control period.

Table II: Pattern of Combination of Microorganisms in 252 Sputum Specimens

Microorganisms	Symptomatic Period	Control Period
H. influenzae + S. aureus	3	–
H. influenzae + H. parainfluenzae	1	–
H. influenzae + B. hemolytic streptococcus	2	–
H. influenzae + S. pneumoniae	2	–
H. influenzae + H. parainfluenzae + β hemolytic streptococcus	1	1
E. coli + H. parainfluenzae	2	1
E. coli + S. pneumoniae	–	1
S. aureus + H. parainfluenzae	–	1
S. aureus + K. oxytoca	–	1
S. aureus + E. coli	1	–
S. aureus + K. pneumoniae	2	–
S. epidermidis + H. parainfluenzae	1	–

Table III: Antibiotic Susceptibility of Microorganisms

	Pseudomonas Aeruginosa (t: 51)				Escherichia Coli (t: 32)				Klebsiella Pneumoniae (t: 27)			
Antibiotic	n	S (%)	S.S (%)	R (%)	n	S (%)	S.S (%)	R (%)	n	S (%)	S.S (%)	R (%)
Ampicillin	–	–	–	–	(32)	–	–	32 (100)	(27)	–	–	27 (100)
Ampicillin-sulbactam	(51)	3 (5)	–	48 (95)	(32)	12 (38)	2 (6)	18 (56)	(27)	6 (22)	6 (22)	15 (56)
TMP-SMX	(51)	5 (10)	1 (2)	45 (88)	(32)	9 (28)	–	23 (72)	(27)	18 (67)	–	9 (33)
Chloramphenicol	(51)	2 (4)	4 (8)	45 (88)	(15)	8 (53)	–	7 (47)	(10)	3 (30)	–	7 (70)
Amikacin	(51)	51 (100)	–	–	(32)	30 (94)	2 (6)	–	(27)	23 (85)	3 (11)	1 (4)
Cefuroxime	(28)	–	3 (11)	25 (89)	(32)	18 (56)	6 (19)	8 (25)	(27)	14 (52)	9 (33)	4 (15)
Cephalothin	–	–	–	–	(18)	5 (28)	5 (28)	8 (44)	(27)	3 (11)	3 (11)	21 (78)
Cefoxitin	(7)	–	–	7 (100)	(–)	–	–	–	(–)	–	–	–
Ceftazidime	(51)	48 (94)	3 (6)	–	(9)	4 (44)	–	5 (56)	(11)	–	–	11 (100)
Ceftriaxone	(51)	11 (22)	18 (33)	23 (45)	(32)	29 (91)	3 (9)	–	(27)	18 (67)	7 (26)	2 (7)
Aztreonam	(20)	11 (55)	4 (20)	5 (25)	(32)	26 (81)	–	6 (19)	(27)	25 (93)	2 (7)	–
Ciprofloxacin	(6)	6 (100)	–	–	(–)	–	–	–	(–)	–	–	–

	Staphylococcus aureus (t: 39)			
Antibiotic	n	S (%)	S.S (%)	R (%)
Ampicillin	(39)	2 (5)	–	37 (95)
Ampicillin-sulbactam	(39)	28 (72)	6 (15)	5 (13)
TMP-SMX	(39)	26 (67)	1 (2)	12 (31)
Chloramphenicol	(27)	23 (85)	–	4 (15)
Amikasin	(39)	34 (87)	3 (8)	2 (5)
Erythromycin	(39)	33 (85)	–	6 (15)
Cephalotin	(39)	33 (65)	1 (2)	5 (13)
Cefuroxime	(4)	4 (100)	–	–
Ceftriaxone	(39)	35 (90)	3 (8)	1 (2)

