INTERNAL MEDICINE

□ CASE REPORT □

Importance of Controlling Drug-resistant *Pseudomonas aeruginosa* Infection: Experience from Lung Transplantation in a Cystic Fibrosis Case

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Abstract

Cystic fibrosis (CF) is rare in Japan. We encountered a CF case with drug-resistant *Pseudomonas aeruginosa* infection and successfully performed lung transplant from living related donors. A combination of beta-lactams and aminoglycosides for drug-resistant *P. aeruginosa* infection was administered before lung transplantation. Intravenous colistin was also used immediately before and after transplant surgery. Gram staining of respiratory specimens was performed every day after surgery and it was useful in monitoring infection status. Strict monitoring of infections by the Gram staining and culture of respiratory specimens is considered to be effective in preventing lower respiratory infection in lung transplantation.

Key words: cystic fibrosis, *Pseudomonas aeruginosa*, lung transplantation, ventilator-associated tracheobronchitis

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Introduction

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes a chloride channel in epithelial membranes (1). Although CF is a common autosomal recessive disorder in Caucasians, its frequency is very low in Japan (2). As the most common cause of death in CF cases is respiratory failure, lung or heart-lung transplantation is the ultimate treatment option for patients with CF, particularly those with end-stage lung damage. Here, we report for the first time a CF case with drug-resistant *Pseudomonas aeruginosa* infection that was successfully treated by a lung transplant from living related donors in Japan, and describe in detail how we controlled drug-resistant *P. aeruginosa* infection before and after lung transplant surgery.

Case Report

A 24-year-old male with dyspnea on effort, productive cough and fever was diagnosed as having CF with chronic sinusitis at the age of 15 years. He was the second child of non-consanguineous parents and his mother had systemic lupus erythematous and Graves' disease. His past medical history revealed pseudo-Bartter syndrome and cholangiolitis. Missense mutation Q98R was detected in exon 4 and polymorphic 125C was present in exon 1 on CFTR mutation screening, and sweat chloride concentration was 330 mmol/L (normal range: 0-40 mmol/L), thus confirming CF.

On the day before surgery, his vital signs were as follows: body temperature, 37.5°C; heart rate, 124 beats/minute with

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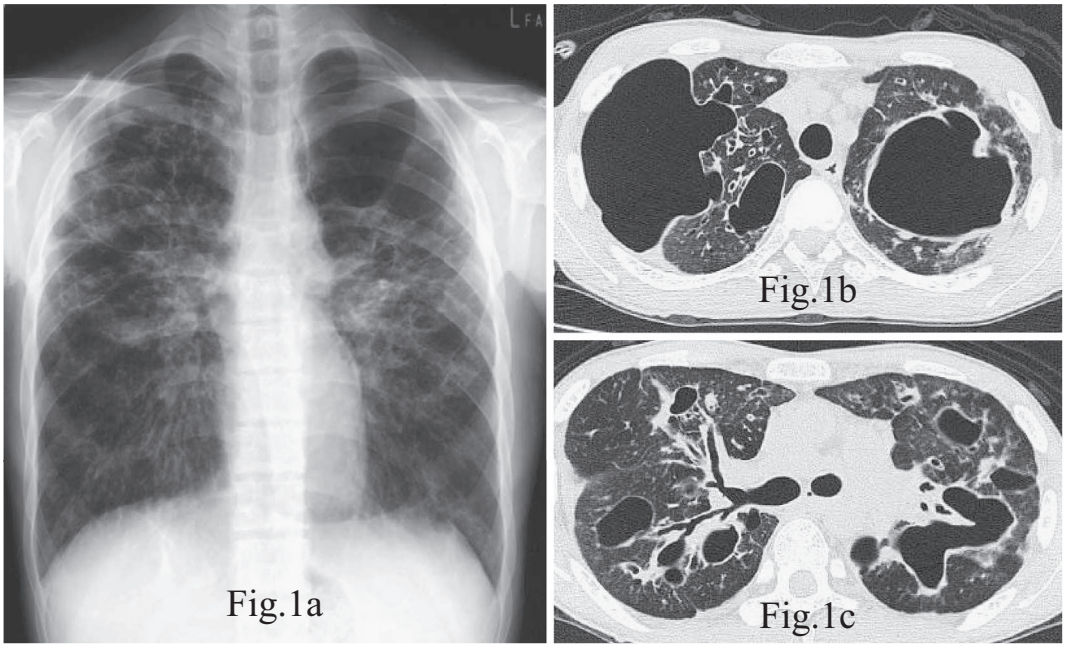


