Unusual respiratory bacterial flora in cystic fibrosis: microbiologic and clinical features

Paul M. Beringer, PharmD, and Maria D. Appleman, PhD

Pulmonary infections continue to be a significant source of morbidity and mortality among patients with cystic fibrosis. Although our understanding of the pathogenesis and clinical consequences of pulmonary infections with *Pseudomonas aeruginosa* has increased greatly in recent years, very little is known about potentially emerging pathogens such as *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans*, and methicillin-resistant *Staphylococcus aureus*. In this review, the authors discuss methods for appropriate identification of these "unusual" organisms and their epidemiologic and clinical features. Multicenter surveillance studies are needed to more clearly establish the pathogenicity of these organisms. Curr Opin Pulm Med 2000, 6:545–550 © 2000 Lippincott Williams & Wilkins, Inc.

*Department of Pharmacy, School of Pharmacy and †Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA.

Correspondence to P. Beringer, PharmD, School of Pharmacy, 1985 Zonal Avenue, Los Angeles, CA 90033, USA; e-mail: beringer@hsc.usc.edu

Abbreviations

CF	cystic fibrosis
MRSA	methicillin-resistant *Staphylococcus aureus*
PCR	polymerase chain reaction

Pulmonary infections are a significant cause of morbidity and mortality in patients with cystic fibrosis (CF). More than one third of all patients have at least one pulmonary exacerbation annually, with an average length of hospitalization of 10 days. In addition, respiratory failure is the leading cause of death, accounting for more than three quarters of all cases [1]. The microbial flora in the lungs of cystic fibrosis patients include many antibiotic-resistant, slow-growing, and often mucoid organisms. The prevalence of the major pathogens within the airways of CF patients changes with age. *Staphylococcus aureus* is the predominant pathogen in children, reaching a prevalence rate of nearly 50% by the age of 10 years. In adolescents, *Pseudomonas aeruginosa* becomes the predominant pathogen and approaches a prevalence rate of 80% in adults [1].

Initial infection with CF pathogens appears to be a result of a breech of host defense mechanisms. Although the exact pathophysiologic mechanisms are still unclear, a number of hypotheses linking the defect of cystic fibrosis transmembrane regulator (CFTR) and an increased susceptibility to bacterial infection have recently been described. In particular, the discovery of antimicrobial peptides secreted within normal airways is receiving much attention, and their pharmacologic development may provide additional therapies for patients with CF [2•].

The clinical consequences of *P. aeruginosa* infection have been well described. Ballman *et al.* [3] recently demonstrated that a significant decline in FEV_1 can be prevented in patients treated with intravenous antibiotics for acute pulmonary exacerbations during the early course of *P. aeruginosa* colonization. However, a significant decline in FEV_1 occurred in patients with chronic mucoid *P. aeruginosa* despite a more aggressive approach to therapy during this stage. These and other similar data suggest that early aggressive therapy against *P. aeruginosa* may be necessary to prevent or delay colonization and the subsequent progressive decline in pulmonary function.

In recent years, the emergence of unusual bacterial pathogens within the airways of patients with CF has been increasingly reported. Data from CF centers in the United States indicate that the following organisms currently have a prevalence rate between 1 and 10%:

Figure 1. Age-specific prevalence of unusual microbiological organisms in patients with cystic fibrosis
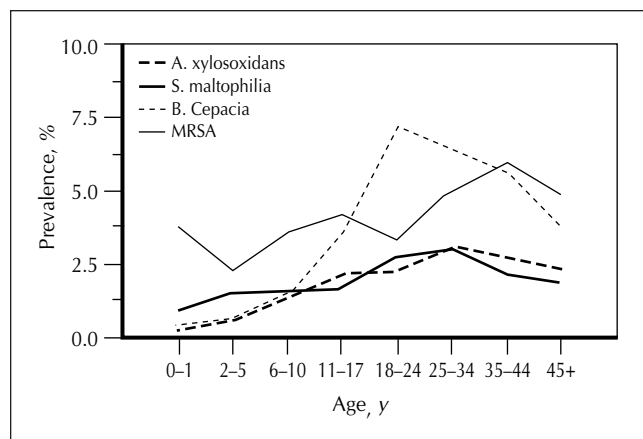
Burkholderia cepacia complex, *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans*, and methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. The prevalence of these organisms increases with advancing age (Fig. 1) and increasing severity of lung disease (Fig. 2) in patients with CF [1]. Although further work is necessary to determine whether a cause-and-effect relationship exists between the presence of these organisms and lung function, this epidemiologic feature has increased the level of concern among many clinicians. This concern is heightened by the fear for selection pressure exerted by the increased use of chronic maintenance antibiotic therapy in CF patients colonized with *P. aeruginosa*. Preliminary data indicate that chronic administration of aerosolized tobramycin does not select for these organisms; however, confirmation of this finding using longitudinal data is necessary [4]. Data from a recent large multicenter controlled trial indicate that the prevalence of these unusual bacteria may in fact be higher due to the difficulty in isolating and identifying these organisms [5]. Laboratories that process respiratory specimens from CF patients must be familiar with the special protocols needed for culture, accurate identification, and accurate susceptibility testing. The importance of careful specimen handling and transport to prevent delays cannot be overemphasized. Moreover, unless selective media and prolonged incubation are used, some of these bacteria may never be detected [6].

