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Case report

Protothecosis algaemia in a patient presenting with septic arthritis: A rare case of *Prototheca zopfii* isolated from Malaysia

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ABSTRACT

Prototheca species have been reported to cause infections in human. Typically, clinical symptoms of protothecosis include cutaneous infection, olecranon bursitis, tenosynovitis and disseminated systemic disease. We report a case of septic arthritis in which *Prototheca zopfii* was isolated from blood. Joint aspirate was also sent for cultures but did not yield any growth. No other organisms were isolated from this patient during his admission. The blood isolate was identified to species level via Polymerase Chain Reaction (PCR) method. The patient improved with administration of intravenous itraconazole.

Introduction

Members of the genus Prototheca are saprophytic, achlorophyllic algae that rarely cause human protothecosis. They are widely distributed in the environment [1,2] and may colonize skin, hair and nails [1]. Clinically, *Prototheca sp* are reported to be associated with cutaneous lesions, olecranon bursitis and systemic disorders in humans [1,3–6]. Risk factors for protothecosis include a background of immunological compromise and trauma [1,5,6]. To date, *Prototheca wickerhamii* is the most common species to cause human disease [1]. We report a case of an immunocompetent elderly gentleman who presented with septic arthritis from which *Prototheca zopfii* was isolated from blood.

Case report

A 52-year-old gentleman presented to the emergency department of a tertiary center with painful unilateral swelling of the right knee for 2 weeks, associated with mild and sporadic fever. The patient, who was a retired government official, had underlying hypertension but was otherwise well and had never been hospitalized prior to this presentation. There was no history of trauma, osteoarthritis, rashes or involvement of other joints. He was also not known to have been diagnosed with gout and no suspicion of a sexually transmitted disease in this patient.

Admission bloods revealed mild thrombocytopenia, with a platelet count of 108,000/μL of blood (reference range, 150,000–400,000/μL of blood), but otherwise other parameters of the full blood count were normal. Blood uric acid was within normal range and there was no evidence of renal impairment, liver dysfunction or metabolic derangement. C - reactive protein levels were slightly raised, but ESR was normal. The patient was then started on intravenous vancomycin and ceftazidime empirically. Synovial fluid aspiration of the right knee yielded 8 mL of pale yellow and clear but rather viscous fluid. The fluid as well as blood was then sent to the microbiology laboratory for cultures. The fluid cultures were then sub cultured into blood, chocolate blood, MacConkey, Schaedler anaerobe agar and thioglycollate broth for enrichment. Biochemical analysis of the fluids revealed no abnormalities. The analysis includes glucose and total protein levels, which were within normal range for synovial fluid. Leukocytes were present on direct fluid examination, but scanty (only 7 per low power field) and further gram stain showed some PMNs and no organisms were seen. Neither crystals nor foreign bodies were seen, and the synovial fluid cultures were negative for growth.

Blood samples were cultured using BD BACTEC™ Aerobic, Anaerobic and MYCO/F Lytic Blood Culture System vials which were then processed in the automated BACTEC FX system. At day 3 of incubation, an alert notification was sent by the Epicenter system that links BACTEC FX to a monitoring computer, signalling that growth was detected in MYCO/F Lytic bottle. Gram stain was done from these vials and shows large Gram-variable structures of about 15–25 um, appearing vesicular, reminiscent of that of a

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morula. These structures are large enough to be viewed at 40 ×. There were no recognizable bacteria, fungal elements or yeasts when viewed at 100x oil immersion.

Subsequently, the positive samples were subcultured onto blood, chocolate blood, MacConkey and Sabouraud dextrose agar. Overnight cultures revealed smooth, moist, yeast-like colonies best viewed on Sabouraud dextrose agar (Fig. 1(a) and (b)), and to a lesser extent on blood agar and chocolate agar. No growth was detected on MacConkey agar. A direct smear of the colonies were made and stained via Lactophenol Cotton Blue dye, which revealed similar large morula-like structures with better clarity, likely to be endospore-forming organism (Fig. 2(a), and (b)). A presumptive identification of *Prototheca sp* was informed to the clinicians at this time.