Figure 1. **Radiological findings. a) Chest X-ray taken before lung transplantation indicates diffuse bilateral cystic and bronchiectatic changes. b) and c) Chest computed tomography shows advanced cystic and bronchiectatic changes in both lungs.**

a regular rhythm; respiratory rate, 33 breaths/minute; and blood pressure, 96/62 mmHg. On physical examination, he was emaciated and coarse crackles were heard in the whole lung, and no signs of systemic lymphadenopathy, hepatosplenomegaly, or pre-tibial edema were noted. Laboratory findings on the day before surgery were: WBC, 7,600/mm^3 (Seg, 71%; Lym, 19%; Mono, 5%; Eo, 4%; Ba, 1%); RBC, $5.09\times10^6/mm^3$; Hb, 11.8 g/dL; Ht, 40.7%; Plt, $19.3\times10^4/mm^3$; CRP, 1.01 mg/dL; TP, 8.1 mg/dL; Alb, 3.8 mg/dL; T-bil, 0.3 md/dL; AST, 65 IU/L; ALT, 58 IU/L; γGTP, 141 IU/L; ALP, 749 IU/L; LDH, 168 IU/L; BUN, 11.0 mg/dL; Cr, 0.55 mg/dL; Na, 138 mEq/L; K, 3.6 mEq/L; and Cl, 95 mEq/L.

Blood gas analysis indicated a pH of 7.378, PCO_2 of 62.6 torr, PO_2 of 78.2 torr, and HCO_3^- of 36.0 mmol/L under 3 L/min O_2 nasal inhalation. Pulmonary function test data were as follows: VC, 1.33 L; %VC, 33.1%; FEV_1, 0.83 L; %FEV_1, 22.1%; and FEV_1%, 61.0%. These data indicated both severe obstructive and restrictive dysfunction. Microbiological tests revealed *P. aeruginosa* at 1×10^7 CFU/mL in sputum and the minimum inhibitory concentrations (MICs) for piperacillin (PIPC), amikacin (AMK), gentamycin (GM), imipenem and cilastatin (IPM/CS) and ciprofloxacin (CPFX) were ≥32, 16, 16, ≥32, and 8.0 μg/mL, respectively, which did not fully satisfy the criteria for multidrug-resistant *Pseudomonas* (MDRP; requires MICs for AMK, IPM/CS and CPFX of ≥32, ≥16, and ≥4 μg/mL, respectively). *P. aeruginosa* was also detected in the paranasal sinus at 2×10^4 CFU/mL and the MICs for GM, IPM/CS and CPFX were 2.0, <0.5, and 8.0 μg/mL, respectively. Chest x-ray films before lung transplant surgery indicated diffuse bilateral cystic and bronchiectatic changes (Fig. 1a). Chest computed tomography showed progressive cystic and bronchiectatic changes in both lungs (Fig. 1b, 1c).

