

CASE STUDIES in the ANTIFUNGAL TREATMENT of HIGH-RISK PATIENTS

Fever and Pulmonary Infiltrates in a Pediatric AML Patient

CASE PRESENTATION:

Fever and Pulmonary Infiltrates in a Pediatric AML Patient

A 12-year-old boy presented with anemia, thrombocytopenia, and a white blood cell count of 65,000/mm³; the majority of cells were blasts with occasional Auer rods. Bone marrow contained a large number of blasts that were myeloperoxidase positive. Flow cytometric analysis demonstrated a discrete population of CD34⁺ cells, which coexpressed the myeloid markers CD13 and CD33. Cytogenetics demonstrated the presence of t(8;21), and the patient was diagnosed with the FAB M2 acute myeloid leukemia (AML).

The patient was treated with the Children's Cancer Group IDA-DCTER (idarubicin-cytarabine, 6-thioguanine, etoposide, dexamethasone) regimen, given over 4 days with a second cycle administered after 6 days of rest per protocol, despite the development of severe aplasia. He became febrile on day 7 and was treated with broad-spectrum antibiotics. Fluconazole was subsequently added on day 14 after the second cycle of IDA-DCTER (daunorubicin given in second cycle per protocol rather than idarubicin [DCTER]). The patient then presented with persistent fever on day 18. Despite negative cultures and scans, therapy was initiated with amphotericin B deoxycholate 0.5 mg/kg on day 21.

After 8 weeks of aplasia, the patient's bone marrow recovered and blasts were less than 5%. At this time, he was given consolidation chemotherapy with IDA-DCTER, again given over 4 days producing bone marrow aplasia. Neutropenia was present by day 7. A second cycle of DCTER was administered after 6 days of rest. During the second cycle, he again became febrile and was treated with piperacillin-tazobactam, vancomycin, and fluconazole; the fever resolved within 3 days. However, by day 24, the patient

developed increasing abdominal pain with elevated aminotransferase levels, thrombocytopenia, mild ascites, and a bilirubin of 4.8 mg/dL, leading to a diagnosis of hepatic veno-occlusive disease.

Although the bilirubin levels eventually decreased to 3.0 mg/dL, the severity of abdominal pain increased. Chest x-ray, abdominal film, and fungal and bacterial cultures from the bloodstream were all negative. A computed tomography (CT) scan of the abdomen was performed and was nondiagnostic; however, a possible lesion was demonstrated at the level of the diaphragm. A subsequent chest CT scan demonstrated a cavitary lesion in the right lower lobe (**Figure, page 2**). Thoracoscopic biopsy and video-assisted thoracoscopic surgery were done with extensive platelet and red blood cell support. The lesion was removed, and histologic examination demonstrated narrow, branching septate hyphae. Immunohistochemistry done with polyclonal antibody and peroxidase techniques using commercially available antibodies was positive for *Aspergillus* species. Treatment was started with amphotericin B lipid complex (ABLC) at 5 mg/kg/day. *Aspergillus fumigatus* was confirmed on culture.

The patient was treated with ABLC for 4 weeks and then switched to itraconazole oral solution once liver function tests and bilirubin levels had returned to normal for more than 1 week. The patient went on to receive a continuation course of HiDARAC (cytosine arabinoside 3 g/m² q12h x 4) and L-asparaginase (10,000 units IM) with no recurrence of fungal infection or acute nonlymphocytic leukemia. The patient received 4 weeks of itraconazole beyond neutrophil recovery and remains alive 4 years later.

In this case study, Joseph M. Wiley, MD, describes a 12-year-old boy who developed invasive pulmonary aspergillosis following intensive chemotherapy. An invasive procedure was required for a prompt and accurate diagnosis, and the patient survived the life-threatening infection with appropriate therapy. In the discussion that follows, Dr. Wiley points out the huge differences in the amount of data on invasive fungal diseases in the pediatric population as compared with that in adults and underscores the urgent need for more data, particularly on the diagnostic and treatment aspects of invasive fungal infections in children. Also highlighted are the differences in the pharmacokinetic profiles of the various classes of antifungal drugs in adults versus those in children.