The isolate was subjected to Matrix assisted Laser Desorption-Ionization Time-of-Flight (MALDI-ToF) via the Bruker Biotyper system, but failed to generate a meaningful database match despite multiple attempts which includes identification by extended extraction technique. Specification of the organism was achieved by PCR method described by Ratna et al. D1/D2 domain of the large subunit (LSU) in rRNA gene regions were amplified using universal primers NL1/NL4 [8]. The PCR products was visualised on 1.5 % agarose gel after electrophoresis. Sequencing was done by a private company, and consensus sequences were obtained via appropriate chromatogram software. The results were submitted into the BLAST sequence comparison algorithm database [9] and revealed a maximum similarity match to *Prototheca zopfii* (Genebank accession number, MG827346.1, KX353638.1) with query cover and identification rate both at 100 %. After a discussion with the primary team, we have decided not to proceed for antifungal sensitivity for this isolate as there are currently no interpretations available.

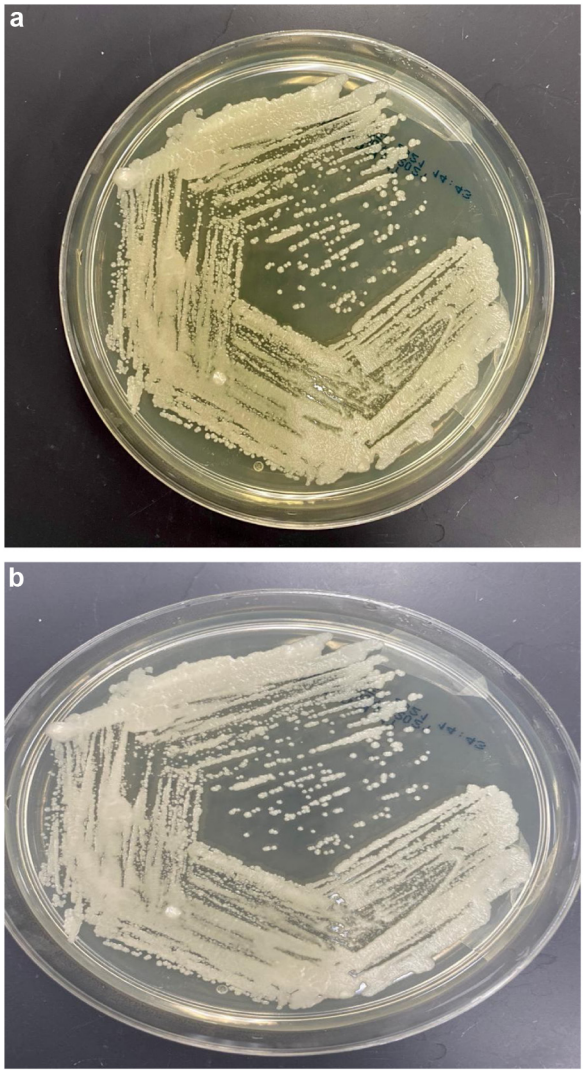

Fig. 1. (a) & (b): Whitish, smooth, moist, yeast like colonies were seen at 28C, day 4 on Sabouraud Dextrose agar, colony size 0.8-1.2 mm.

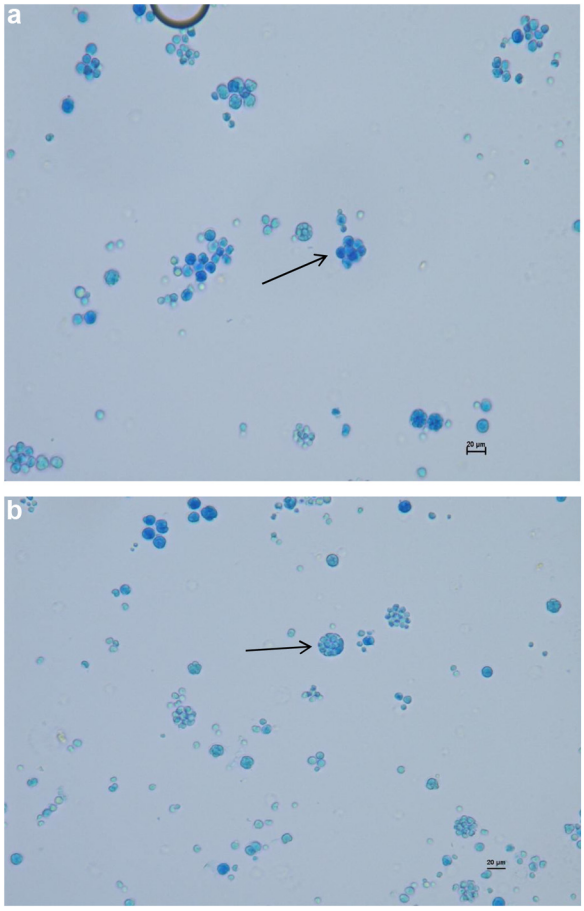

Fig. 2. The above pictomicrographs labelled Fig. 2(a) and (b) were taken from a direct colony smear stained with Lactophenol cotton blue dye and viewed at 40x magnification. As shown by arrows are large sporangia (theca) which contains small sporangiophores or endospores. No budding cells are present.