After a diagnosis of CF at age 15 years, macrolide therapy was initiated. *Staphylococcus aureus* was initially detected at 3 years after CF diagnosis and cefazolin was routinely used during exacerbation, and was effective. The frequency of exacerbation increased gradually during the clinical course and *S. aureus* was replaced by *P. aeruginosa*, and was continuously detected during exacerbation of chronic respiratory infection (Fig. 2). Drug susceptibility tests for *P. aeruginosa* indicated that it was gradually becoming resistant to beta-lactam, quinolone and aminoglycoside antibiotics. After 60 admissions due to exacerbation of chronic airway tract infection, severe respiratory failure, and poor general condition, lung transplantation from living relatives was planned. As control of the *P. aeruginosa* infection prior to transplant surgery was considered to be critical, management was carefully and strictly executed. A break-point checkerboard plate was used to select a combination of antibiotics in order to maximize the reduction of *P. aeruginosa* in the damaged lung (3). The combination of beta-lactams such as meropenem, ceftazidime, aztreonam (AZT) and PIPC with AMK showed synergistic effects on break-point checkerboard plate analysis (Fig. 3). Isolated *P. aeruginosa* was also susceptible to colistin.

As *S. aureus* was also isolated with *P. aeruginosa*, a combination of AZT and arbekacin (ABK) was administered after admission. Although *S. aureus* was found to be methicillin sensitive after several days of isolation, ABK was continued because it was potentially effective for *P. aeruginosa*. The triple regimen of AZT, AMK and rifampicin (RFP) was administered for exacerbation that occurred 3 months before

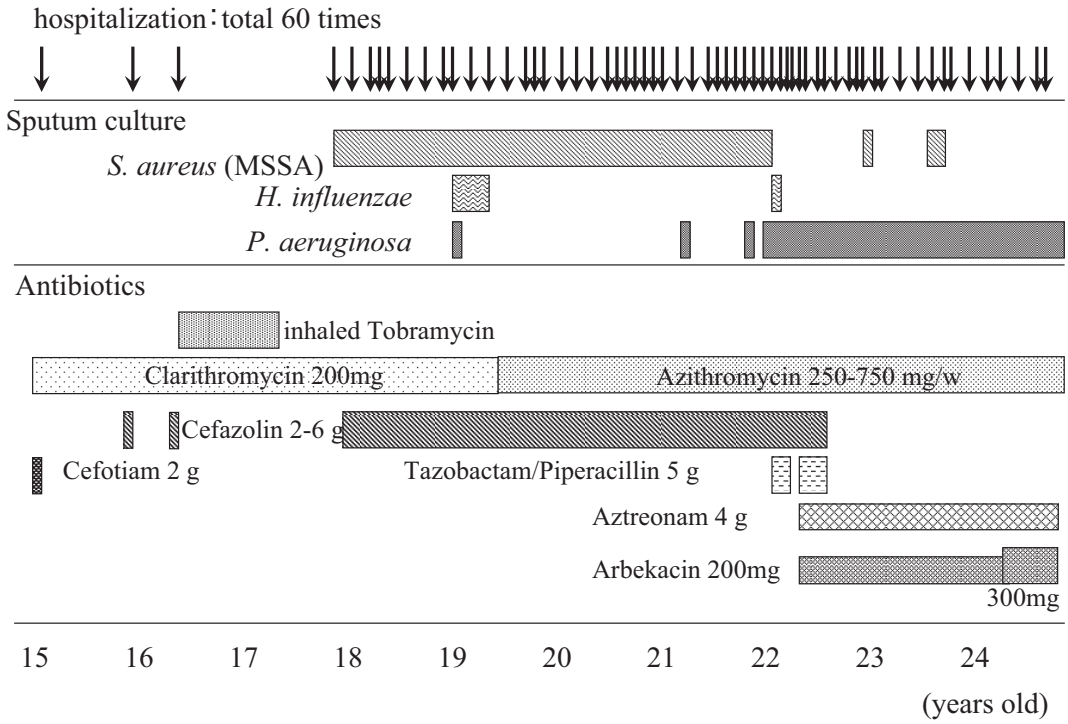


Figure 2. Clinical course from the year of diagnosis to lung transplantation surgery.

