

Human Protothecosis

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INTRODUCTION

TAXONOMY

Human protothecosis is a rare infection caused by members of the genus *Prototheca*. These organisms are generally considered to be achlorophyllic algae and are ubiquitous in nature (55). The first description of human infection attributed to *Prototheca* species was made by Davies and colleagues in 1964 (36). Most such infections are probably caused by traumatic inoculation into subcutaneous tissues. Olecranon bursitis and localized cutaneous infections are more commonly developed in immunocompetent patients, whereas dissemination and visceral involvement mainly affect patients with compromised host immunity (80). Such conditions have commonly been summarized as “defects in cell-mediated immunity,” although the specific circumstances under which *Prototheca* infection develops are not always predictable. *Prototheca* species are rare but often endemic in cattle, with bovine mastitis being the most reported infection (57, 135).

Prototheca spp. exist in the environment as ubiquitous detritus inhabitants and contaminants of various substrates (100). Their role as pathogens causing human diseases is largely unknown. Given the increasing numbers of immunocompromised individuals throughout the world, the incidence of infection caused by unusual organisms is bound to increase. This review provides a summary of the literature addressing biological, clinical, and epidemiological aspects of human *Prototheca* infection.

The taxonomic position of *Prototheca* has been disputed for a long time. Currently, it is classified among the lower algae, the *Chlorophyceae*, based on its ultrastructure, the occurrence of plastid-like granules in plasma, and its asexual method of reproduction through free cell formation (Fig. 1).

In 1894, Krüger described two microorganisms isolated in Germany from mucous flux of *Tilia* and *Ulmus* spp., namely, *Prototheca moriformis* and *Prototheca zopfii* (72). Although the generic diagnosis indicated these organisms to be fungi, they could not be connected with saccharomycetes or the lower phycomycetes. In 1913, Chodat reclassified them as algae because their spores are produced internally in a manner identical to that of the green algae *Chlorella* (29). In 1930, Ashforth et al. (4) isolated *Prototheca portoricensis* and *P. portoricensis* var. *trispurus* from cases of human sprue in tropical areas and confirmed the ability of this genus to grow on chemically defined media. Ciferri (30) reclassified them as saccharomycetes in 1957. It is now generally considered that the genus developed from *Chlorella* at some point in evolution (14, 46, 60, 121) and evolved differences in cell wall composition, physiology, and the ability to survive environmental stress (14, 36, 37).

In 1972, Arnold and Ahearn (3) investigated the carbohydrate and alcohol assimilation patterns of *Prototheca* and considered the validity of the following five species: *Prototheca filamenta*, *P. moriformis*, *Prototheca stagnora*, *Prototheca wick-erhamii*, and *P. zopfii*. Nadakavukaren and McCracken (97) studied the ultrastructure of *P. filamenta* and pointed out that this *Prototheca* species was misclassified. Subsequently, King and Jong (67) proposed the new genus *Sarcinosporon* and suggested *P. filamenta* to synonymous with *Sarcinosporon inkin*. Thereafter, Pore et al. (122) carried out comprehensive mor-

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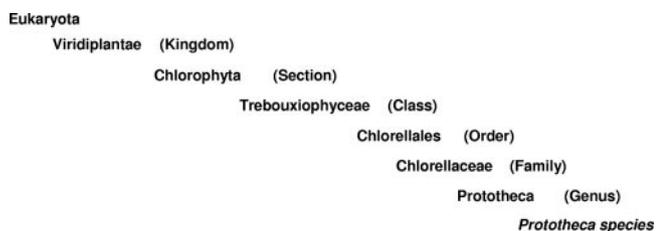


FIG. 1. Taxonomy of the genus *Prototheca*. (Reprinted from the website of the Prototheca Group, Institute for Animal Hygiene and Veterinary Public Health of the University Leipzig [http://www.prototheca.com], with permission.)

phological and physiological studies on *P. filamenta*. The genus *Fissuricella* was proposed to accommodate *P. filamenta*, with the binomial *Fissuricella filamenta*. Morphological, physiological, and immunofluorescence studies by Sudman and Kaplan (143) demonstrated *P. moriformis*, *Prototheca chlorellioides*, *Prototheca pastoriensis*, *Prototheca trispora*, and *Prototheca ubrizysi* to be synonymous with *P. zopfii*. The following three valid species in the genus *Prototheca* were suggested: *P. stagnora* Cooke 1968, *P. wickerhamii* Tubaki and Soneda 1959, and *P. zopfii* Krüger 1894.

In 1985, Pore (120) reviewed the taxonomy of the genus and considered *P. wickerhamii*, *P. moriformis*, *P. zopfii*, and *P. stagnora* to be valid species. Currently, *P. zopfii*, *P. wickerhamii*, *P. stagnora*, *Prototheca ulmea*, and *Prototheca blaschkeae* sp. nov. (3, 126, 153) are assigned to the genus. The species *P. moriformis* is not generally accepted (126, 153), as it is genetically and biochemically very similar to *P. zopfii*; otherwise, there is a marked heterogeneity between strains of *P. moriformis*. The transfer of *P. wickerhamii* to *Auxenochlorella* or to a new genus, as suggested by Ueno et al. (154), is also in progress. So far, *P. wickerhamii* and *P. zopfii* have been reported to cause infections in humans, with *P. wickerhamii* being the more common of the two (12, 56, 143, 150). Synonyms for *P. zopfii* Krüger 1894 are *P. chlorellioides* Beijerinck 1904; *P. portoricensis* Ashford, Ciferri, and Dalmau 1930; *P. trispora* Ciferri, Montemartini, and Ciferri 1951 (formerly *P. portoricensis* var. *trisporus*); *Prototheca ciferrii* Negroni and Blaisten 1941; *Prototheca segbwema* Davies, Spencer, and Waklein 1964; *Prototheca ubrizysi* Zsolt and Novak 1968; and *Prototheca hydrocarbonea* Kockova-Kratochvilova and Havelkova 1974 (69, 120).

Recently, a novel thermotolerant strain of *P. zopfii* was isolated from a hot spring (155). Its taxonomic characteristics coincided with those of *Prototheca zopfii* var. *hydrocarbonea*, and phylogenetic analysis based on a small-subunit (SSU) rRNA gene sequence also revealed a close relationship between the two strains.

BIOLOGY

Prototheca species are spherical unicellular organisms ranging from 3 to 30 μm in diameter. These organisms do not possess glucosamine, a specific fungal cell wall component, or muramic acid, a specific component found in bacterial cell walls (69, 83, 159). *Prototheca* species are distinguished from other algae, such as *Chlorella*, by their lack of chloroplasts and

the presence of a two-layered, instead of three-layered, cell wall on electron microscopy (40, 60, 100).

The achloric *Prototheca* species are heterotrophic and require external sources of organic carbon and nitrogen (70). Their life cycle is similar to that of algae from the genus *Chlorella* (40, 120).