The patient was given intravenous itraconazole, although the primary team continued to administer vancomycin and ceftazidime to cover for bacterial infections. The patient responded well to this regime and his symptoms relieved. Eventually, the patient was discharged after 2 weeks of treatment.

Discussion

Prototheca is a genus of algae in the family *Chlorellaceae*. They are asexually producing, spherical, unicellular organisms ranging from 3 to 30 um in diameter. All the species within this genus, even though classified as 'green algae', have forfeited their photosynthetic ability and have switched to parasitism, requiring external sources of organic carbon and nitrogen [10]. Unlike plants, they do not possess chloroplasts nor pyrenoids and their survival are dependent on saprophytic behaviour [10,11]. Glucosamine, a specific fungal cell wall component, is also not present in these organisms. They are ubiquitously occurring organisms [2] and can be found in soil, sewage, animal waste, fresh water and sea water [1,12]. Interestingly, they are also capable of colonizing skin, hair and nails in humans without causing disease [1].

The first reported case secondary to *Prototheca sp* was by Davies et al. in 1964 [3]. *Prototheca wickerhamii* is the most common species documented to cause human disease, followed by *Prototheca zopfii* [3,5]. Other associated members include *Prototheca stagnora*, *Prototheca ulmea*, and *Prototheca blaschkeae* sp nov [1]. A usual clinical picture that accompanies infection by *Prototheca sp* include cutaneous lesions, bursitis and invasive disease [13]. Occasionally, some infections by *Prototheca sp* have been described to involve synovial tendon sheaths [14–18]; at least one of these affected patients was reported to present with symptoms of arthritis [16]. The pathogenesis of infection by *Prototheca sp* is largely unknown to this day, but common belief dictates that traumatic inoculation with the algal spores are a potential cause [1]. Humans with primary immunological defects, particularly of the cellular arm, are at greater risk for protothecosis, and it has been postulated that quantitative and qualitative defects in neutrophil function play an important role in the host defence against *Prototheca sp* [1]. No history of trauma nor immunological deficiency was elicited from our patient.

Identification of this organism is possible via cultures of appropriate samples on conventional agar i.e blood agar, Sabouraud dextrose agar [1]. Otherwise, histopathological diagnosis can also be attained by visualizing *Prototheca* sporangia containing sporangiospores on tissue biopsy samples, stained by hematoxylin and eosin [1]. Other modalities of diagnosis include Matrix assisted Laser Desorption-Ionization Time-of-Flight (MALDI-ToF) [2], as well as PCR [7,8,19]. At the time of writing, there are no official guidelines for the performance, interpretation, or quality control of in vitro susceptibility tests for *Prototheca sp*. [20]. To date, amphotericin B, itraconazole, posaconazole and voriconazole have all been tried and was shown good clinical outcome [20]. In a case of human cutaneous protothecosis [11], a good outcome was observed following selection of itraconazole which yielded the same result as in our present case.

Although rare and documented to have low virulence, protothecosis should not be ignored as an emerging infection. It would be beneficial for clinicians as well as laboratory personnel to be aware of the potential pathogenicity of this organism to cause human disease. Although thorough clinical history, particularly of immunological status and trauma, serves a great importance in placing clinical value should *Prototheca sp* be isolated in clinical samples, we cannot rule out the possibility of infection in immunocompetent patients. Further studies to determine antifungal sensitivity breakpoints are of paramount importance.

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Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Ethical approval

The Malaysian Ethical Committee has given permission to publish the case report of the patient with accompanying images.

Author contribution

Prem Ananth Palaniappan: Writing - reviewing and editing, validation. Cassandra Anne binti Abot: Writing- original draft, reviewing and editing. Ratna Mohd Tap: Investigation and resources. Fairuz Amran: Supervision, review.

Declaration of Competing Interest

All the authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.idcr.2021.e01121.

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