	Staphylococcus aureus (t: 39)			
Antibiotic	n	S (%)	S.S (%)	R (%)
Ampicillin	(28)	13 (46)	8 (29)	7 (25)
Ampicillin-sulbactam	(28)	28 (100)	–	–
Amoxcillin-clavulanate	(28)	28 (100)	–	–
TM-SMX	(28)	21 (75)	4 (14)	3 (11)
Chloramphenicol	(20)	17 (85)	–	–
Cefuroxime	(28)	28 (100)	–	–
Ceftazidime	(17)	17 (100)	–	–
Ceftriaxone	(28)	28 (100)	–	–
Aztreonam	(28)	28 (100)	–	–

S: Sensitive
S S. Semi-sensitive
R: Resistant

t: Total number of microorganisms isolated from sputum cultures
n: Number of cultures to which antibiotic was applied.
TM-SMX: Trimethoprim-sulfamethoxazole

Discussion

Despite the importance of evaluating the sputum cultures of CF patients, it is often difficult to obtain these cultures in infants and young children. The most appropriate methods for obtaining sputum samples are probably by the transtracheal approach or broncho-alveolar lavage, but it is quite difficult to use these routinely. As shown in previous comparative studies, samples taken from the oropharynx in CF patients give information about lower respiratory tract flora, although this method is less valid for some microorganisms in the normal flora of the oropharynx[6,7].

Pseudomonas aeruginosa was the most common of the isolated microorganisms found during symptomatic periods. According to studies from various countries, there has been a gradual increase in the isolation of P. aeruginosa and the other pseudomonas species over the years[8,9]. The cause of this increases could be linked to patients' common activities, such as summer camps, as well as to the use of selective media for isolation[10,11]. In our study we did not detect P. cepacia in any culture despite selective media. The fact that such activities are rare in Turkey may explain the lack of P. cepacia in our CF patients.

Staphylococcus aureus was reported to be the most commonly isolated microorganism in the early years of CF. In the 1962 study by Iacoca et al.[6] in which they evaluated the sputum cultures of 34 CF children. S. aureus was isolated in 85 percent of cultures. On the other hand, in the study by Mearns et al.[12] it was shown that S. aureus infections were decreasing gradually over the years.

Haemophilus influenzae is an important microorganism that is particularly common in CF patients. Corey et al.[9] showed that the role of H. influenzae infections is gradually increasing in CF patients. In the literature, the incidence of H. influenzae infections in CF patients has been described as 10 to 20 percent[6,7,13,14]; the same incidence was noted in our patients. Two H. influenzae strains which were isolated in control periods were serotype b, but no serotype b isolate was detected during smptomatic periods.

It has recently been proposed that E. coli strains forming mucoidal colonies play a role in lower respiratory tract infections of CF patients[15]. In the same study E. coli ranked fourth and third in symptomatic and control periods, respectively. The isolation rate for Klebsiella pneumoniae was eight percent in symptomatic periods, and 14 percent in control periods. In the study by Bauernfeind et al.[14], however, E. coli and K. pneumoniae were isolated in 0.9 and 0.4 percent of cases, respectively. These percentages were much lower than our findings.

Streptococcus pneumoniae was detected in seven percent of cultures taken in symptomatic periods, and 12 percent in control periods. This high rate may be

related to the use of oropharyngeal cultures in the current study. Although S. pneumoniae has not frequently been detected in the suputum cultures of CF patients. It was detected in 11.8 percent of the specimens in our study, similar to the findings of Bauernfeind et al.[14]. Beta-hemolytic streptococcus was also isolated frequently, especially during smyptomatic periods. In this study, although P. aeruginosa, S. aureus, H. influenzae and E. coli infections were seen in all age-groups. K. pneumoniae was generally seen in the first six months of life. Some reports regarding serum antibacterial precipitins have shown that S. aureus precipitins were gradually increasing. P. aeruginosa precipitins which were high in young age-groups were gradually decreasing and H. influenzae precipitins were increasing with age[16]. Mearns et al.[12] reported that P. aeruginosa precipitins were high in all age-groups and S. aureus and H. influenzae precipitins were low in young children but gradually incerased with age.