Reproduction is asexual, and during cell maturation, the cytoplasm undergoes a process of cleavage to form endospores (143). These spores increase in size upon release from the mother cell and go through an assimilative stage. About 2 to 20 endospores develop that are initially irregular in shape, and the sporangia (mother cells) break under pressure from the enlarging spores; release of the spores is passive (143), and their number and size vary among the species (105). Spore release takes place every 5 to 6 h in the presence of adequate nutrients (60). The sporangia of *P. wickerhamii* (3 to 10 μm in diameter) are generally smaller than the sporangia of *P. zopfii* (7 to 30 μm in diameter). Another trait of *P. wickerhamii* is the morula form of its sporangia, with endospores arranged symmetrically like a daisy; other species, including *P. zopfii*, do not form these multiple septated structures.

P. zopfii provides an experimental system for studying cytokinesis and daughter cell number variation in multiple fission (124). Although the mean daughter cell number increases linearly with the growth rate and this dependency is genetically controlled, pedigree analysis showed that daughter cell number variation is not under direct genetic control. In a population growing in steady-state balanced growth, each cell has a given probability of dividing into 2, 4, 8, 16, or 32 daughter cells. These probabilities are independent of the division number of the cell in the preceding generation and can be altered by changes in the culture medium.

Nutritional traits possessed by all species of *Prototheca* include the utilization of salts of ammonium but not of nitrate (72, 121). *Prototheca* species assimilate glucose, fructose, and galactose; disaccharides are not metabolized. All species require thiamine and oxygen for growth, but light does not promote growth (2, 72). Irradiation with blue light inhibits the respiratory capacity of *P. zopfii* (43). Little is known about the fermentation behavior of *Prototheca* under anoxic conditions. *P. zopfii* converts 1 mol of glucose to 2 mol of lactic acid, like homolactic fermenting bacteria (5), and it was thought that *Prototheca* lacks the ability to produce gas from glucose anaerobically (120). The novel thermotolerant strain of *P. zopfii* var. *hydrocarbonea* was found to produce an appreciable amount of ethanol and CO_2 from glucose under anoxic conditions at 25 and 40°C. This type of alcohol fermentation has not yet been reported for the genus *Prototheca*. *P. zopfii* var. *hydrocarbonea* also creates gas from sucrose at 40°C (155). D-Lactic acid, ethanol, CO_2 , and a trace of acetic acid are produced from glucose, but L-lactic acid, formic acid, and H_2 are not. At 25°C, D-lactic acid and ethanol are produced in approximately equimolar amounts under $\text{N}_2\text{-H}_2\text{-CO}_2$, whereas ethanol production is predominant under N_2 . More ethanol is produced at 40°C than at 25°C, irrespective of the gas composition of the atmosphere. Walker et al. (160, 161) described *n*-hexadecane utilization and crude oil degradation by *P. zopfii*.

Several phenotypic studies support the recognition of distinct clusters within *Prototheca*, which have been named "variants" (10). *P. zopfii* has consistently been divided into three

variants or biotypes (I to III) (127). Auxanographic and biochemical investigations of various isolates revealed that all bovine mastitis isolates showed delayed assimilation of galactose, and thus they were assigned to variant II. Isolates from swine farms (variant III) were not able to assimilate glycerol (127, 130). Comparative investigations by means of Fourier transform infrared spectroscopy (FTIR) showed distinct differences between variant III and the other two variants. Discrimination was not possible between strains assigned to variants I and II (132). However, serological typing by immunoblotting revealed major differences in the patterns of immunogenic structures between the three *P. zopfii* biotypes (127). Based on sequence analysis of the 18S rRNA gene and determination of cellular fatty acids, Roesler et al. (126) suggested that biotype III is a novel species, *P. blaschkeae* sp. nov. In addition, two novel genotypes, types 1 and 2, were proposed for the current biotypes 1 and 2 of *P. zopfii*.

PATHOGENESIS

Algae were previously not considered pathogens in humans; *Prototheca* species isolated from previously damaged skin, blood, or feces have been interpreted as contaminants in the majority of cases. *P. filamenta* was isolated from a case of athlete's foot and was thought to be a skin saprophyte (3). Sonck and Koch (140) recovered *P. wickerhamii* from five patients with dermatologic diseases but suggested *Prototheca* to be a skin saprophyte. The organism is of low virulence in patients with intact immune systems (31), and infection usually spreads indolently in local areas (109). In general, *Prototheca* species have rarely been described as pathogens in malignancy and human immunodeficiency virus (HIV) disease, implying a low pathogenic potential in this population (28, 41, 150, 164).

The pathogenesis of protothecosis is largely unknown. It is believed that *Prototheca* species may infect humans through contact with potential sources or by traumatic inoculation with the algae (28, 45, 59, 75, 80). Systemic and local predisposing factors can be identified in nearly all patients. Humans with cellular deficiency are at risk for protothecosis (56), and it has been postulated that quantitative (112) and qualitative (15) defects in neutrophil function play an important role in the host defense against *Prototheca* species (12, 24, 157). Human polymorphonuclear neutrophils (PMNs) ingest and kill *P. wickerhamii*. Ultrastructural studies revealed digestion of the organism by PMNs 60 min after engulfment, and optimal killing required the presence of both specific immunoglobulin G antibody and heat-stable serum opsonins (112). Individuals with neutrophils incapable of killing *P. wickerhamii* (15) suffer from protothecosis. In contrast, for cancer patients, neutropenia does not appear to be an important risk factor, as only 2 of 13 patients with protothecosis were neutropenic (150). The fact that there are relatively few cases of protothecosis in AIDS patients suggests that a type of immunodeficiency other than that caused by AIDS contributes to susceptibility to protothecosis (15). One patient did not develop dissemination despite a CD4 count of 38 cells/mm³ (115). Tyring et al. (152) also suggested a role for natural killer cell activity in the pathogenesis of protothecal infections. Other host factors have not been identified.

In general, *Prototheca* spp. also seem to have low virulence

in animal experiments. Phair et al. (112) were unable to induce *P. wickerhamii* infections in neutropenic guinea pigs or athymic mice. Animals displayed local reactions at the point of injection despite high inoculum doses, and only a few cases of protothecosis have been caused in laboratory animals. In contrast, *P. zopfii* is lethal for immunosuppressed mice when used as an inoculum of 10⁶ CFU. Overall, pathogenicity and virulence are moderate, and *Prototheca* species are considered rare opportunistic pathogens (55).

Krcméry, Jr., et al. (71) reviewed 108 cases of human protothecosis and suggested a 2.2% attributable mortality rate, which is a much lower rate than that for candidemia.

