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# Association of *Pseudomonas cepacia* with Chronic Granulomatous Disease

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*Pseudomonas cepacia* was recovered from a number of infected sites in three patients with chronic granulomatous disease of childhood. The organisms were identified on the basis of their oxidative utilization of a variety of carbohydrates and their positive β-galactosidase and oxidase activities. They were resistant to most antimicrobial agents and moderately susceptible to chloramphenicol. Peripheral blood leukocytes isolated from two siblings with chronic granulomatous disease, including one of the patients in this series, failed to kill *P. cepacia* in vitro. Prolonged prophylactic and antimicrobial therapy may well have played a significant role in the colonization and infection of these patients with *P. cepacia*.

Definitive taxonomic and biochemical clarification of the genus *Pseudomonas* has led to a greater awareness of infection caused by unusual *Pseudomonas* species. As a result, *Pseudomonas putrefaciens*, *Pseudomonas stutzeri*, and *Pseudomonas maltophilia* have recently been incriminated in a variety of infectious processes (7, 18, 25).

*Pseudomonas cepacia*, variously described under the names EO-1 (eugonic oxidizer group 1) (1, 3, 6, 15), *P. kingii* (13), and *P. multivorans* (1), is another species that has come under investigation since documentation of its virulence as a cause of infections primarily of nosocomial origin (1, 2, 10, 16, 18). *P. cepacia* has been recovered from a variety of human clinical sources, including blood, cerebrospinal fluid, joint fluid, and abscess cavities, wherein its role as the primary etiological agent is unquestionable (3, 7, 9, 13, 18, 20, 22). This report describes the recovery of *P. cepacia* from three patients with chronic granulomatous disease (CGD) of childhood. The clinical and biochemical features that may lead to the colonization and infection of patients with CGD by *P. cepacia* are discussed.

## CASE REPORTS

**Case 1—PA.** From 11 months of age, recurrent furuncles occurred. At 19 months, peritonitis due to *Serratia marcescens* and *Streptococcus faecalis* was successfully treated. At 27 months of age, the diagnosis of CGD was established (24) and, because of recurrent staphylococcal cervical adenitis, therapy with chloramphenicol was instituted and continued for 1 year. At 44 months of age, bilateral pneumonia developed with a right pleural effusion unresponsive to penicillin and, subsequently, to intravenous chloramphenicol. Blood cultures were repeatedly negative. However, *P. cepacia* was grown from fluid aspirated from the right pleural space. The isolate was sensitive in vitro only to 15 μg of chloramphenicol and to 250 μg of sulfadiazine per ml. Intravenous sulfisoxazole (2 g daily) was added, and fever, pulmonary infiltrates, and pleural effusion resolved. Daily oral administration of sulfisoxazole (2 g) and chloramphenicol (750 mg) was continued for 2 months.

One year later, the patient developed relapsing pneumonitis and empyema. *P. cepacia* was repeatedly cultured from the pleural fluid. In spite of intensive therapy with appropriate antibiotics and multiple transfusions of HL-A compatible leukocyte concentrates obtained from the patient's father and paternal aunt, he died 3 months later without showing improvement. Autopsy revealed severe necrotizing bilateral pneumonia. *P. cepacia* was recovered from both lung and spleen.

**Case 2—BL.** This 6-year-old white male had a life-long history of recurrent skin, lymph node, and eyelid infections which responded to antibiotic therapy. At the age of 3, when the diagnosis of CGD was established, he was begun on oral chloramphenicol and remained well for 1 year. Two months prior to admission, daily fever to 105 F (40.6 C) commenced, unresponsive to oral chloramphenicol. One week before admission, left cervical adenitis became apparent which, after hospitalization, was treated by surgical excision. The remainder of the admission physical examination was normal except for slight hepatosplenomegaly.

Histological examination of the excised node revealed hyperplasia with multiple abscesses and granulomas. Direct-touch preparations from the tissue showed rare slender, gram-negative rods with rounded ends which subsequently proved to be *P. cepacia*,

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sensitive in vitro only to chloramphenicol (15 μg/ml). Smear and cultures for mycobacteria were negative. Chloramphenicol therapy (250 mg intravenously four times daily) was instituted with slow defervescence over 8 days. Oral chloramphenicol (250 mg four times daily) and sulfisoxazole (750 mg four times daily) were continued for 3 months after discharge without recurrence of *P. cepacia* infection over a 3-year period, although recurrent staphylococcal infections of the eyelids and perianal area have continued.