the scheduled surgery. A combination of tazobactam/piperacillin (TAZ/PIPC) and isepamycin (ISP) with or without colistin was also used 1 month before surgery in order to minimize drug-resistant *P. aeruginosa*, as residual bacteria may have an influence on the transplant surgery. Figure 4 summarizes the clinical course and the antibiotics used over the 3 months before surgery. Although the inhalation route for antibiotic therapy was considered, it was not used due to severe hemoptysis induced by inhaled stimulants. Bilateral endoscopic maxillectomy was performed prior to lung transplant surgery for the management of chronic sinusitis, and daily nasal washing with 0.9% saline was initiated and continued until lung transplant surgery. These procedures were completed immediately before transplant surgery. The lower lobes of the right and left lungs from the uncle and father of the patient, respectively, were then resected and transplanted.

Pulmonary edema and bleeding in the left lung and thorax were seen at day 1 after surgery. Primary graft dysfunction was suspected and replacement of the left lung lobe with the left lower lobe from the patient's sister was performed immediately. Strict monitoring of infection by Gram staining and culture of intratracheal specimens recovered by bronchofiberscopy were started. Swabs of the pharynx and secretions of the paranasal cavity were also monitored. On post-operative days 8 and 9, Gram-negative rod bacteria phagocytized by neutrophils were repeatedly seen on Gram staining of the lower respiratory specimen. Because the patient remained under mechanical ventilation and no evidence of pneumonia was noted on chest x-ray, ventilator-associated tracheobronchitis (VAT) was diagnosed, and inhalation of tobramycin was immediately initiated. Gram-stained rod-shaped bacteria were no longer seen from the following day and no bacteria were isolated on culture tests. The nasogastric tube was removed as it may have been the entry route of infection. Local administration of tobramycin in the paranasal cavity was also performed against *P. aeruginosa* colonized in the paranasal cavity. *P. aeruginosa* was no longer detected in lower respiratory specimens, or in swabs of the pharynx and paranasal cavity after administration of tobramycin to both the lung and paranasal cavity. On post-operative day 13, *Enterococcus faecium* was isolated from the indwelling central venous catheter and intratracheal sputum, but not from blood. Linezolid was temporality administered for *E. faecium* infection, despite the strain being sensitive to vancomycin, as its distribution in deep tissue is favorable, and the patient exhibited renal impairment. The nasoduodenal tube was removed on post-operative day 21 (Fig. 5). Mechanical ventilation was discontinued on post-operative day 23. Monitoring of infection in both the upper and lower respiratory system continued and the patient was discharged at 4 months after surgery without severe complications. He remains in good condition with no restrictions on daily living at 18 months after surgery.

Discussion

CF is a fatal autosomal recessive hereditary disease with an incidence in Caucasian populations of around 1 in 2,500 (1). In contrast, the incidence of CF in Japan is extremely low; it is estimated to be 1 in 350,000 (2). To date, a total of approximately 150 cases have been reported in Japan. The cause of death in CF cases is typically lung diseases due to respiratory failure. In recent years, aggressive management of lung disease in CF has resulted in great im-