EPIDEMIOLOGY

Hospital-acquired cases of protothecosis have been reported in association with surgery and orthopedic procedures (53, 54, 94, 100, 139, 150). Infection may also occur by penetration of the agent when a skin injury comes in contact with contaminated water (45, 59, 161). *Prototheca* spp. have been found to colonize the human skin, fingernails, respiratory tract, and digestive system (10, 125, 133, 140, 157, 163). Up to now, 117 cases of protothecosis have been described in the literature, of which 66% ($n = 77$), 19% ($n = 22$), and 15% ($n = 18$) were associated with cutaneous infection, systemic infection (defined as the presence of *Prototheca* species in noncontiguous organs), and olecranon bursitis, respectively. Among patients with dissemination, 59% were cured.

Prototheca infection is exogenous or endogenous and usually nontransmissible (55, 75, 100, 150); it is believed that *Prototheca* species may infect humans through contact with potential sources, such as contaminated soil or water, by traumatic inoculation with the algae, or even through insect bites (22, 75, 139, 163). Also, rare cases of onychoprotothecosis have been described (47, 168). Endogenous infections may arise in colonized patients with predisposing factors (65, 78, 148, 150).

Prototheca spp. are globally ubiquitous (123) and can be isolated from various reservoirs, such as the environment, animals, and food (55, 100, 123). Typical sources of *Prototheca* species are the slime flux of trees, grass, fresh and salt water, wastewater, animals such as cattle, deer, and dogs, stables, animal buildings, excrement (22, 123, 142), and food items such as butter, potato peels, cow's milk, soil, and bananas (72, 99, 100, 117, 119, 135, 166). Since chlorination is not uniformly effective in eliminating potentially pathogenic *Prototheca* species from the effluents of sewage water and household waste, the algae survive and return to the environment (163). Each species or strain has a different susceptibility to commercial chlorinating agents (89, 123).

The skin is the organ most frequently involved in protothecal infection; protothecal infections are common in patients with underlying immunosuppression or several underlying diseases (Table 1). Patients with steroid use, hematologic or solid-tissue malignancy, or diabetes mellitus are somewhat at risk for protothecosis. Of all the immunosuppressive drugs prescribed, glucocorticoids are the most widely used and the most specifically associated with the onset of *Prototheca* infection. Infection has resulted, e.g., from locally injected steroids (66, 80, 109), systemic steroids (78, 109, 152), oral steroids (12), and even the application of topical steroid cream (45). Infection

TABLE 1. Main underlying diseases and comorbid conditions of patients with protothecosis

Underlying factor	No. of patients	References
Local or systemic steroid use	24	12, 27, 32, 45, 50, 52, 56, 66, 80, 84, 88, 92, 100, 109, 146, 151, 152, 162
Hematologic malignancy or cancer	16	15, 39, 53, 65, 74, 78, 85, 100, 144, 149, 150, 163
Diabetes mellitus	13	48, 56, 79, 88, 100, 149, 164, 165
AIDS	7	15, 62, 77, 107, 115, 116, 166
Organ transplantation	6	35, 56, 144, 146, 164, 165
Surgery	6	54, 56, 94, 100, 139, 149
Alcoholism	4	64, 100, 109
Peritoneal dialysis	3	48, 102, 130

has also resulted in diseases that are themselves treated with steroids, e.g., lupus erythematosus (147), lymphoma (53), rheumatoid arthritis (27, 100), and myasthenia gravis (92). One likely explanation for the ability of glucocorticoids to induce infection is their acute suppression of lymphocyte activation and impairment of PMNs and macrophages (138). However, no experimental work has been done to support this hypothesis.

Protothecosis occurs globally and has been reported on every continent except Antarctica (100). Cases have been reported from Europe (61, 77, 78, 93, 129), Asia (28, 85, 86, 96, 145, 147, 169), Africa (36), Oceania (55), and South and North America (45, 90, 166, 167), particularly the southeast United States (45, 55, 158, 166). In Taiwan, the rural region is most affected, displaying a high population of farmers and elderly people (27).

The majority of patients with protothecosis are over 30 years of age or elderly, but cases have also been described for children and neonates (53, 56, 61, 150, 151, 165).

The incubation period for protothecal infection is not well documented (166). Periods of weeks to months have been suggested (27, 150), but in most cases, patients were not able to remember the time of trauma and thus the length of time the organisms were harbored. Reports have confirmed incubation times of 10 days to 4 months. A local or systemic immunosuppressive factor is given in half the cases of protothecosis (80).

Workers in rice paddies, fishermen (146), farmers (27), handlers of raw seafood, and aquarium staff (12) are especially at risk for exposure to *Prototheca* species.

CLINICAL FINDINGS

The occurrence of protothecosis can be local or disseminated and acute or chronic, with the latter being more common. Protothecosis has been classified in three clinical forms, namely, (i) cutaneous lesions, (ii) olecranon bursitis, and (iii) disseminated or systemic infections (55, 56, 80).

At least half of protothecosis cases are simple cutaneous infections, and the majority of these infections occur in individuals who are compromised by immunosuppressive therapy. Individuals presenting with olecranon bursitis are usually not immunocompromised but report penetrating or nonpenetrating trauma to the affected elbow (39, 100). Dissemination

occurs in individuals who are severely immunocompromised. Uncommon presentations, such as urinary tract protothecosis (156), colpitis (61), respiratory tract protothecosis (56), chorioiditis (51), intestinal protothecosis (125), ungual infections (47, 168), and meningitis (62, 144), have been documented in the literature. Also, three cases with lung involvement have been reported, including one case of probable and two cases of autopsy-proven pulmonary infection (78, 150).

The chronic presentation of protothecosis is typical for skin lesions and olecranon bursitis, yet in one patient protothecal meningitis persisted for more than 6 years despite treatment with various antifungal agents (144). Acute and fatal infections are rare and usually occur in severely immunosuppressed patients (65, 78). In one case, the use of infliximab for treatment of steroid-refractory graft-versus-host disease likely played a role in the fatal outcome of protothecosis (65).

Cutaneous Infections

Cutaneous protothecosis includes cases of infection coincident with trauma and consequent to defects in skin and mucosal surfaces (such as postoperative wounds) but also encompasses situations with no clear compromise of mucosal integrity (56, 65, 80). Manifestations develop slowly and usually show no spontaneous dissolution (134, 165).

The most common presentation of cutaneous protothecosis is usually a vesiculobullous and ulcerative lesion with purulent discharge and crusting (142, 150, 157). However, the spectrum of cutaneous lesions can take various other forms, including erythematous plaques, pustules, papules, nodules, verrucous lesions, pyodermic and herpetiform lesions, vesicles, ulcers, and hypopigmented or atrophic lesions (27, 50, 56, 88, 164). Manifestations of postoperative infection include nodular lesions, synovitis, tendosynovitis, and chronically draining wounds (54, 94).

It is believed that the incubation period is several weeks and that the algae penetrate the skin following posttraumatic damage (45, 59, 80). The lesions generally remain localized; immunocompromised patients, particularly those with cellular immunodeficiency, show a trend toward dissemination (56). The cutaneous lesions are located mainly in exposed areas, such as the extremities and the face. Over one-half of documented cases of protothecosis concern cutaneous or subcutaneous manifestations, which are often preceded by skin or wound infections (148).