Executive Editor:

Pranatharbi Chandrasekar, MD

Professor of Medicine
Director, Fellowship Program

Division of
Infectious Diseases

Wayne State University
School of Medicine

Detroit, Michigan



Contributing Author:

Joseph M. Wiley, MD

Chief, Division of Pediatric
Hematology-Oncology

Chairman, Department of
Pediatrics

The Children's Hospital
at Sinai

Baltimore, Maryland



COMING SOON!

Newsletters by Michael Kleinberg, MD;
Dimitrios Kontoyiannis, MD; and Ramon Del Busto, MD

Educational Objectives

- Determine how therapeutic choices for invasive fungal infections can be most appropriately employed in high-risk patients
- Explore methods to improve patient outcomes, considering antifungal efficacy, spectrum of activity, safety, and cost-effectiveness
- Compare and understand the clinical uses of available antifungal agents

CME Information

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of Wayne State University School of Medicine and the MedEd Group, LLC.

The Wayne State University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians. The Wayne State University School of Medicine designates this educational activity for a maximum of 1.0 AMA PRA Category 1 credit(s)[™]. Physicians should only claim credit commensurate with the extent of their participation in the activity.



To receive documentation of your participation in this CME activity, complete the newsletter posttest with a passing score of 70% or higher. Full instructions for submission are included on the posttest accompanying this CME newsletter. After passing the posttest, you will be issued a CME certificate for 1.0 credit. Certificates of completion will be issued within 2-3 weeks of receipt of the posttest and evaluation.

Disclosure Information

The disclosure policy of Wayne State University School of Medicine requires that the faculty participating in a CME activity disclose to the audience any significant relationship they may have with a pharmaceutical or medical equipment company, product, or service that may be mentioned as part of their presentation, as well as any relationship with the commercial supporter of this activity.

Pranathbarthi Chandrasekar, MD, is a consultant for Enzon Pharmaceuticals, Inc., and has received honoraria/travel expenses, or other financial or material support from Enzon Pharmaceuticals, Inc., Pfizer, Inc., Merck & Co., Inc., and Schering-Plough.

Joseph M. Wiley, MD, is a consultant for Enzon Pharmaceuticals, Inc., and Merck & Co., Inc., and has received honoraria/travel expenses or other financial or material support from Enzon Pharmaceuticals, Inc., and Merck & Co.

This CME activity is supported by an educational grant from Enzon Pharmaceuticals, Inc.

QUESTION & ANSWER

What is the prevalence of invasive fungal infections (IFIs) in the pediatric population? What are the major fungal pathogens?

The pediatric patient groups at highest risk of developing IFIs are those with cancer, in particular acute leukemia, and transplant recipients, especially those undergoing bone marrow transplantation. As in the adult population, fungal infections are a serious and increasingly frequent problem in immunocompromised children, in part related to the aggressive and more potent chemotherapeutic regimens as well as the use of broad-spectrum antibiotics. In one retrospective study, rates of fungal infection in children with cancer or hematologic disease (excluding transplant recipients) increased by 0.89% each year over a 5-year period.¹ The overall incidence of fungal infection was 4.9%. Although patients with acute leukemia represented only 36% of the population, they represented a disproportionately higher proportion of fungal infection cases (67%).

Currently, *Candida* and *Aspergillus* are the two most common infecting pathogens in neutropenic pediatric and adult patients. There is a trend toward a greater rate of *Candida* infections occurring early in the course of chemotherapy treatment. In pediatric acute myeloid leukemia (AML), it may be that many patients will not have proven aspergillosis by the time they recover from the first cycle, but they will harbor the organisms, which will grow and evolve in the second cycle. In a recent nationally based retrospective cohort study, the incidence of invasive aspergillosis was 3.7% in children with AML.² However, the mortality rate with invasive aspergillosis is a concern, as these patients had a fivefold greater risk of death as a result of the infection than did patients with AML who did not have the infection.

Which risk factors does this patient have for a fungal infection?