The purpose of this article is to discuss the difficulties and recent advances in the isolation, identification, and susceptibility testing of these unusual bacterial pathogens. In addition, the clinical significance of these organisms in the airways of patients with CF will be discussed where data are available.

Burkholderia cepacia complex
Microbiology
The term *Burkholderia cepacia* complex has been used to describe a cluster of five related species originally referred to as *B. cepacia* genomovars I through V. Genomovars are phenotypically similar but genotypically distinct strains. Today the complex includes *B. cepacia* genomovar I, *B. multivorans* (formerly *B. cepacia* genomovar II), *B. cepacia* genomovar III, *B. stabilis* (formerly *B. cepacia* genomovar IV), and *B. vietnamiensis* (formerly *B. cepacia* genomovar V). Genomovar I is a plant pathogen but contains the type strain, thus retaining the name *B. cepacia*. Genomovar III and *B. multivorans* account for the majority of isolates obtained from patients with CF [7•].

Burkholderia cepacia selective agar, or oxidation-fermentation polymyxin-bacitracin-lactose, agar separates *B. cepacia* complex from the other gram-negative bacteria present in the respiratory secretions. The sensitivity of these media is approximately 96% and 100%, respectively, but their specificity is less than 100%. Therefore, the detection of growth on these media is considered only an initial screening test [8•].

Most microbiology laboratories depend on commercially available identification kits instead of conventional biochemicals to identify isolates from the selective plates. van Pelt *et al.* [8•] compared API 20NE (bioMerieux Vitek, Hazelwood, MO), Vitek GNI (bioMerieux Vitek), and the RapID NF Plus system (Remel Laboritories, Lenexa, KS) for *B. cepacia* identification and found the API 20NE to be the better system. None of the commercial kits were 100% sensitive or specific. The difficulty in making the correct identification may be due to the fact that *B. cepacia* is actually a

Figure 2. Relation between prevalence of *Burkholderia cepacia* and pulmonary function
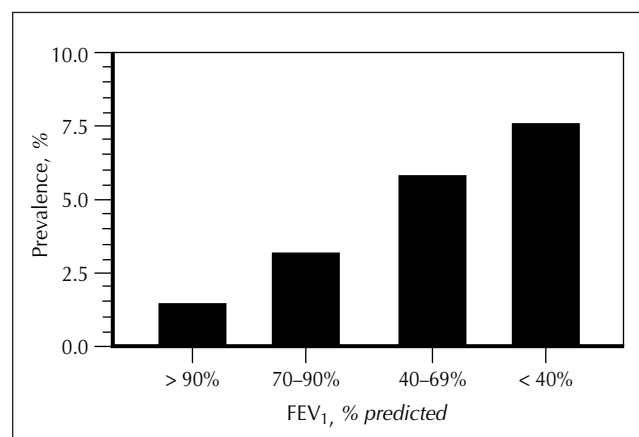
FEV, forced expiratory volume.

complex of five genomovars, not one single species. The final accurate identification of any isolate depends on species-specific polymerase chain reaction (PCR) assays, and any isolate identified as *B. cepacia* in a clinical laboratory should be sent for identity confirmation. In the United States, isolates should be sent to the CFF *Burkholderia cepacia* Research Laboratory and Repository (John J. LiPuma, MD, University of Michigan Medical Center, 1150 W. Medical Center Drive, Ann Arbor, MI 48102) [7•,8•,9,10].