The first human case of protothecosis was diagnosed in 1964 on the foot of a barefoot rice farmer from Sierra Leone (36). The lesion began on the inner side of the right foot as a depigmented area that had been injured several times by the patient's walking barefoot. Within 3 years, it had become a papule with a raised edge covering two-thirds of the foot. In tissue sections and culture, the grouped, rounded bodies of *Prototheca* species were observed. A skin biopsy showed hyperkeratosis and pseudoepitheliomatous hyperplasia. The organism was seen in the epidermis and in the papillary and reticular dermis. Because *Prototheca* species had been noted to be sensitive to pentamidine, a total of 4.9 g was given to the patient, but without benefit. At last report, the lesion was advancing and the organism was found in the lower femoral lymph nodes. The isolate was named *P. segbwema* after the

TABLE 2. Unusual presentations of human protothecosis

Patient no.	Site(s) of infection	<i>Prototheca</i> sp. detected	Treatment ^a	Outcome	Reference
1	Skin, blood	<i>P. wickerhamii</i>	AmB, transfer factor	Cured	34 ^b
2	Skin, fistula	<i>P. wickerhamii</i>	Tetracycline	Death from bacterial sepsis	35 ^b
3	Intestine	<i>P. wickerhamii</i>	None	Not given	24 ^{b,c}
4	Peritoneum	<i>P. wickerhamii</i>	AmB (intravenous and intraperitoneal)	Cured	102 ^b
5	Skin (extensive)	<i>P. wickerhamii</i>		Death from cancer	100 ^b
6	Gall bladder, liver	<i>P. wickerhamii</i>	AmB, ketoconazole	Cured	25 ^b
7	Peritoneum	<i>P. wickerhamii</i>	AmB (intravenous), doxycycline	Cured	130 ^b
8	Peritoneum	<i>P. wickerhamii</i>	AmB, 5-flucytosine	Cured	48 ^b
9	Respiratory tract	<i>P. wickerhamii</i>	AmB, excision	Cured	56 ^b
10	Blood, catheter	<i>P. wickerhamii</i>	AmB	Cured	53 ^b
11	Meninges	<i>P. wickerhamii</i>	AmB, 5-flucytosine	Death	62 ^b
12	Intestine, liver	<i>P. wickerhamii</i>	AmB, fluconazole	Not given	85 ^b
13	Intestine, lymph nodes	<i>P. wickerhamii</i>	AmB	Not given	85 ^b
14	Blood, urinary bladder	<i>P. zopfii</i>	Fluconazole	Death	76 ^b
15	Liver, meninges	<i>P. wickerhamii</i>	AmB, miconazole, fluconazole	Not eradicated in 6 yr	144 ^b
16	Blood, skin	<i>P. wickerhamii</i>	AmB, liposomal AmB	Cured	92 ^b
17	Endocardium	<i>Prototheca</i> spp.	AmB, excision	Cured	13 ^b
18	Skin, internal organs	<i>P. wickerhamii</i>	Fluconazole	Death	84 ^b
19	Ileum, duodenum	<i>Prototheca</i> spp.	AmB, itraconazole, gamma interferon	Cured	125 ^b
20	Skin, internal organs	<i>Prototheca</i> spp.	AmB	Death	163 ^b
21	Respiratory tract	<i>Prototheca</i> spp.	Fluconazole	Death	150
22	Blood, catheter	<i>Prototheca</i> spp.	Liposomal AmB	Cured	150
23	Skin, internal organs	<i>P. zopfii</i>	AmB, voriconazole	Death	78
24	Skin, blood	<i>Prototheca</i> spp.	AmB	Death	65
25	Skin, arthritis	<i>P. wickerhamii</i>	Itraconazole, AmB	Death	107

^a AmB, amphotericin B.

^b Reviewed in reference 12.

^c Possible case of protothecosis.

location of the hospital in which the organism was isolated, but the name has been suggested to be synonymous with *P. zopfii*. Over the following years, the number of documented cases of protothecosis rose continuously, with about four new cases being diagnosed every year over the past decade.

Olecranon Bursitis

Infections of the bursa subcutanea olecrani, which are generally preceded by injuries or grazing of the elbow, are clinically significant in this respect (1, 98, 101, 148). The reason for the predilection for the olecranon bursa as a site of *Prototheca* infection is unclear but may reflect the predisposition of this area to repeated trauma. Signs and symptoms appear gradually several weeks following the trauma and include mild induration of the bursa accompanied by tenderness, erythema, and production of variable amounts of serosanguinous fluid (39, 100).

Reports of olecranon bursitis have also included cases of wound contamination, such as when a *Prototheca* species was probably introduced into a preexisting wound during cleaning of a contaminated tank, cases without penetrating trauma, and cases occurring in the setting of chronic bursitis (1, 56).

Systemic Infections

Disseminated protothecosis occurs in individuals undergoing cancer treatment (150) or solid organ transplantation (56, 80) or in those with AIDS (56, 80). The organs most commonly affected in dissemination are the skin, subcutaneous tissue, gut, peritoneum, blood, and spleen. Overall, 23 cases of dissemi-

nated infection have been described. The dissemination cases were observed in immunocompromised individuals; in all but two cases, the species involved was *P. wickerhamii*.

What may be termed a disseminated opportunistic infection by a *Prototheca* species was first reported by Klintworth et al. (68) in 1968. The patient was diabetic and had widespread metastases of breast cancer; *P. wickerhamii* was isolated from several ulcerating papulopustular lesions on the leg. It was concluded that the patient died of the carcinoma, but no autopsy was performed. The first clear case of multiorgan systemic protothecal infection was described by Cox et al. in 1974 (34). The patient was a 29-year-old man who had an unknown defect in cellular immunity. Multiple lesions were found in the peritoneal cavity, lymph nodes, skin, and blood. A similar case of visceral protothecosis was later described in 1990 by Chan et al. (25); the infection mimicked sclerosing cholangitis. The patient had multiple peritoneal nodules that resembled metastatic cancer but were in fact manifestations of protothecosis. The authors recommended that protothecosis be considered in the differential diagnosis of hepatic and biliary inflammatory diseases of uncertain etiology (34).

Four catheter-related cases of protothecosis included three episodes of peritonitis complicating continuous ambulatory peritoneal dialysis (48, 102, 130) and one episode of mixed infection of a Hickman catheter with *P. wickerhamii* and *Torulopsis* sp. (53). Central venous catheter-related algemia has been reported, with accompanying fever, chills, and sepsis syndromes (148, 150). In the meantime, several other cases of disseminated protothecosis have been described (Table 2). Recovery of *Prototheca* spp. from the blood occurred in 47% ($n =$

11) of cases with dissemination. The manual lysis centrifugation method was superior to other methods in detecting *P. wickerhamii* in blood from HIV patients (7). Not that uncommonly, *Prototheca* spp. are associated with copathogens, such as *Candida glabrata* (164), *Staphylococcus aureus* (15), herpes simplex virus (147), *Enterococcus faecalis* (150), *Leuconostoc* spp. (150), *Klebsiella pneumoniae* (65), *Cryptococcus* spp. (48), *Pseudomonas aeruginosa* (164), and *Escherichia coli* (150).