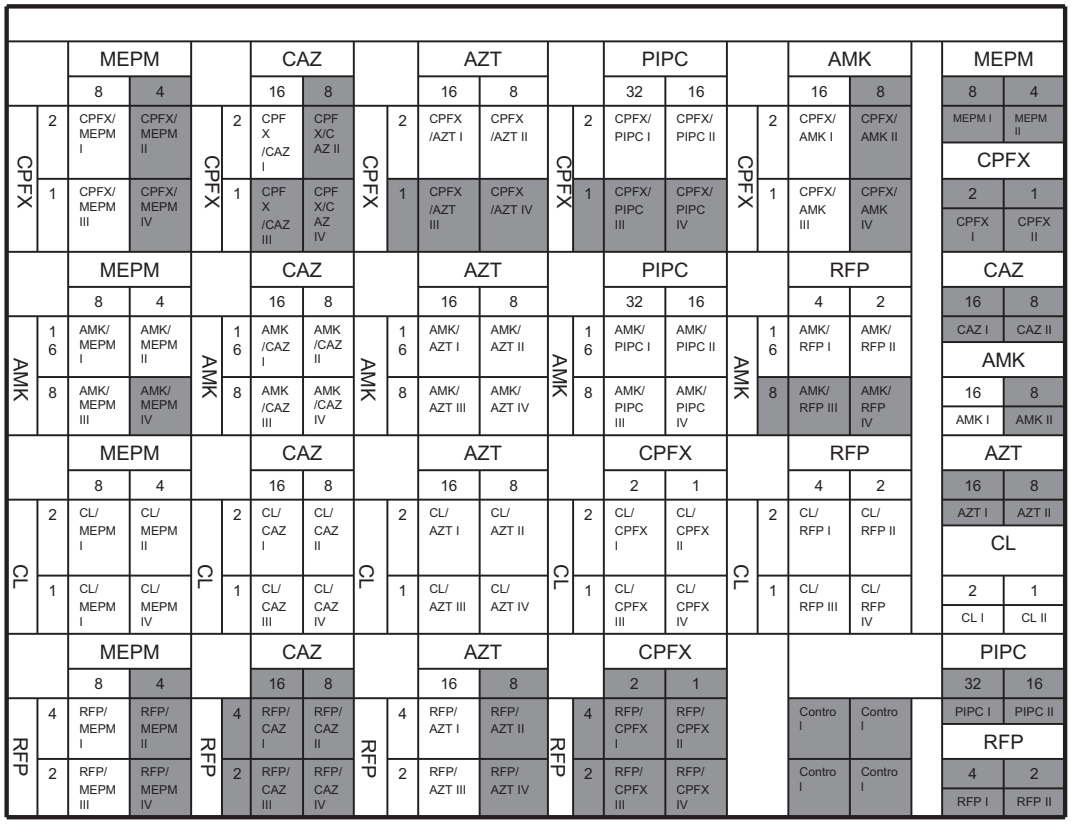
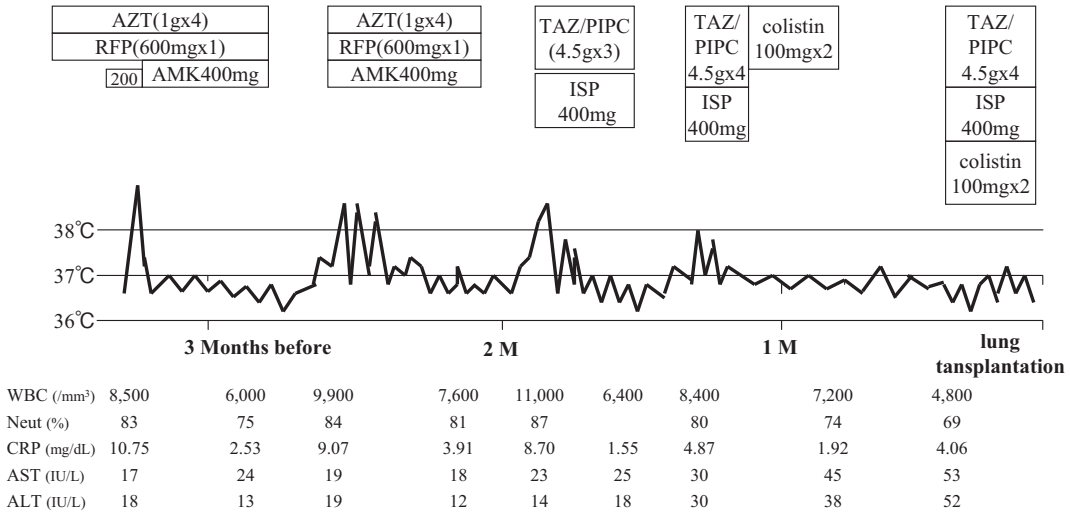