Ultimately, the PCR methodology will be applied directly to respiratory specimens for truly rapid as well as specific results. Whitby *et al.* [11] found that PCR assays may be more accurate than culture, since they were able to detect the organisms in culture-negative specimens collected from patients with intermittent colonization.

Epidemiology
The national mean prevalence rate is 3.5% with a range of 0 to 14% [1]. A contributing factor to the prevalence rate is the ease of transmissibility of some strains between patients that have lead to outbreaks at some centers. Predisposing factors for colonization include hospitalization and having a colonized sibling [12].

Clinical features
Whether the severity of pulmonary disease predisposes individuals to colonization with *B. cepacia* complex or whether *B. cepacia* complex contributes to the decline in pulmonary function and increased mortality remains controversial. Muhdi *et al.* [13] reported their experience with *B. cepacia* complex in 18 patients over a 4-year period following an epidemic at their center. Significantly worsened FEV_1 ($P < 0.02$) and FVC ($P < 0.01$) were noted in patients who were colonized with *B. cepacia* complex when compared with matched control subjects. In contrast, Frangolias *et al.* [14] recently reported their experience in 36 patients colonized with *B. cepacia* complex with a follow-up interval of nearly 5 years. In contrast to this finding, a comparison of pulmonary function with matched control subjects failed to demonstrate any significant differences in the rate of change at 2 or 4.5 years after acquisition of *B. cepacia* complex. However, a significantly higher mortality rate was observed in *B. cepacia* complex cases over the entire study period ($P < 0.05$). Owing to concerns for reinfection following transplantation, many centers consider *B. cepacia* complex colonization a contraindication to transplantation. Despite this concern, patients colonized with multidrug-resistant organisms, including *B. cepacia* complex, have been transplanted successfully [15].

Susceptibility
Burkholderia cepacia complex isolates are intrinsically resistant to aminoglycoside antibiotics and are often multidrug resistant. Analysis of multidrug-resistant isolates (n = 652) by the CF Referral Center for Susceptibility and Synergy Studies (Lisa Saiman, MD, College of Physicians & Surgeons of Columbia University, 650 West 169th Street, Black Building 4/413, New York, NY 10032) demonstrated that minocycline and meropenem were the most active agents, with 33% and 28% of strains susceptible to these agents, respectively [16]. Combinations of chloramphenicol/minocycline and chloramphenicol/ceftazidime most consistently demonstrated synergistic activity against these isolates (49 and 26%, respectively) [17].

Burkholderia gladioli
Burkholderia gladioli and *B. cepacia* complex are genotypically distinct but phenotypically similar; separation of the two species using conventional tests is very difficult [18]. Clode *et al.* [19], Whitby *et al.* [20], and Bauernfeind *et al.* [21] describe PCR assays that used various specific primers to discriminate reliably between *B. gladioli* and related species. Unlike *B. cepacia* complex, infections with *B. gladioli* are not associated with poor prognosis, and the organism is typically not antibiotic resistant [22]. Since the presence of *B. cepacia* complex has prognostic and therapeutic implications, the identification of either species by phenotypic methods should be confirmed by PCR using species-specific primers.

Ralstonia species
Ralstonia is a new genus that includes former members of *Burkholderia* species (*B. pickettii* and *B. solanacearum*), *Alcaligenes eutrophus* and CDC group IVc-2. These organisms have been renamed as *R. picketti*, *R. solanacearum*, *R. eutropha*, and *R. paucula*, respectively. The genus was separated from *Burkholderia*, *Pseudomonas*, and *Alcaligenes* by phenotypic characteristics, cellular lipid and fatty acid analysis, rRNA-DNA hybridization, and phylogenetic analysis of 16S rDNA sequences [7•,23]. These organisms are gram-negative, oxidase-positive, nonfermentative bacilli considered as opportunistic pathogens that can cause serious infections in immunocompromised patients. They can be identified by biochemicals in the clinical laboratory.