DIAGNOSIS

General Features

Protothecosis is generally not suspected clinically, and patients are subjected to various treatment modalities for long periods without satisfactory results. The definitive diagnosis of infection usually depends on morphological identification of the organisms in wet slide preparations of cultures and/or direct identification in tissue specimens. The combination of microbiological and histopathological tests is recommended for suspicious cases. *Prototheca* species react to the periodic acid-Schiff stain (PAS) and reveal yeast-like colonies on Sabouraud dextrose agar (28, 143) but differ from fungi as they lack glucosamine in their cell walls (26, 28, 60, 101). Species identification is usually based on macroscopic and microscopic examination, growth temperature, and sugar and alcohol assimilation patterns (42). Microscopic examination of the organism in culture reveals the same structures as those observed in tissue (i.e., spherical sporangia containing multiple endospores) (56). This gives the organism the appearance of a spoked wheel. Intracellular spores are characteristic for *Prototheca* species, yet the number and size are influenced by the culture medium (124) and incubation time (79).

Pathological Findings

Prototheca is a large nonbudding cell readily seen in tissue. It is spheroid, ovoid, or elliptical with a prominent wall, and the round cell (theca) contains several thick-walled autospores (108, 120). No budding is found. *Prototheca* species are not easily apparent in hematoxylin- or eosin-stained smears but stain well with Gridley fungus stain, Grocott's modification of Gomori methenamine silver, or PAS, with or without diastase (Fig. 2) (64, 75). The lack of characteristic endospores causes *Prototheca* to resemble nonsporulating cells of *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, and some stages of *Coccidioides immitis*, *Pneumocystis jiroveci*, *Rhinosporidium seeberi*, and the agent causing chromomycosis (8, 38, 88, 114, 139, 140, 149, 162); thus, diagnosis of *Prototheca* species infection by histopathology can be difficult. The size of the sporangia is helpful in distinguishing *Prototheca* from other fungi. The sporangia of *Coccidioides* are 10 to 100 times larger than those of *Prototheca* species, and the individual endospores of *Coccidioides* are smaller.

In tissue, *Prototheca* organisms may appear morphologically similar to green algae (30, 33, 131). Light microscopy revealed not only similarities in size, shape, and mode of reproduction but also a striking difference between the *Prototheca* organisms and green algae. Unlike *Prototheca* species, the green algae contained abundant cytoplasmic starch granules that were PAS

negative following diastase digestion. PAS is particularly useful for differentiating the green algae from *Prototheca* cells in tissue (26, 58). Also, electron microscopy showed chloroplasts to be absent from *Prototheca* spp.

Histopathologic studies describe a variety of host tissue responses, ranging from severe granulomatous necrosis to a total absence of inflammatory changes (142). The histologic characteristics of cutaneous/subcutaneous and postoperative lesions have been variously described as granulomatous inflammation with necrosis; giant cells; a mixed infiltrate with plasma cells, lymphocytes, and histiocytes; hyperkeratosis; focal parakeratosis; pseudoepithelialization; hyperplastic lymphoid tissue; a dense chronic inflammatory cell infiltrate; and the presence of abundant organisms (56, 91, 107, 109). Organisms are usually in the mid- to papillary dermis, although some involvement in the epidermis was reported earlier (27, 162).

The histologic features of olecranon bursitis lesions consist of granulomatous inflammation with giant cells, epithelioid cells, lymphocytes, and plasma cells as well as organisms in tissue (52, 56, 61). In dissemination, tissues show significant eosinophilia and fibrosis in the gall bladder, duodenum, and hepatic portal areas (24, 25, 56).

Microbiological Tests

Diagnostic procedures for the identification of *Prototheca* spp. should include the evaluation of characteristic micromorphology and specific assimilation patterns (105, 114). Identification should not rely on the assimilation pattern alone, as *Prototheca* sp. profiles can be identical to those of other yeasts.

The failure to isolate *Prototheca* species may be explained by the fact that they are readily overgrown by bacteria and fungi when culture is attempted from contaminated sources. *Prototheca* species have simple nutritional requirements and grow readily on a variety of synthetic media (143). Yet many commonly used culture media are unsatisfactory and contain unsuitable nutrients or inhibitors (118), such as cycloheximide, which is present in many selective fungal media (18). Other media that may be useful include beef infusion broth, blood agar, and brain heart infusion agar (6, 73). Pore (118) suggested *Prototheca* isolation medium for selective cultivation. The combination of flucytosine and potassium hydrogen phthalate inhibits most bacteria and fungi. *Prototheca* isolation medium allows *Prototheca* isolation from densely contaminated sources, such as sewage, soil, or stream water. Incubation at 30°C for 72 h is adequate for most *Prototheca* species, while some slow-growing strains require incubation at 25°C for up to 7 days. Growth is optimized between 25 and 37°C, and organisms usually proliferate within 48 h as soft, wet, yeast-like, white-to-light-tan colonies. The organism can be either aerobic or microaerophilic (121). *P. wickerhamii* and *P. zopfii* can be distinguished from *P. stagnora*, which grows only at 30°C (85).

Round-oval *Prototheca* organisms with endospores can be unambiguously identified in native specimens (148). A wet mount of the culture material may be stained with lactophenol cotton blue or calcofluor white to reveal the characteristic endospore-forming sporangia (the so-called morula form) (Fig. 2). *P. wickerhamii* and *P. zopfii* differ in that *P. wickerhamii*