Figure 3. **Results of break-point checkerboard plate analysis. The grey columns indicate bacterial growth. Numbers in the figure indicate the concentration of each antibiotic (μg/mL). AMK: amikacin, AZT: aztreonam, CAZ: ceftazidime, CL: colistin, CPFX: ciprofloxacin, MEPM: meropenem, PIPC: piperacillin, RFP: rifampicin**



WBC (/mm³)	8,500	6,000	9,900	7,600	11,000	6,400	8,400	7,200	4,800
Neut (%)	83	75	84	81	87		80	74	69
CRP (mg/dL)	10.75	2.53	9.07	3.91	8.70	1.55	4.87	1.92	4.06
AST (IU/L)	17	24	19	18	23	25	30	45	53
ALT (IU/L)	18	13	19	12	14	18	30	38	52

Figure 4. **Clinical course 2. The figure shows the clinical course and antibiotics administered. AZT: aztreonam, AMK: amikacin, ISP: isepamycin, RFP: rifampicin, TAZ/PIPC: tazobactam/piperacillin**

provements in length and quality of life, and the median expected survival age has reached 36 years (4).

We recently reported three CF cases with confirmed CFTR mutations in Nagasaki, Japan (5). The case described in this report has the longest follow-up period of these cases. The respiratory failure progressed gradually over the last 10 years, and several factors and conditions, such as FEV_1 below 30% of predicted FEV_1, oxygen-dependent respiratory failure, hypercapnia, and pulmonary hypertension, fulfilled the indications for lung transplantation (6).

CF patients in advanced stages of lung disease ultimately require lung transplantation; however, drug-resistant *P.*

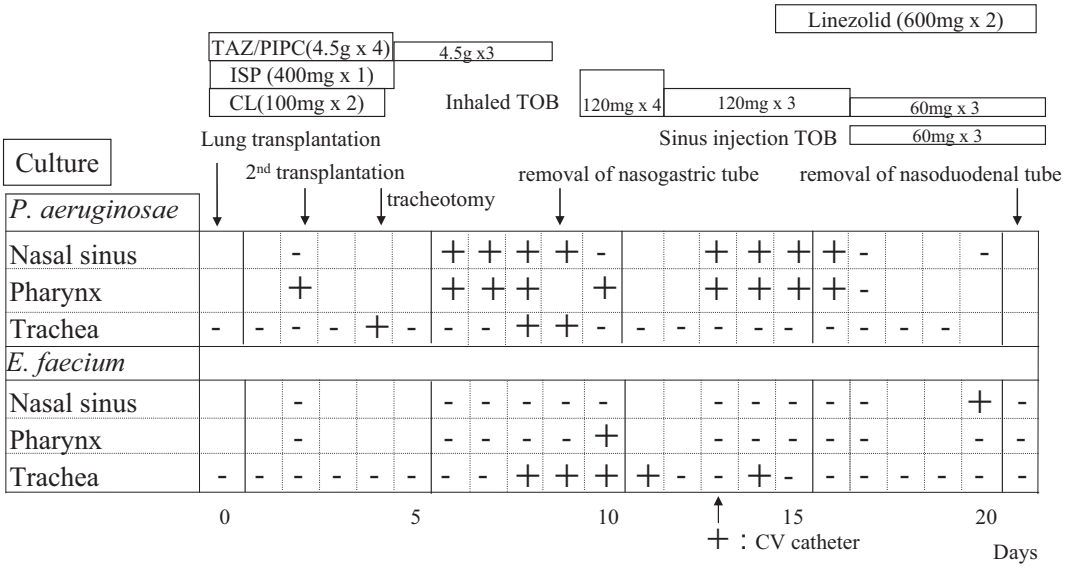


Figure 5. Clinical course 3. The figure shows the post-operative clinical course.

aeruginosa infection is a major obstacle to completing lung transplantation (7). Needless to say, control of this infection during the perioperative period is critical to patient outcome. Pre-transplant colonization with MDRP, however, is not a contraindication to transplantation, as it has no significant influence on short-term survival (6). In the present case, *P. aeruginosa* was isolated from about 2 years before surgery, and the drug susceptibility profile of isolates temporarily showed MDRP.