Stenotrophomonas maltophilia
Microbiology
Infections with *Stenotrophomonas maltophilia* may place the CF patient at increased risk for morbidity and mortality because of the organism's inherent resistance to multiple antibiotics. Carroll *et al.* [24] compared the following susceptibility testing methods for *Stenotrophomonas*: disk diffusion, E-test (AB Biodisk, Solna, Sweden), Alamar colorimetric broth microdilution, Vitek, MicroScan (Baxter Microscan, West Sacramento, CA), and an in-house microdilution minimum inhibiting concentration

(MIC) method. Vitek, MicroScan, and the in-house microdilution methods generated inconsistent results for all antibiotics except trimethoprim-sulfamethoxazole. Disk diffusion and E-test results had the closest correlation. The rate of resistance to all antibiotics was greater when results were interpreted at 48 hours than at 16 to 18 hours of incubation. Only trimethoprim-sulfamethoxazole, doxycycline, and minocycline demonstrated consistent activity against *S. maltophilia* independent of the method of testing used.

Epidemiology

Stenotrophomonas maltophilia has a mean prevalence of 5.4%. Prior antibiotic exposure is a leading predisposing factor [25]. Acquisition of *S. maltophilia* was not found to be associated with recent hospitalization or having a sibling who was colonized with this organism [26]. Persistence of the organism in the airways varies greatly among culture-positive patients with 80% transiently colonized (< 12 months), 9% persistently colonized (> 12 months but subsequently returned to culture negative), and 11% chronically colonized [26].

Clinical features

The effect of *S. maltophilia* colonization on pulmonary function and mortality over a 12-year period was reported recently [26]. No obvious deleterious effect on pulmonary function was noted over a 2-year period following acquisition of *S. maltophilia*. In addition, the 5-year survival rate was greater than 90% for patients with initial mild to moderate pulmonary disease without regard for colonization status. In contrast, for those patients with initially severe pulmonary disease, the 5-year survival rate was lower in culture-positive compared with culture-negative patients (40% *vs* 72%). However, the number of patients was too small to allow statistical comparisons.

Susceptibility

Stenotrophomonas maltophilia isolates are instrinsically resistant to multiple antibiotics because of involvement of a multidrug efflux system [27]. Doxycycline was found to be most active agent against 78% of 263 multidrug-resistant strains tested at a referral laboratory [16]. In addition, the following combinations were also found to exhibit the most consistently synergistic activity against 52, 46, and 45% of the multidrug-resistant isolates in the same study: trimethoprim/sulfamethoxazole and ticarcillin/clavulanate, doxycycline and ticarcillin/clavulanate, and trimethoprim/sulfamethoxazole and doxycycline [16]. High-dose colistin (concentrations achievable with aerosolization) also demonstrated activity against 61% of the isolates. This therapy, in conjunction with trimethoprim/sulfamethoxazole, resulted in improvement of pulmonary function in a patient who demonstrated clinical infection with this organism [28].

Alcaligenes xylosoxidans
Microbiology

Alcaligenes xylosoxidans is an oxidase-positive, motile, gram-negative bacillus that has been isolated from the gastrointestinal tract of humans and from various hospital and environmental water sources. It can be mistaken for nonpigmented strains of *P. aeruginosa* or *B. cepacia*. *A. xylosoxidans* can be identified with the Vitek GNI (bioMerieux), API NFT (bioMerieux), API 20NE (bioMerieux), and the RapID NF Plus system (Innovative Diagnostics Systems Inc., Atlanta, GA). Susceptibility testing can be performed using agar dilution, broth dilution, disk diffusion, and E-test (AB Biodisk).

Epidemiology

The prevalence of airway colonization with *A. xylosoxidans* was reported to be 8.7%, according to results from a recent cross-sectional survey of 595 patients [5]. This number is higher than that reported by the CFF registry data (2%), possibly because of selection biases [29]. Although data on acquisition of *A. xylosoxidans* in patients with CF are limited, it appears that patient-to-patient spread is not the primary means of transmission of this organism [30,31].

Clinical features

Alcaligenes xylosoxidans has been reported to be associated with acute pulmonary exacerbations in CF patients in one report; however, all were concomitantly colonized with *P. aeruginosa* [31]. Long-term colonization does not appear to result in any obvious decline in clinical status, although the number of patients evaluated in recent reports is quite small (n = 10) [30,32].

Susceptibility

Strains of *A. xylosoxidans* are typically multiply resistant to available agents. Ninety percent of isolates obtained from CF patients enrolled in a recent cross-sectional study were resistant to tobramycin [5]. Analysis of 94 multidrug-resistant isolates by a referral laboratory showed that imipenem and piperacillin were the most active agents, with 44 and 40% of strains susceptible, respectively [33]. Combinations of imipenem/amikacin and timentin/tobramycin exhibited the most consistently synergistic activity against 30 and 29% of isolates, respectively [33].