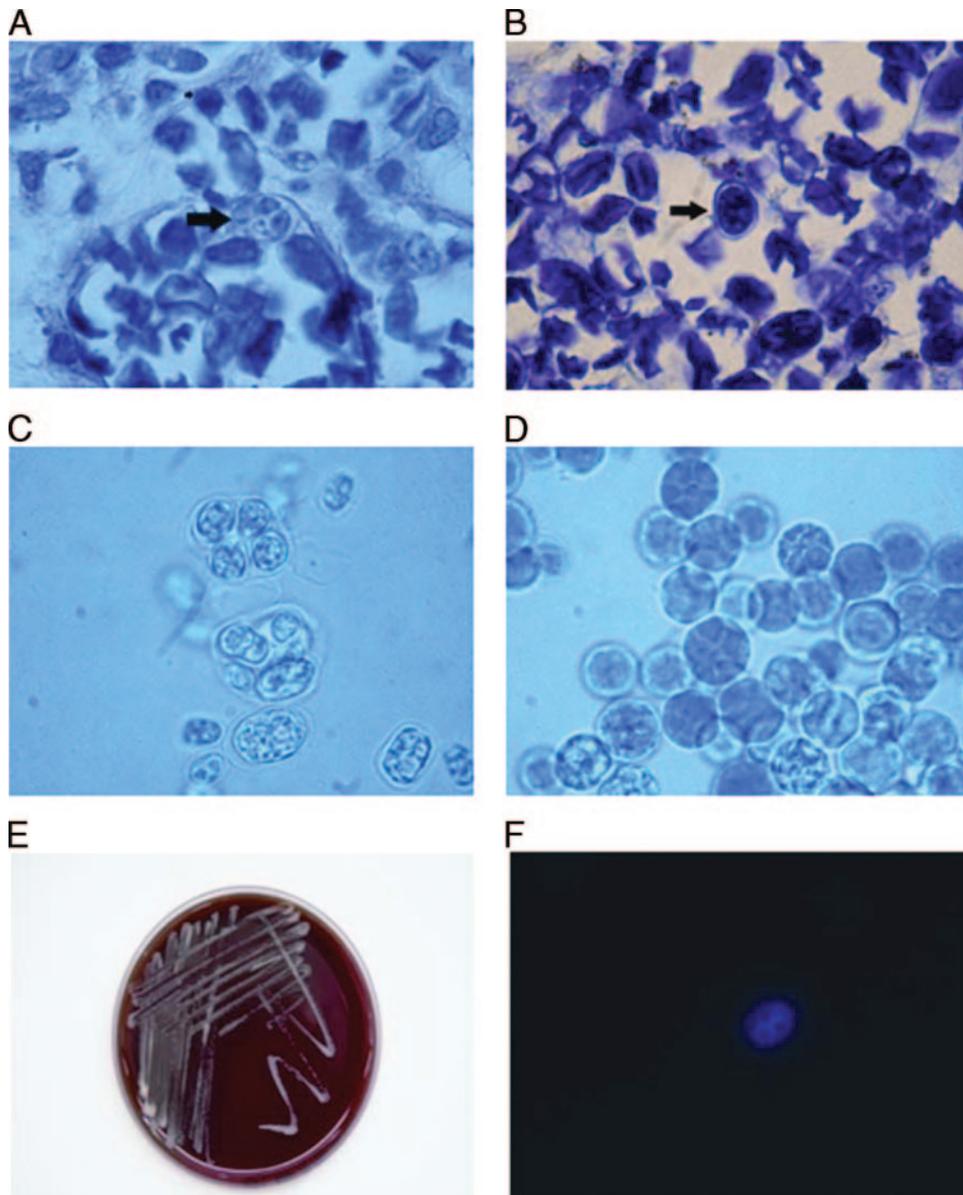


FIG. 2. Protothecosis and *Prototheca* spp. (A) Histopathology by hematoxylin and eosin staining of cutaneous protothecosis shows morula-like structures (large arrow) and endospores (small arrow). Magnification, $\times 1,000$. (B) Typical morphology is best observed with PAS. (C and D) Wet mount preparation with lactophenol cotton blue shows *P. zopfii*, with asymmetrical morula-like structures (C), and *P. wickerhamii*, with symmetrical morula-like structures (D). (E) Smooth, creamy, white, yeastlike colonies of *P. zopfii* on Columbia blood agar after incubation at 37°C for 24 h. (F) Staining of bronchoalveolar lavage fluid with calcofluor white also yields typical morula-like structures of *P. zopfii*.

tends to form symmetrical morula forms, whereas *P. zopfii* exhibits more random internal segmentation (121).

The colony morphology of *Prototheca zopfii* Krüger 1894 on Sabouraud glucose agar at 25°C is dull white and yeast-like in consistency (Fig. 2). The cells are variable in size and shape, being 8.1 to $24\ \mu\text{m}$ by 10.8 to $26.9\ \mu\text{m}$. The autospores are spherical and 9 to $11\ \mu\text{m}$ in diameter (Table 3). Morphology varies depending on the medium employed for growth (3, 72, 120).

Colony morphology for *P. wickerhamii* Tubaki and Soneda 1959 on Sabouraud glucose agar at 25°C is moist and cream-colored. Growth is optimal at 30°C , and the cells are similar to

those of *P. zopfii* in shape but are somewhat smaller. The cells vary from 8.1 to $13.4\ \mu\text{m}$ by 10.8 to $16.1\ \mu\text{m}$ when grown on glucose-containing media. The autospores are smaller (4 to $5\ \mu\text{m}$ in diameter) and more numerous (up to 50 per theca) (Table 3) (3, 72, 120).

P. stagnora Cooke 1968 is mucoid, owing to the capsular material it produces (Table 3). Unlike *P. zopfii* and *P. wickerhamii*, it does not grow well at 37°C (3, 72, 120).

Prototheca organisms can be identified to the species level by using the API strip series (bioMérieux, Marcy l'Etoile, Paris, France) applicable for yeasts (105), the Vitek yeast identification database (bioMérieux, Marcy l'Etoile, Paris, France) (42),

TABLE 3. Key for *Prototheca* differentiation^a

Feature	<i>P. wickerhamii</i>	<i>P. zopfii</i> ^b	<i>P. blaschkeae</i>	<i>P. stagnora</i>
Colony morphology	Hemispheric, with smooth margin	Flat, rough, central button, corrugated margin	Flat, smooth, central button, corrugated margin	Flat, with smooth margin
Diameter of cells (μm)	3–10	7–30		7–14
Glycerol assimilation	+	+	–	+
Growth on:				
Sucrose	–	–		+ ^c
Trehalose	+ ^d	–	–	–
<i>n</i> -Propanol	–	+		–
Arginine	+	+	–	+
Dextrose	+	+	+	+
Galactose	±	±		+
Clotrimazole (50 μg)	– ^e	+		+
Growth at 37°C	+	+	+	–
Capsule	–	–	–	+

^a Adopted according to the data in references 119, 125, 126, and 141. +, positive; –, negative; ±, strain variation.

^b Currently includes two genotypes.

^c Assimilation within 14 days.

^d Assimilation within 7 days.

^e Production of inhibition zone.

the Vitek 2 test (bioMérieux, Marcy l'Etoile, Paris) (78), and the RapidID Yeast Plus test (Remel, Santa Fe, NM) (44, 78). The API 20C clinical yeast system is a ready-to-use micro-method permitting the performance of 19 assimilation tests for the identification of most clinically significant yeast species. Biochemical reactions are complete after 72 h of incubation. The API 20C system provides an opportunity to determine the assimilation patterns of *P. stagnora*, *P. wickerhamii*, and *P. zopfii* (92, 105). Yet both the API 20C and Vitek databases include only *P. wickerhamii* for identification.

The API 50 system is also a ready-to-use micromethod that permits the assimilation patterns of 50 carbohydrates to be studied (105). The system is not available commercially. Pal et al. (106) described a new staining solution named "PHOL" for studying the morphology of clinical and environmental isolates of fungi and *Prototheca* species. The solution has shown a good ability to stain isolates of fungi and *Prototheca* and has the potential to stain the young as well as old isolates. Urease activity can be determined on Christensen urea agar at 30°C for 7 days, as *Prototheca* species fail to hydrolyze urea (105). For this examination, isolates of *Cryptococcus albidus* and *Candida albicans* should be used simultaneously as positive and negative controls, respectively.