Patients with AML have a wide variety of risk factors. Our case study involves a child with AML who was treated with dose-intensive chemotherapy that resulted in prolonged neutropenia. Current treatment protocols for AML include treatment with up to five cycles of intensive chemotherapy, making these patients severely neutropenic for prolonged periods of time. In a retrospective single-institution analysis of 115 consecutive children with newly diagnosed or relapsed leukemia, 72 (62.6%) developed fever and neutropenia during induction therapy. Of these, 15 (21%) developed proven IFI (10 *Candida*, 4 *Aspergillus*, and 1 *Rhizopus*).³ This was the first study to demonstrate that pediatric patients with leukemia are at very high risk for the development of new IFI during induction therapy. Our patient had persistent fever in the first cycle and received broad-spectrum antibiotics and azole

prophylaxis early in the course of treatment. All these factors increased this child's risk of fungal colonization and subsequent infection. Other risk factors for IFI include hospitalization and use of corticosteroid therapy.⁴

What evidence or signs suggested a possible mold infection, and what led to the diagnosis of invasive aspergillosis? Why was a galactomannan assay not done?

This case study highlights the challenges of diagnosing an IFI but also makes the point that a series of diagnostic tests are oftentimes needed to make a definitive diagnosis. Importantly, clinical signs of invasive aspergillosis are generally non-specific (eg, fever) as is the appearance of pulmonary consolidations or infiltrates. Other diagnostic tools such as bronchoscopies, serum and blood cultures, and microbiologic tests often have limited value.⁵

This patient was afebrile following antibiotic treatment and fluconazole prophylaxis during recovery in the second period, and results on chest x-ray, abdominal film, and cultures were all negative. In this case, there were no clear signs of IFI. The major issue then was the increasing abdominal pain. We therefore conducted a CT scan of the abdomen, which turned out to be nondiagnostic, but it did show a possible lesion at the level of the diaphragm (Figure). A subsequent chest CT revealed a cavitory lesion, and we conducted video-assisted thoracoscopic surgery to remove the lesion and ultimately confirmed the presence of *Aspergillus fumigatus*. Therapy was then initiated with amphotericin B lipid complex (ABLIC). A microbiologic confirmation of the infection is important to guide treatment. As an example, if *Aspergillus terreus* had been cultured, we would have initiated a different antifungal class, as *A terreus* is not well treated with polyenes. Alternatively, *Zygomycetes* and *Scedosporia* may present in identical fashion, and presuming *Aspergillus* can lead to potential problems if some of the newer triazoles or echinocandins are used, as these agents have modest or no activity against many of the emerging pathogens.

In our case study, a galactomannan assay was not done to test for the presence of *Aspergillus*. Although the clinical specificity and sensitivity of this test in hematology and oncology adult patients are high,⁶ the utility of galactomannan as a screening tool in children is not yet known. Another issue is that our patient was receiving antibiotic treatment with piperacillin-tazobactam, and piperacillin-tazobactam has resulted in false positives



Figure. CT scan demonstrating a cavitory consolidation of the left lower lobe with an "air crescent" sign.

on the galactomannan assay.^{7,8} This phenomenon may be related to the presence of galactomannan of *Penicillium* spp origin introduced during production of the drug^{9,10} and has resulted in the Food and Drug Administration issuing a warning to this effect in February 2004.

What is the relevance of hepatic veno-occlusive disease?

During the second treatment cycle, our patient developed hepatic veno-occlusive disease as a result of chemotherapy. Veno-occlusive disease is microvascular damage to the liver that produces intrahepatic venous obstruction and cholestasis, and has been described with a number of chemotherapeutic agents.¹¹ The major pathophysiologic event is the development of vasculitis with fibrin deposition in the centrilobular veins. Clinical signs include increasing weight gain, enlarged liver, and elevated levels of bilirubin, alkaline phosphatase, and glutamyl transpeptidase. A critical aspect of this disease is that it substantially increases the risk for other organ systems to fail.

In terms of antifungal therapy and veno-occlusive disease, it is important to recognize that therapy with certain azole antifungals, such as voriconazole, can cause hepatotoxicity. In addition, since the azoles are metabolized by hepatic cytochromes, hepatic dysfunction may impair metabolism of the drug and increase the potential for drug-drug interactions. For this reason, hepatic function should be monitored closely during treatment with azoles, especially in patients taking chemotherapeutic regimens that also pose a hepatic risk. Cases of serious hepatic reactions have been observed in patients with hematologic malignancies treated with voriconazole.¹²

What are the data for the different antifungal therapies for the treatment of IFI in the pediatric population?

There are few studies that evaluate the use of antifungal therapy in the pediatric population, and most of these studies are limited by their small sample size, inclusion of adult patients, or evaluation in largely empiric therapy trials. Consequently, management of IFIs in children has been based largely on experience with adult

patients, which is not the best approach and suggests the need for more pediatric studies.