Methicillin-resistant *Staphylococcus aureus*
Microbiology

Although MRSA strains possess the genetic determinants for resistance, not all strains express resistance in a culture; special methods must be used to detect resistance in clinical specimens. These methods include incubation at a lower temperature (30–35°C), media

with 2 to 4% sodium chloride, incubation for 24 hours, and use of an inoculum of 5×10^5 cfu/mL prepared from an agar plate culture rather than broth [34].

Testing for methicillin susceptibility can be done using the disk diffusion technique with a 1 mg oxacillin disk, the E-test, or a 4% sodium chloride agar plate containing 6 µg/mL of oxacillin. Automated susceptibility testing methods are not reliable [35,36].

Molecular methods have been developed for the rapid detection of MRSA in clinical specimens. A new method for detecting methicillin resistance is to use a DNA probe to detect the *mecA* gene [37]. Another method is a slide latex agglutination kit detecting PBP 2a using a monoclonal antibody [38]. Both of these rapid tests are efficient and effective in detecting *S. aureus* resistance to methicillin.

Epidemiology

According to the CFF patient registry, the national mean prevalence rate of MRSA is 3.2% with a range of 0 to 17% [1]. In a study involving 595 patients from 69 CF centers, 6.1% of patients were colonized with MRSA [5]. Other investigators reported a higher incidence of MRSA carriage. The difference in incidence may be a function of the procedures used by the various institutions to detect MRSA [6]. Nosocomial transmission from the general hospital population rather than from other CF patients is thought to be the source of acquisition [39]. Prolonged hospitalization or surgical procedures may predispose to the acquisition of MRSA [40]. In addition, a comparison of pulmonary function with matched controls (FEV_1 29% *vs* 47%, *P* = 0.0034) at the time of acquisition indicates that poor pulmonary function is a risk factor for MRSA colonization [41]. Colonization appears to be transient (< 1 month) in 35% of cases [41].

Clinical features

The impact of MRSA colonization in adult CF patients at one center was recently reported. A total of 26 patients were identified over a 15-year period. MRSA colonization was thought to be a contributing factor in only 1 of 12 deaths that occurred during the study. Therefore, colonization does not appear to have an affect on mortality.

Because of insufficient data on outcomes associated with acute pulmonary exacerbations involving MRSA, some centers choose to treat both *P. aeruginosa* and MRSA when both are present whereas others treat only the *P. aeruginosa* isolate [40].

Although limited data suggest no adverse clinical consequences to MRSA colonization in patients with CF, there are social implications. Isolation of the colonized patient is necessary during hospitalization and at the clinic setting. In addition, like *B. cepacia*, many centers consider colonization with MRSA a contraindication to transplantation.

Susceptibility

Glycopeptide antibiotics such as vancomycin have been the drug of choice in treating infections involving MRSA and have been utilized successfully in patients with CF [40,41]. Due to the increasing prevalence of vancomycin-resistant enterococci and recent reports of vancomycin intermediately susceptible strains of *S. aureus*, indiscriminate use of this vital antibiotic class must be avoided. Two new agents with activity against MRSA have been approved by the Food Drug Administration this year, quinupristin/dalfopristin (Synercid) and linezolid (Zyvox), which expand the therapeutic options for the management of infections involving this organism.

Conclusions

Significant progress has been made over the past few decades in improving the survival of patients with cystic fibrosis. No longer is CF considered strictly a pediatric disease. Much of this improvement can be attributed to advances in the medical management of the disease in particular treatment of pulmonary infections. Although *P. aeruginosa* remains the most common organism present within the airways of these patients in adulthood, new organisms (*S. maltophilia*, *A. xylosoxidans*, *B. cepacia* complex, MRSA) are increasingly being reported. Advances in the isolation and identification of these organisms have contributed to a better appreciation of the prevalence of these organisms. Additional multicenter surveillance studies are necessary to firmly establish whether colonization with these organisms has prognostic significance. Since these organisms are typically resistant to many of the conventional antibiotic therapies available, treatment options may be limited. Synergy testing can be of assistance in determining potentially useful therapies in the setting of multidrug resistance; however, outcome correlations with the synergy data in many cases has not been described. Until data are available to address these critical issues, strict infection control measures may be the key to limiting the spread of such organisms among patients with CF.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
•   Of special interest
••   Of outstanding interest

1   Cystic Fibrosis Foundation: Patient Registry 1998 Annual Data Report. Bethesda, Maryland, September 1999.

2   Bals R, Weiner DJ, Wilson JM: The innate immune system in cystic fibrosis
•   lung disease. J Clin Invest 1999, 103:303–307.
This article provides an excellent summary of current theories on the pathogenesis of pulmonary infections in patients with cystic fibrosis.