Casal et al. (21) showed colonies of *Candida parapsilosis* and *Prototheca* spp. growing on CHROMagar *Candida* medium (CHROMagar Company, Paris, France) that were similar in color (cream) and texture after 48 to 72 h of growth, with *Prototheca* colonies being slightly smaller than those of *C. parapsilosis*. Given the increasing incidence of *C. parapsilosis* in clinical processes and the possible occurrence of *Prototheca* species in clinical samples, caution is warranted in using this medium. Also, CHROMagar *Candida* medium allows no differentiation between *P. wickerhamii*, *P. zopfii*, and *P. stagnora*, as all produce similar colonies.

Arnold and Ahearn (3) developed a method for identification of the *Prototheca* species, using carbohydrate and alcohol assimilation tests with the application of sucrose, trehalose, lactose, inositol, *n*-propanol, and xylose as carbon sources. This auxanographic method is reliable but time-consuming. It may

take up to 2 weeks to make a definitive identification. The absence of growth on trehalose is a main diagnostic feature for differentiation between *P. wickerhamii* and *P. zopfii* (Table 3).

The fluorescent antibody technique (56, 143) is a helpful tool for detection of *Prototheca* organisms at the genus level.

An aggregation test distinguishes the *Prototheca* genus from various types of the *Candida* genus (95) but is not available for commercial use. FTIR has been reported to be a suitable and efficient method for distinguishing and characterizing human-pathogenic yeasts and animal-pathogenic algae. Also, FTIR allows differentiation between *P. zopfii* and *P. wickerhamii* (132). However, for routine diagnosis, more conventional methods, such as cultivation and microscopic examination, seem to be sufficient (148). The detection of antibodies proved to be useful in the diagnosis of clinical and subclinical protothecal mastitis (11, 128) in cattle. Yet a serologic survey of *P. wickerhamii* in wastewater workers yielded negative results (31).

Another simple and rapid method for differentiating *Prototheca* species from *Candida* was described by Casal and Gutierrez (17, 18). The algacide ribostamycin inhibits growth of *Prototheca* species but not that of *Candida* species or other yeasts at 60 μg ribostamycin per lamella. Also, clotrimazole is useful in separating *P. wickerhamii* from *P. zopfii*, using 50-μg clotrimazole disks. *P. zopfii* tested in a study was resistant, and *P. wickerhamii* was susceptible (20). Susceptibility to neomycin is helpful in differentiating *P. wickerhamii* and *P. zopfii* from *P. stagnora* (16).

IN VITRO SUSCEPTIBILITY

Only a few studies are available on susceptibility tests and susceptibility patterns of *Prototheca* species. There are no official guidelines for performance, interpretation, or quality control of in vitro susceptibility tests for these algae (137). Currently, it is known that MIC testing is not always reproducible, nor do the results always correlate with clinical success (56). Nevertheless, susceptibility testing is not necessary to

TABLE 4. MIC ranges for various antimicrobials against *Prototheca* spp.^a

Drug	MIC (μg/ml)	Reference(s)
Gentamicin	0.2–0.9	137
Tetracycline ^b	>100	75, 79, 88, 157
Fluconazole	8–>200	15, 82, 92, 144, 169
Itraconazole	0.39–>100	15, 82, 92, 144, 169
Ketoconazole	1–60	15, 82, 88, 157, 169
Miconazole ^c	0.1–>100	136, 141
Clotrimazole	5–6	34, 141
Polymyxin B	0.39–100	87, 88, 141
Flucytosine ^c	>100	64, 79, 80, 92, 157
Amphotericin B ^d	0.15–12.5	15, 64, 80–82, 88, 92, 94, 136
Voriconazole	0.15–>16	78, 82
Nystatin	1–>100	136
Pentamidine	4	34

^a Data were compiled from multiple published studies in which various methodologies were used.

^b Tetracycline is synergistic with amphotericin B.

^c Strain variations.

^d *P. zopfii* seems less susceptible than other species to amphotericin B.

guide treatment of *Prototheca* infections. Testing is recommended if clinical treatment is not successful.

In Vitro Susceptibility Tests

Etest (AB Biodisk, Solna, Sweden) (9, 81), broth and agar dilution (82, 136, 137), and agar disk diffusion (79) tests have been adopted for determination of MICs for *Prototheca* spp. Several authors suggested the Etest as an accurate method for susceptibility testing of these algae (9, 78, 81), as it is easy to perform and provides MICs similar to those obtained with other well-documented techniques. A large study tested the in vitro susceptibilities of *Prototheca* spp. to gentamicin, using broth macro- and microdilution methods (137). Excellent agreement was demonstrated between the macro- and broth microdilution data, with 87% correlation. Tetracycline and gentamicin sensitivities of *P. wickerhamii* were assessed with the broth dilution method and disk diffusion assay (79). Results were similar for both methods. For tetracycline and minocycline, moderate resistance was demonstrated, but the precise zone was difficult to measure.

Sabouraud glucose agar (20), yeast nitrogen broth (136), Mueller-Hinton broth (79), modified susceptibility test medium (137), RPMI 1640 (82), and Casitone medium (9, 81) were satisfactory for susceptibility testing of these algae. The checkerboard method (79) was satisfactory to investigate the synergy of amphotericin B and tetracycline.

Overall, it seems that many aspects of susceptibility testing of *Prototheca* species are identical to those for procedures that were developed and standardized for yeasts. Conditions for protothecal growth have to be considered in optimizing testing for these organisms. It is not clear whether susceptibility tests for antifungal agents are reliable for evaluating algae.

Susceptibility Profiles

A summary of the in vitro activities of several antimicrobials against *Prototheca* species is shown in Table 4. In general, in vitro susceptibility testing has demonstrated *Prototheca* spp. to

be sensitive to amphotericin B (15, 80, 81, 130, 136) and variable in susceptibility to azoles, such as fluconazole, itraconazole, and voriconazole (9, 15, 78, 130, 136). *Prototheca* spp. are also variable in susceptibility to a wide range of antibacterial agents, such as tetracycline, gentamicin, and amikacin (15, 19, 79, 136, 137, 169).

A potential reason for the susceptibility to polyene and azole agents is the presence of ergosterol in the neutral lipid fraction of the cell membranes of *Prototheca* species (141). In particular, *P. wickerhamii* exhibits a membrane ergosterol content of 4%. The sensitivity of *Prototheca* to polymyxin B is related to the organism's phospholipid components. The free-fatty-acid fraction of the cell membrane is probably responsible for sensitivity to imidazoles. *Prototheca* species show marked variability in their susceptibility to imidazoles.