**Case 3—MH.** Right perinephric abscess due to *Escherichia coli* was surgically drained in this patient at age 4 months. Multiple furuncles, skin abscesses, and cervical adenitis due to *Staphylococcus aureus* continued to be a problem. At age 8 years, he developed severe pneumonia and multiple purulent skin abscesses from which *P. cepacia* was recovered. Four months of therapy with chloramphenicol and sulfisoxazole produced clinical cure. However, he experienced continued staphylococcal pyoderma during the following 4 years of observation.

## RESULTS

**Intraleukocytic bactericidal activity versus P. cepacia.** Intracellular killing of *P. cepacia* by leukocytes was studied by the method of Keusch et al. using unfractionated whole blood and a bacteria-phagocyte ratio of approximately 1:500 (14). At 0, 15, 60, and 120 min, the number of viable organisms was quantitated by standard pour-plate technique after hypotonic lysis of the leukocytes to release viable intracellular bacteria. Since the organism was serum resistant, reduction in viable count was considered to represent intracellular bacterial killing. No antibiotics were added during incubation to kill extracellular bacteria.

Intracellular killing of *P. cepacia* by leukocytes from patient 3, his brother, who also is affected with CGD, and a normal control is shown in Fig. 1. In contrast to the control, which showed greater than 90% kill during the study, the CGD leukocytes failed to reduce the viable colony count of *P. cepacia*. Similar results were obtained when *S. aureus* was utilized as the test organism.

**Bacteriology.** In all instances, the isolates recovered were pale-staining, gram-negative bacilli displaying some tendency toward pleomorphism, ranging from short coccobacilli to more elongated slender rods.

These actively motile organisms grew well on 5% sheep blood agar (BBL) and on MacConkey and Endo agars. Two of the three strains developing on the latter medium produced a deep lavender discoloration which became more pronounced with prolonged incubation. None of the strains grew on *Salmonella-Shigella* agar (Difco) or hektoen-enteric agar (Pfizer).

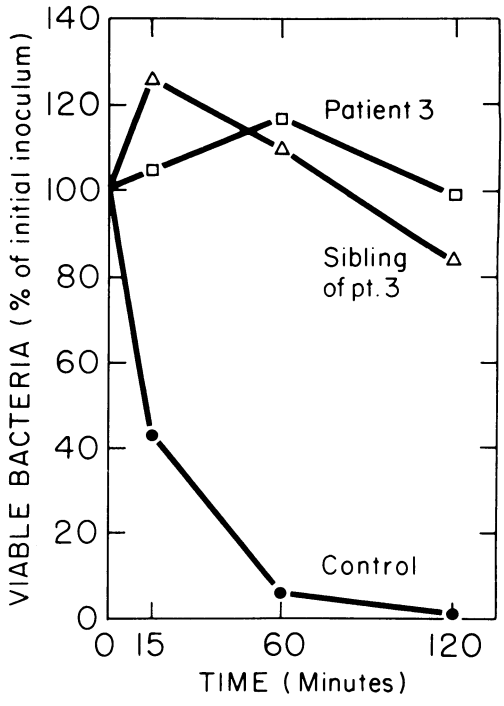


FIG. 1. *Intracellular killing of P. cepacia by leukocytes from a CGD patient and sibling at various time intervals as compared to control leukocytes.*

TABLE 1. *Distinguishing characteristics of P. cepacia*

| Test[a] | Result |
|---|---|
| TSI | |
| Slant | No change |
| Butt | No change |
| Pigment | Yellow |
| Motility | Positive |
| Oxidation | |
| Glucose | Positive |
| Lactose | Positive |
| Maltose | Positive |
| Mannitol | Positive |
| Sucrose | Positive |
| Growth | |
| 6.5% NaCl | Negative |
| Endo agar | Positive |
| H-E agar | Negative |
| MacConkey agar | Positive |
| SS agar | Negative |
| β-Galactosidase (ONPG) | Positive |
| Catalase | Positive |
| Citrate utilization | Positive |
| Nitrate reduction | Positive |
| Oxidase | Positive |