3   Balman M, Rabsch P, von der Hardt H: Long term follow up of changes in FEV$_1$ and treatment intensity during *Pseudomonas aeruginosa* colonization in patients with cystic fibrosis. Thorax 1998, 53:732–737.

4   Burns JL, Van Dalfsen JM, Shawar RM, et al.: Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J Infect Dis 1999, 179:1190–1196.

5   Burns JL, Emerson J, Stapp JR, et al.: Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clin Infect Dis 1998, 27:158–163.

6   Shreve MR, Butler S, Kaplowitz HJ, et al.: Impact of microbiology practice on cumulative prevalence of respiratory tract bacteria in patients with cystic fibrosis. J Clin Microbiol 1999, 37:753–757.

7   Vandamme P, Mahenthiralingam E, Holmes B, et al.: Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). J Clin Microbiol 2000, 37:1042–1047.
This article contains a discussion of the genomovars of the *B. cepacia* complex.

8   van Pelt C, Verduin CM, Goessens WHF, et al.: Identification of *Burkholderia* spp. in the clinical microbiology laboratory: comparison of conventional and molecular methods. J Clin Microbiol 1999, 37:2158–2164.
In this article, the authors discuss the difficulties in the identification of *Burkholderia* spp. and of the need to confirm the species identity by molecular methods.

9   LiPuma JJ, Dulaney BJ, McMenamin JD, et al.: Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. J Clin Microbiol 1999, 37:3167–3170.

10   Bauernfeind A, Schneider I, Jungwirth R, et al.: Discrimination of *Burkholderia multivorans* and *Burkholderia vietnamiensis* from *Burkholderia cepacia* genomovars I, III, and IV by PCR. J Clin Microbiol 1999, 37:1335–1339.

11   Whitby PW, Dick JL, Campbel PW III, et al.: Comparison of culture and PCR for detection of *Burkholderia cepacia* in sputum samples of patients with cystic fibrosis. J Clin Microbiol 1998, 36:1642–1645.

12   LiPuma JJ: *Burkholderia cepacia*: management issues and new insights. Clin Chest Med 1998, 19:473–486.

13   Mudhi K, Edenborough FP, Gumery L, et al.: Outcome for patients colonized with *Burkholderia cepacia* in a Birmingham adult cystic fibrosis clinic at the end of an epidemic. Thorax 1996, 51:374–377.

14   Frangolias DD, Mahenthiralingam E, Rae S, et al.: *Burkholderia cepacia* in cystic fibrosis: variable disease course. Am J Respir Crit Care Med 1999, 160:1572–1577.

15   Kanj SS, Tapson V, Davis DR, et al.: Infections in patients with cystic fibrosis following lung transplantation. Chest 1997, 112:924–930.

16   San Gabriel P, Liu Z, Whittier S, et al.: Antibiotic susceptibility and synergy testing of *Burkholderia cepacia* and *Stenotrophomonas maltophilia*. North American Cystic Fibrosis Conference 1998; Toronto, Canada.

17   Burns JL, Saiman L: *Burkholderia cepacia* infections in cystic fibrosis. Pediatr Infect Dis J 1999: 18:155–156.

18   Wilsher ML, Kolbe J, Morris AJ, et al.: Nosocomial acquisition of *Burkholderia gladioli* in patients with cystic fibrosis. Am J Respir Crit Care Med 1997, 155:1436–1440.

19   Clode FE, Kaufmann ME, Malnick H, et al.: Evaluation of three oligonucleotide primers sets in PCR for the identification of *Burkholderia cepacia* and their differentiation from *Burkholderia gladioli*. J Clin Pathol 1999, 52:173–176.

20   Whitby PW, Pope LC, Carter KB, et al.: Species-specific PCR as a tool for the identification of *Burkholderia gladioli*. J Clin Microbiol 2000, 38:282–285.