Overall, all *Prototheca* spp. are resistant to 5-flucytosine (64, 136); *P. zopfii* and *P. wickerhamii* are sensitive to miconazole, while other species are not. For all species tested, griseofulvin shows in vitro resistance. Sud and Feingold (141) tested *P. wickerhamii* ATCC 16529 and observed sensitivity to miconazole, clotrimazole, amphotericin B, and polymyxin B. Several *P. wickerhamii* strains previously examined are resistant to imidazoles (87, 136). *P. wickerhamii* and *P. zopfii* are variably resistant to fluconazole and itraconazole (9, 78). In contrast, voriconazole shows superior activity against *P. wickerhamii* (82, 110). However, there was a significant correlation between voriconazole and fluconazole MICs, and the authors did not exclude cross-resistance.

Shahan et al. (61, 137) have shown *Prototheca* spp. to be susceptible to gentamicin, with MICs of between 0.3 and 0.9 μg/ml. Since these MICs are well within the therapeutically achievable serum levels of 4 to 10 μg/ml, gentamicin was suggested for effective treatment. However, cases in which gentamicin was used failed treatment (52). Synergistic activation of amphotericin B and tetracycline has been observed in vitro (79). Five patients with cutaneous protothecosis received this combination and were successfully treated (52, 61, 152, 157, 166). Takaki et al. (144) reported induction of secondary resistance during a 3-year period of therapy and clinical failure. Amphotericin B and fluconazole MICs increased from 0.39 to 3.13 and from 50 to 200 μg/ml, respectively. This extent of clinically significant acquired resistance to any drug is generally believed to be uncommon. Casal et al. (23) showed the existence of a beta-lactamase in *P. zopfii* capable of inactivating several compounds.

In general, *Prototheca* species show various susceptibility profiles, and there is no direct correlation between in vitro activity and clinical response, with the exception of a few cases (15, 64, 78, 169). Thus, in vitro susceptibility testing is not indicated for routine patient management purposes for infections of the skin or bursae.

TREATMENT

Treatment of protothecal infections remains controversial, and various treatment regimens have been attempted, but there has been no consistency in the clinical responses. Data on potential therapy are drawn from isolated case reports, limited case series, and in vitro studies. No prospective clinical studies have been published comparing specific treatments for proto-

thecosis. Antifungals such as ketoconazole, itraconazole, fluconazole, conventional amphotericin B, and liposomal amphotericin B are the most commonly used drugs to date. Among them, amphotericin B displays the best activity against *Prototheca* spp. Usually, treatment involves medical and surgical approaches; treatment failure is not uncommon. Infection is indolent, with no apparent tendency toward self-healing (56, 85).

Successful options for cutaneous lesions have included total excision (27), topical therapy with amphotericin B (61, 77, 125), ketoconazole (75, 85, 109), itraconazole (45, 80, 103, 113, 145), fluconazole (45, 66, 80), transfer factor (34), topical amphotericin B with systemic tetracyclines (52, 152, 157), systemic amphotericin B, with or without excision (66, 87, 115, 116, 150), and oral tetracyclines (61, 139). Failed treatments involved tetracycline (52), itraconazole (15, 86, 125), fluconazole (84, 115), flucytosine (48, 104), and ketoconazole (88, 94, 115). Various success rates were reported for systemic penicillin, griseofulvin, and emetine as well as for topical therapies such as peroxide, chlorhexidine, potassium permanganate, copper sulfate, picric acid, ammonium compounds, Castellani's paint, and potassium iodide (56, 148).

Excision of infected small localized tissue may be acceptable in superficial infections, as evidenced by the success of this approach in several previously reported cases (27, 49, 54, 56, 80, 150). Persistent or deeper infection may require systemic therapy plus excision (162). According to Boyd et al., patients with protothecosis should receive intravenous amphotericin B with oral tetracycline. If oral therapy is indicated, an azole antifungal agent should be considered (12). However, the data on azoles are inconsistent. Itraconazole (400 mg/day for 6 weeks) failed as therapy, and treatment with fluconazole at 200 mg/day improved the patient's condition (80, 86). Fluconazole appears to provide clinical efficacy somewhat superior to that of itraconazole despite the high MICs obtained in vitro (15, 92, 144). In accordance with the results of drug susceptibility tests, one patient was treated with amikacin combined with tetracycline and responded well to this therapy (169). The duration of treatment varies from days to weeks (45).

Successful treatment of olecranon bursitis has focused on bursectomy; repeated drainage has failed (150). Drainage coupled with local instillation of amphotericin B has been curative (32, 39). According to Boyd et al. (12), patients with olecranon bursitis should undergo bursectomy, and patients ineligible for surgery should receive intrabursal amphotericin B. The role of systemic ketoconazoles (162), fluconazoles (162), and other imidazoles is unclear (56). Itraconazole treatment should be administered for at least 2 months (63).

Systemic diseases have been treated with amphotericin B, and all catheter-related events were treated by removal of the catheter and systemic administration of either amphotericin B (53, 102), amphotericin B plus oral doxycycline (130), or fluconazole (48). In two cases of peritonitis complicating continuous ambulatory peritoneal dialysis, intraperitoneal amphotericin B was used (48, 102). The removal of a foreign body or the excision of an infected site in combination with antifungal therapy remains the most prudent option for serious protothecal infections (65). The utility of azoles is questionable, as most treatment failures have been associated with their use (78, 150). Administration of amphotericin B appears to be the most effective treatment modality for sys-

temic protothecosis, although five patients failed therapy. Overall, these patients had a combination of profound immunosuppression and widespread infection (62, 65, 78, 107, 125). In two cases, amphotericin B therapy consisted of only 5 days, a period that is probably too short to clear a fungal infection (65, 78). One patient with intestinal protothecosis failed therapy with amphotericin B (total dose of 5 g during a 4-month period) and itraconazole (200 mg twice a day for another 4 months) yet responded to itraconazole plus gamma interferon for 6 months (125). The optimal dose and duration of therapy are uncertain. So far, amphotericin B therapy is recommended as first-line therapy in cases of dissemination and for patients with severe underlying illness or with immunosuppression (63).

Breakthrough infections with fluconazole (15), voriconazole (78), or itraconazole (150) treatment have been observed and display the moderate activity of azole drugs against *Prototheca* species.

CONCLUSIONS

Prototheca species cause a wide range of infections in humans, including cutaneous infections, olecranon bursitis, and disseminated disease. These infections can occur in both immunocompetent and immunosuppressed patients, although more severe and disseminated infections tend to occur in immunocompromised individuals (80). Usually, treatment involves medical and surgical approaches; treatment failure is not uncommon. Various treatment regimens have been attempted. Antifungals such as ketoconazole, itraconazole, fluconazole, and amphotericin B are the most commonly used drugs to date. Among them, amphotericin B displays the best activity against *Prototheca* spp. Diagnosis is largely made upon detection of characteristic structures observed on histopathologic examination of tissue (111).

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