The lipid amphotericin ABLC has the most extensive database in pediatric patients. Currently, the largest study in the pediatric population is based on data from the Collaborative Exchange of Antifungal Research (CLEAR) registry, which enrolled 548 children and adolescents (0 to 20 years of age).¹³ These were predominantly cancer patients or transplant recipients, with AML patients comprising 19.0% of the total population. In the 255 patients with documented IFI (mostly *Candida* or *Aspergillus*), a complete (cured) or partial (improved) response was achieved in 54.5% of patients, with an additional 16.9% of patients having a stable outcome (Table 1). Among patients with proven *Aspergillus* infection, the rate of complete or partial response was 39.1%. In terms of safety, ABLC was relatively renal sparing, with no significant difference observed between the rates of new hemodialysis versus baseline hemodialysis; 24.8% of patients had a greater than 1.5-fold increase in serum creatinine levels from baseline values, and 8.8% of patients had a greater than 2.5-fold increase from baseline values. The latter group represented a greater proportion of patients in the 12- to 20-year age group. Additionally, the study found that a greater percentage of patients with no prior therapy had a greater than 1.5-fold increase from baseline in serum creatinine levels than that of patients with prior therapy, suggesting that de novo treatment with this agent may cause an initial increase in serum creatinine levels but that these levels stabilize over time.

Two smaller studies have also evaluated ABLC. In an emergency-use program, Walsh et al¹⁴ enrolled 111 pediatric patients with IFI who were intolerant of or refractory to conventional antifungal therapy. In the 54 patients evaluated for efficacy, a complete or partial response was achieved in 70% of patients following ABLC therapy. For the entire patient population, there were no significant changes in serum creatinine levels during the study. Similarly, in a retrospective study of 46 pediatric patients with IFI, Herbrecht et al¹⁵ found that 83% of

patients responded to therapy with no significant changes in serum creatinine levels.

Few pediatric studies have been conducted with other antifungal agents. There is a very small retrospective analysis of the lipid product liposomal amphotericin B involving 14 transplant recipients (1 to 16 years of age) with documented IFI who were mostly infected with *Candida* spp.¹⁶ Results showed a clinical response rate of 86%. The second-generation triazole voriconazole has been evaluated in a small open-label trial involving 58 patients between 9 months and 15 years of age with proven or probable IFI.¹⁷ Overall response was achieved in 45% of patients; response rates were 43% in patients with aspergillosis. However, patients with hematologic malignancies demonstrated a response rate of 33%. A retrospective study by Franklin et al¹⁸ demonstrated the safety of caspofungin in 25 immunocompromised pediatric patients. However, efficacy could not be evaluated in this study because of the large number of patients receiving concomitant antifungal agents and the fact that caspofungin was administered in some patients for only 1 day. With micafungin, a recent trial that enrolled 225 patients, including 85 (37.8%) pediatric patients, showed a favorable response (complete or partial) in 35.6% of patients, when used as monotherapy or in combination.¹⁹ These were patients with invasive aspergillosis who had failed to respond to prior therapy or who were unable to tolerate alternative therapy.

Why was this patient initiated on ABLC rather than another antifungal agent for treatment of invasive aspergillosis?

At the time this case occurred, clinicians were transitioning away from conventional amphotericin B deoxycholate (AmBd) to the lipid products. For this patient, we initiated therapy with ABLC because of its extensive efficacy and safety data in pediatric patients, as previously discussed. Few data are available with other agents.

Voriconazole was not yet commercially available at that time. However, either itraconazole or voriconazole probably would not have been a safe choice, at least initially, because of the

Table 1. CLEAR Results: Clinical Response According to Age in Evaluable Patients With Documented IFI (N=255)¹³

Clinical Response	All (n=255)	0-3 mo (n=32)	4 mo-1 y (n=19)	2-11 y (n=87)	12-20 y (n=117)
Cured	74 (29.0)*	19 (59.4)	6 (31.6)	23 (26.4)	26 (22.2)
Improved	65 (25.5)	4 (12.5)	6 (31.6)	24 (27.6)	31 (26.5)
Stable	43 (16.9)	3 (9.4)	5 (26.3)	19 (21.8)	16 (13.7)
Deteriorated	73 (28.6)	6 (18.8)	2 (10.5)	21 (24.1)	44 (37.6)
Cured + improved	139 (54.5)	23 (71.9)	12 (63.2)	47 (54.0)	57 (48.7)
Cured + improved + stable	182 (71.4)	26 (81.3)	17 (89.5)	66 (75.9)	73 (62.4)

*Numbers in parentheses, percent.