21   Bauernfeind A, Schneider I, Jungwirth R, et al.: Discrimination of *Burkholderia gladioli* from other *Burkholderia* species detectable in cystic fibrosis patients by PCR. J Clin Microbiol 1998, 36:2748–2751.

22   Graves M, Robin T, Chipman AM, et al.: Four additional cases of *Burkholderia gladioli* infection with microbiological correlates and review. Clin Infect Dis 1997, 25:838–842.

23   Moissenet D, Goujon CP, Garbarg-Chenon A, et al.: CDC Group IV c-2: a new *Ralstonia* species close to *Ralstonia eutropha*. J Clin Microbiol 1999, 37:1777–1781.

24   Carroll KC, Cohen S, Nelson R, et al.: Comparison of various in vitro susceptibility methods for testing *Stenotrophomonas maltophilia*. Diagn Microbiol Infect Dis 1998, 32:229–235.

25   Ballestero S, Virseda I, Escobar H, et al.: Stenotrophomonas maltophilia in cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 1995, 14:728–729.

26   Demko CA, Stern RC, Doershuk CF: *Stenotrophomonas maltophilia* in cystic fibrosis: incidence and prevalence. Pediatr Pulmonol 1998, 25:304–308.

27   Zhang L, Li XZ, Poole K: Multiple antibiotic resistance in *Stenotrophomonas maltophilia*: involvement of a multidrug efflux system. Antimicrob Agents Chemother 2000, 44:287–293.

28   Taylor RF, Gaya H, Empey DW: A new approach to the treatment of *Xanthomonas maltophilia* respiratory infection in a patient with cystic fibrosis. Resp Med 1998, 92:363–364.

29   Cystic Fibrosis Foundation: Patient Registry 1996 Annual Data Report. Bethesda, Maryland, September 1997.

30   Moissenet D, Baculard A, Valcin M, et al.: Colonization by *Alcaligenes xylosoxidans* in children with cystic fibrosis: a retrospective clinical study conducted by means of molecular epidemiological investigation. Clin Infect Dis 1997, 24:274–275.

31   Dunne WM, Maisch S: Epidemiological investigation of infections due to *Alcaligenes* species in children and patients with cystic fibrosis: use of repetitive-element-sequence polymerase chain reaction. Clin Infect Dis 1995, 20:836–841.

32   Peltroche-Llacsahuanga H, Haase G, Kentrup H: Persistent airway colonization with *Alcaligenes xylosoxidans* in two brothers with cystic fibrosis. Eur J Clin Micro Infect Dis 1998, 17:132–134.

33   Liu Z, Chen Y, San Gabriel P, et al.: *Alcaligenes* species: misidentification and antibiotic resistance. North American Cystic Fibrosis Conference, 1998; Toronto, Canada.

34   Mulligan ME, Murray-Leisure KA, Ribner BS, et al.: Methicillin-rersistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. Am J Med 1993, 94:313–328.

35   Dillard SC, Waites KB, Brookings ES, et al.: Detection of oxacillin-resistance in *Staphylococcus aureus* by MicroScan MIC panels in comparison to four other methods. Diagn Microbiol Infect Dis 1996, 24:93–100.

36   Ribeiro J, Vieira FD, King T, et al.: Misclassification of susceptible strains of *Staphylococcus aureus* as methicillin-resistant *S. aureus* by a rapid automated susceptibility testing system. J Clin Microbiol 1999, 37:1619–1620.

37   Kwok YY, Citron DM, Fiorentino N, et al.: Evaluation of a new rapid test to detect the mecA gene in *Staphylococcus* aureus. Abstract 0868. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 1999, San Francisco.

38   Cavassini M, Wenger A, Jaton K, et al.: Evaluation of MRSA-screen, a simple antiPBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 1999, 37:1591–1594.

39   Givney R, Vickery A, Holliday A, et al.: Methicillin-resistant *Staphylococcus aureus* in a cystic fibrosis unit. J Hosp Infect 1997, 35:27–36.

40   French GL: MRSA in cystic fibrosis. J Hosp Infect 1998, 40:179–191.

41   Thomas SR, Gyi KM, Gaya H, et al.: Methicillin-resistant *Staphylococcus aureus*: impact at a national cystic fibrosis centre. J Hosp Infect 1998, 40:203–209.