H. influenzae was observed to be the microorganism most commonly isolated together with other bacteria in the specimens.

The standard regimens of treatment for P. aeruginosa in CF patients have previously consisted of a combination of a antipseudomonal penicillin and an aminoglycoside. However, increasing resistance to antipseudomonal penicillins and adverse drug reactions to aminoglycosides have created the need for other treatment regimens. Ceftazidime or imipenem plus aminoglycosides have been recommended as new antibiotic regimens for P. aeruginosa infections in CF patients[17-24]. Aminoglycosides are known to be very effective in CF patients[25,26], and in our study a high susceptibility to amikacin (100%) was detected in all of the P. aeruginosa isolates in in-vitro conditions. Ciprofloxacin, which has gained importance because of its oral antipseudomonal effect[27,28], was used in only six patients considering their young age, and the P. aeruginosa strains isolated from these patients were found to be susceptible to ciprofloxacin (100%). In-vitro response to ceftazidime was also high (94%). Aztreonam seems to be a valid antibiotic, as the results showed 55 percent susceptibility and 20 percent moderate susceptibility. In-vitro resistance was detected in most of the P. aeruginosa strains against ceftriaxone, trimethoprim-sulfamethoxazole, chloramphenicol, cefuroxime and cefoxitin.

In S. aureus infections high susceptibility rates were detected to cefuroxime, ceftriaxone, amikacin, chloramphenicol and erythromycin. Ampicillin resistance was found in 95 percent of the S. aureus isolates.

Haemophilus influenzae was found susceptible in in-vitro conditions to ampicillin-sulbactam, amoxicillin-clavulanate, aztreonam, cefuroxime, ceftriaxone and ceftazidime. On the other hand, susceptibility to ampicillin, which is recommended in classical H. influenzae treatment, was determined to be only 46 percent. It

is known that H. influenzae resistance to ampicillin has gradually increased in recent years[29,30]. One might think that the widespread use of ampicillin in our country would play an important role in these results. Trimethoprim-sulfamethoxazole and chloramphenicol were other recommended antibiotics in the classical treatment of H. influenzae infections, and susceptibilities to these two agents were 75 and 85 percent respectively.

In the current study, high in-vitro susceptibility was found to amikacin and aztreonam in K. pneumoniae and to amikacin, aztreonam and ceftriaxone in E. coli.

Although advanced microbiological methods were not used in this study, it is the first on this subject in Turkey, and the results were similar to those obtained from studies in Europe and North America.

P. aeruginosa was the most commonly isolated microorganism, especially in symptomatic periods. S. aureus infections are still important for CF children in our country, and P. cepacia is not. The in-vitro antibiotic susceptibility results obtained in our study were not significantly different from those of similar studies in the literature.

REFERENCES

1. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontopheresis. Pediatrics 1959; 23: 545-549.
2. Chapin KC, Doern GV. Selective media for recovery of Haemophilus influenzae from specimens contaminated with upper respiratory tract microbial flora. J Clin Microbiol 1983; 17: 1163-1165.
3. Welch DF, Muszynski JM, Pai CH, et al. Selective and differential medium for recovery of Pseudomonas cepacia from the respiratory tracts of patients with cystic fibrosis. J Clin Microbiol 1987; 25: 1730-1734.
4. Baron JE, Finegold SM. Bailey and Scott's Diagnostic Microbiology (6th ed). St Louis: CV Mosby Co; 1990.
5. National Committee for Clinical Laboratory Standards: Performance Standards for Antimicrobial Disk Susceptibility Tets (4th ed). Approved standard M2-A4. National Committee for Clinical Laboratory Standards: Villanova; 1990.
6. Iacocca VF, Barbero GJ. Respiratory tract bacteriology in cystic fibrosis. Am J Dis Child 1963; 106: 315-324.
7. Ramsey BW, Wentz KR, Smith AL, et al. Predictive value of oropharyngeal cultures for identifying lower airway bacteria in cystic fibrosis patients. Am Rev Respir Dis 1991; 144: 331-337.
8. Kulczycki LL, Murphy TM, Bellanti JA. Pseudomonas colonization in cystic fibrosis. JAMA 1978; 240: 30-34.
9. Corey M, Allison L, Prober C, Levison H. Sputum bacteriology in patients with cystic fibrosis in a Toronto hospital during 1970-1981. J Infect Dis 1984; 149: 283.
10. Goldmann DA, Klinger JD. Pseudomonas cepacia: biology mechanisms of virulence, epidemiology. J Pediatr 1986; 108: 806-812.
11. Gilligan PH. Microbiology of airway disease in patients with cystic fibrosis. Clin Microbial Rev 1991; 4: 35-51.