CLEAR, Collaborative Exchange of Antifungal Research; IFI, invasive fungal infection. Wiley JM, Seibel NL, Walsh TJ. Efficacy and safety of amphotericin B lipid complex in 548 children and adolescents with invasive fungal infections. *Pediatr Infect Dis J*. 2005;24:167-174. Reprinted with permission.

Table 2. Preferred Pediatric Dosing of Approved Systemic Antifungal Agents^{20,21}

Antifungal Drug	Preferred Adult Dosing	Preferred Pediatric Dosing
Polyene		
Amphotericin B deoxycholate	1-1.5 mg/kg/day	Same as adult
Amphotericin B lipid complex	5 mg/kg/day	
Amphotericin B colloidal dispersion	5 mg/kg/day	
Liposomal amphotericin B	5 mg/kg/day	
Triazole		
Fluconazole	100-800 mg/day 3-6 mg/kg/day	6-12 mg/kg/day
Itraconazole	200-400 mg/day	2.5-5 mg/kg/dose bid
Voriconazole	Load: 6 mg/kg/dose bid x 1 day Maintenance: 3-4 mg/kg/dose bid	Load: same as adult Maintenance: 4-8 mg/kg/dose bid* (infants†: 8 mg/kg q12h x 1 day, then 6 mg/kg q12h)
Echinocandin		
Caspofungin	Load: 70 mg qd x 1 day Maintenance: 50 mg qd	Load: 70 mg/m ² qd x 1 day Maintenance: 50 mg/m ² qd (neonates: 1 mg/kg/day x 2 doses, then 2 mg/kg/day)
Micafungin	50-150 mg/day	3 mg/kg/day†
Anidulafungin	Load: 100-200 mg Maintenance: 50-100 mg/day	None established

Reprinted from *Pediatr Clin N. Am.* 2005;52:895-915. Steinbach WJ. Antifungal agents in children, with permission from Elsevier.

*Suggested dosing by the author; exact pediatric dosing for voriconazole not yet determined. †More studies need to be done to determine optimal dosing.

potential added risk of hepatotoxicity in a child who was diagnosed with veno-occlusive disease. It would have been possible to switch to an azole therapy, however, when his bilirubin levels returned to normal. Also, itraconazole or voriconazole may have been a good choice when transitioning to outpatient therapy, since they are available in oral dose form. The issue to consider, however, is that in the next course of chemotherapy, the patient may present again with liver toxicity.

Treatment with an echinocandin is also a possibility. Caspofungin has been shown to be effective for the treatment of invasive aspergillosis in patients who are refractory to or intolerant of other antifungal therapies, including amphotericin B. Micafungin, although not indicated for the treatment of invasive aspergillosis, has demonstrated efficacy in this setting.¹⁹ In considering that the patient may undergo another cycle of chemotherapy, these agents would be less toxic than voriconazole or itraconazole, but these parenteral drugs are more difficult to use on an outpatient basis.

What are the differences, if any, in the pharmacokinetic profiles of different antifungal agents in adults versus children? How do these differences affect dosage?

The pharmacokinetics of the three lipid amphotericin products (ie, ABLC, liposomal amphotericin B, and amphotericin B colloidal dispersion [ABCD]) do not appear to be different between adults and children, and dosing is the same (Table 2).^{20,21} However, for conventional AmBd, the volume of dis-

tribution, when corrected for body weight, is smaller and the clearance rate is faster in children than adults.²² Premature neonates, in particular, have extreme individual variation in the distribution and clearance of AmBd. When using AmBd, dosing in children should be individualized based on therapeutic drug monitoring.²² Overall, the tolerability of children is higher than that of adults, and the younger the child, the greater the tolerability. However, to reduce the risk of nephrotoxicity during early life, which can have long-term consequences, use of a lipid amphotericin product should be considered over the conventional formulation.^{20,22}

With the azoles, there are differences in pharmacokinetics between children and adults. Generally, serum concentrations are lower and clearance rate is faster in children, necessitating higher dosages.²⁰ As an example, in adults, voriconazole is metabolized in a nonlinear fashion, with an approximate threefold increase in the area under the concentration-time curve after a 33% increase in dosage. In contrast, elimination of voriconazole appears to be linear in children after dosages of 3 or 4 mg/kg every 12 hours.²³ The differences are more apparent at 12 years of age and become more substantial the younger the child.