The Cystic Fibrosis Foundation as well as the European Cystic Fibrosis Society strongly recommend the chronic use of inhaled tobramycin to improve lung function and reduce the frequency of exacerbation for patients with CF aged 6 years or older who have moderate to severe lung disease and *P. aeruginosa* persistently present in airway cultures (4, 8). Furthermore, we have previously reported the efficacy of inhaled tobramycin in cases with bronchiectasis (9). Although it is reasonable to begin inhaled treatment when *P. aeruginosa* is isolated, inhalation therapy was not used in this patient because of severe hemoptysis induced by inhaled stimulants, including 0.9% saline.

Antibiotic susceptibility testing is recommended to be repeated at regular intervals while patients are on the waiting list to ensure that a recently tested antibiotic combination is administered at the time of transplant surgery (6). We therefore used the break-point checkerboard plate, developed for screening appropriate antibiotic combinations against drug-resistant organisms, such as MDRP (3). Although the checkerboard plate method has not been standardized in the clinical setting, it is a potentially favorable method to select two antibiotics with maximum efficacy against drug-resistant bacterial infection. We selected a triple regimen of AZT, RFP and AMK, followed by TAZ/PIPC with ISP. These were selected based on the result of checkerboard analysis, while TAZ/PIPC and ISP were used instead of PIPC and AMK. TAZ/PIPC was selected because the dose of PIPC can be increased further than with single administration of PIPC, and ISP was used in order to avoid prolonged exposure to AMK, which may lead to further drug resistance. These treatments were effective and the patient recovered from exacerbation before transplantation. Colistin, a classic polymyxin, was recently applied to treatment of MDRP and other drug-resistant Gram-negative bacteria (10, 11). Although colistin has not yet been approved in Japan, it was used before and after transplant surgery in this case with the approval of both the patient and the Internal Review Committee of Nagasaki University Hospital. We used colistin for only short periods before and after surgery because we wanted to reduce drug-resistant *P. aeruginosa* levels as much as possible while minimizing serious complications due to its toxicity, and to prevent the production of colistin-resistant *P. aeruginosa*. As it was apparent that colistin was unable to completely eliminate all *P. aeruginosa* in the destroyed lung, lung transplantation was the only option for ultimately eliminating drug-resistant *P. aeruginosa*. The first priority before surgery in this case was for the patient to receive surgery without unexpected complications or conditions. Longer usage of colistin may cause severe adverse effects, such as impaired kidney and central nervous function, and such effects would have delayed the surgery. All approaches were effective and no severe exacerbation was observed for a month before transplant surgery, and the surgery was performed as scheduled.

Another important aspect of this case was that *P. aeruginosa* colonizing the paranasal cavity may enter the lower respiratory airway via nasogastric and nasoduodenal tubes under mechanical ventilation. We confirmed that the drug-susceptibility profiles were the same in *P. aeruginosa* isolated from the paranasal cavity before surgery and from the lower respiratory tract after surgery. This mode of infection is known as VAT, as recently proposed by Craven et al (12). VAT is characterized as localized disease with clinical signs

(fever, leukocytosis, and purulent sputum), microbiologic information, and the absence of new infiltrates on chest radiograph, and VAT may progress to ventilator-associated pneumonia (VAP) in selected patients. Earlier administration of inhaled tobramycin, as suggested by Gram staining findings, followed by injection of tobramycin into the paranasal cavity were considered to be effective for the prevention of VAP and acquiring a good clinical outcome. Taken together, the information acquired from sequential Gram staining of specimens from the respiratory tract during the perioperative period was important for the prevention and early detection of VAT, as well as for selection of antibiotics to use in the management of infection after transplant surgery.

In conclusion, strict monitoring of clinical specimens from the respiratory tract by Gram staining is effective in preventing lower respiratory infection during the acute phase following lung transplant surgery in CF cases.

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