12. Mearns MB, Hunt GH, Rushworth R. Bacterial flora of respiratory tract in cystic fibrosis. 1950-1971. Arch Dis Child 1972; 47: 902-907.
13. Rayner RJ, Miller EJ, Ispahani P, Baker M. Haemophilus infection in cystic fibrosis. Arch Dis Child 1990; 65: 255-258.
14. Bauernfeind A, Bertele RM, Harms K, et al. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. Infection 1987; 15: 270-277.
15. Macone AB, Pier GB, Pennington JE, Matthews WJ Jr, Goldmann DA. Mucoid Escherichia coli in cystic fibrosis. N Engl J Med 1981; 304: 1445-1449.
16. May JR, Herrick NC, Thompson D. Bacterial infection in cystic fibrosis. Arch Dis Child 1972; 47: 908-913.
17. Mastella G, Agostini M, Barlocco G, et al. Alternative antibiotics for the treatment of Pseudomonas infections in cytic fibrosis. J Antimicrob Chemother 1983; 12 (Suppl A): 297-311.
18. Dodge J, Zamiri I, Goodchild M, Ingram P. Experience with ceftazidime in cystic fibrosis. J Antimicrob Chemother 1983; 12 (Suppl A): 325-329.
19. Mischler EH. Treatment of pulmonary disease in cytic fibrosis. Semin Resp Med 1985; 6: 271-284.
20. Blumer JL, Stern RC, Yamashita TS, Myers CM, Reed MD. Cephalosporin therapeutics in cystic fibrosis. J Pediatr 1986; 108: 854-860.
21. Krilov LR, Blumer JL, Stern RC, Hartstein AI, Iglewski BN, Goldmann DA. Imipenem/cilasation in acute pulmonary exacerbations of cystic fibrosis. Rev Infec Dis 1985; 7 (Suppl 3): S482-489.
22. British Thoracic Society Research Committee. Ceftazidime compared with gentamicin and carbenicillin in patients with cystic fibrosis, pulmonary pseudomonas infection, and an exacerbation of respiratory symptoms. Thorax 1985; 40: 358-363.
23. Strandvik B. Antibiotic therapy of pulmonary infections in cystic fibrosis. Dosage schedules and duration of treatment. Chest 1988; 94: 1465-1495.
24. Neijens HJ. Strategies and perspectives in treatment of respiratory infections. Acta Pediatr Scand Suppl 1989; 363: 66-73.
25. Wientzen R, Prestidge CB, Kramer RI, McCracken GH, Nelson JD. Acute pulmonary exacerbations in cystic fibrosis. A double-blind trial of tobramycin and placebo therapy. Am J Dis Child 1980; 134: 1134-1138.
26. Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Respir Dis 1985; 132: 761-765.
27. Hodson ME, Roberts CM, Butland RJ, Smith MJ, Batten JC. Oral ciprofloxacin compared with conventional intravenous treatment for Pseudomonas aeruginosa infection in adults with cystic fibrosis. Lancet 1987; i: 235-237.
28. Jensen T, Pedersen SS, Nielsen CH, Hiby N, Koch C. The efficacy and safety of ciprofloxacin and ofloxacin in chronic Pseudomonas aeruginosa infection in cystic fibrosis. J Antimicrob Chemother 1987; 20: 585-594.
29. Howard AJ, Williams HM. The prevalence of antibiotic resistance in Haemophilus influenzae in Ireland. J Antimicrob Chemother 1989; 24: 963-971.
30. Jacobs NF Jr, Jerris RC. Haemophilus influenzae resistance in a community hospital. South Med J 1991; 84: 730-732.