For the echinocandins, the overall pharmacokinetic profile does not appear to be very different between children and adults. However, with caspofungin, it is recommended to dose children by body surface area instead of weight (as in adults). This is based on results of a pharmacokinetic study conducted by Walsh et al.²³ The weight-based approach in children

(2 to 17 years of age) resulted in suboptimal plasma concentrations, whereas the 50-mg/m²/day dosage provided concentration-time curves that were comparable to those of adults treated with the standard regimen of 50 mg/day. Micafungin demonstrates linear pharmacokinetics in children, which is consistent with that of adults, but there is increasing clearance in those less than 9 years of age.²⁴ With the newest antifungal anidulafungin, a small pharmacokinetic study showed that pediatric patients receiving 0.75 or 1.5 mg/kg/day have drug concentration profiles similar to those of adult patients receiving 50 or 100 mg/day, respectively.²⁵

In summary, pediatric patients with acute leukemia, including AML, are a group at high risk for IFI due to periods of prolonged neutropenia as a result of chemotherapy. A definitive diagnosis of IFI is challenging, and screening tests are less established in the pediatric population than the adult population. In our case study, both a surgical biopsy and culture were paramount in establishing disease and initiating appropriate antifungal therapy. Surgery to remove the infected tissue may improve the outcome of invasive aspergillosis, but this has not been well studied. There are few large trials evaluating antifungal therapy in the pediatric population, and extrapolating data from adult studies is not optimal. Future research will help clinicians to better understand the pharmacodynamics and pharmacogenetics of children that determine outcome in infection rather than looking at just the specific drug or disease.

References

- Rosen GP et al. *J Pediatr Hematol Oncol.* 2005;27:135-140.
- Zaoutis TE et al. *Pediatrics.* 2006;117:e711-e716.
- Wiley JM et al. *J Clin Oncol.* 1990;8:280-286.
- Abbasi S et al. *Clin Infect Dis.* 1999;29:1210-1219.
- Reichenberger F et al. *Eur Respir J.* 2002;19:745-755.
- Hope WW et al. *Lancet Infect Dis.* 2005;5:609-622.
- Sulharian A et al. *N Engl J Med.* 2003;349:2366-2367.
- Viscoli C et al. *Clin Infect Dis.* 2004;38:913-916.
- Walsh TJ et al. *J Clin Microbiol.* 2004;42:4744-4748.
- Marchetti M et al. *Antimicrob Agents Chemother.* 2005;49:3984-3985.
- Kumar S et al. *Mayo Clin Proc.* 2003;78:589-598.
- Vfend [package insert]. New York, NY: Pfizer Roerig; 2006.
- Wiley JM et al. *Pediatr Infect Dis J.* 2005;24:167-174.
- Walsh TJ et al. *Pediatr Infect Dis J.* 1999;18:702-708.
- Herbrecht R et al. *Eur J Clin Microbiol Infect Dis.* 2001;20:77-82.
- Ringden O et al. *Pediatr Transplant.* 1997;1:124-129.
- Walsh TJ et al. *Pediatr Infect Dis J.* 2002;21:240-248.
- Franklin JA et al. *Pediatr Infect Dis J.* 2003;22:747-749.
- Denning DW et al. *J Infect.* 2006; May 5 [Epub ahead of print].
- Steinbach WJ. *Pediatr Clin North Am.* 2005;52:895-915.
- Pannaraj PS et al. *Pediatr Infect Dis J.* 2005;24:921-922.
- Goldman RD, Koren G. *J Pediatr Hematol Oncol.* 2004;26:421-426.
- Walsh TJ et al. *Antimicrob Agents Chemother.* 2005;49:4536-4545.
- Seibel NL et al. *Antimicrob Agents Chemother.* 2005;49:3317-3324.
- Benjamin DK Jr et al. *Antimicrob Agents Chemother.* 2006;50:632-638.