[a] TSI, Triple-sugar iron agar; H-E, hektoen-enteric agar; SS, *Salmonella-Shigella* agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

Biochemically, the isolates were characterized as members of the genus *Pseudomonas* by their oxidation, but not fermentation, of glucose (11), cytochrome oxidase activity, and lack of reactivity on triple-sugar iron agar and Kligler's iron agar slants. Definitive identification as *P. cepacia* was based upon oxidation of lactose, maltose, mannitol, and sucrose; positive reactions for catalase and β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside tablets, Key Scientific, Los Angeles, Calif.); nitrate reduction to nitrite; citrate utilization (Simmons); and failure to grow in the presence of 6.5% NaCl. An additional characteristic aiding identification was the production by the isolates of a yellow pigment after 48 h on triple-sugar iron agar and 24 h on Kligler's iron agar slants at 37 C (Table 1).

In vitro antimicrobial susceptibility studies performed by the two-tube broth dilution method (21) showed the three isolates to be resistant to ampicillin (10 μg/ml), carbenicillin (100 μg/ml), cephalothin (15 μg/ml), colistin (5 μg/ml), gentamicin (10 μg/ml), neomycin (20 μg/ml), streptomycin (20 μg/ml), sulfadiazine (250 μg/ml), and tetracycline (4 μg/ml). With chloramphenicol, exact minimal inhibitory concentrations demonstrated that one of the isolates required 6 μg/ml and two required 9 μg/ml for inhibition. Two isolates tested by the agar disk-diffusion method were susceptible to a combination of trimethoprim and sulfamethoxozole (1:20) using a 25-μg (total content) disk.

## DISCUSSION

CGD is a hereditary disorder seen predominantly in male children and characterized by recurrent suppurative infections and granuloma formation. In vitro studies have demonstrated the failure of neutrophiles and monocytes from CGD patients to kill certain ingested microorganisms. This marked impairment in bactericidal activity is in part attributed to the inability of the phagocyte to produce adequate amounts of hydrogen peroxide (12), which in normal phagocytes is an essential part of a potent intracellular bactericidal system consisting of the enzyme myeloperoxidase, $H_2O_2$, and a halide ion. As a result, phagocytes are unable to destroy catalase-producing microorganisms such as *S. aureus*, *Enterobacteriaceae*, *Pseudomonas* spp., *Nocardia*, and fungi, and patients are frequently infected with these species (8, 12). Although these organisms are readily engulfed by CGD leukocytes, they survive intracellularly and thus may be widely distributed throughout the body, usually resulting in multiple abscesses. The present study demonstrated that *P. cepacia*, another catalase-producing bacterium, can survive within CGD leukocytes and in this regard behaves very much like *S. aureus*. The patient with CGD is thus primed for infection with this organism.

*P. cepacia* exists in nature as a plant pathogen or saprophyte and, because of its low invasiveness, is not considered to be a primary human pathogen (5). In our patients, however, prolonged prophylactic antimicrobial therapy, particularly with chloramphenicol and sulfonamide, agents to which the isolates were not highly sensitive in vitro, may well have played a significant role in colonization and infection of these patients with *P. cepacia*. According to Weinstein and co-workers (26), unless high doses of chloramphenicol are used, patients may continue to shed the organism despite continued treatment.

Infections with *P. cepacia* occur in compromised patients (5) and are usually of nosocomial origin. Although this microbial species had not been recovered from other hospitalized patients during the admissions of the three described patients, it is still conceivable that they may have been exposed to this microorganism during one of their numerous hospital admissions. Prolonged prophylactic therapy between these admissions may then have enhanced colonization with *P. cepacia*.

Regarding susceptibility to various antimicrobials, *P. cepacia* is distinguished by its usual resistance to those agents frequently utilized to treat *Pseudomonas* infections, including carbenicillin, gentamicin, and colistin or polymyxin. In other studies it has been sensitive to chloramphenicol and sulfonamide (5, 7, 16, 26). In the present study, however, the three isolates tested were only moderately sensitive to chloramphenicol and resistant to sulfonamide, although two patients responded to this combination of drugs. Recently, sulfonamide and trimethoprin in combination (trimethoprin-sulfamethoxazole) have been shown to be efficacious in the treatment of *P. cepacia* infections (9, 18) and may be a useful combination of agents against this species in patients with CGD.

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