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Respiratory multiple infections by bacteria, viruses, fungi, and parasites in a COPD patient: A case report

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ABSTRACT

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SARS-CoV-2 is the causative agent of COVID-19, an infection that can manifest with mild to severe respiratory symptoms. A 70-year-old man with COVID-19 and COPD presented to a hospital complaining of breathing difficulties. A sample was taken, leading to the finding of the Acanthamoeba parasite. Stenotrophomonas maltophilia, a bacterium known for its resistance to most antibiotics and its significance as a nosocomial pathogen, was identified. Furthermore, for the first time, the Gloeotinia fungus was discovered as an endosymbiont of Acanthamoeba. The patient underwent successful treatment and was discharged from the hospital. Immunocompromised people should be concerned about the increasing incidence of nosocomial infections. The presence of Acanthamoeba should not be overlooked in respiratory disorders, as it has the potential to carry numerous pathogenic microorganisms as endosymbionts.

Keywords: SARS-CoV-2, COPD, *Acanthamoeba*, Endosymbiont, *Stenotrophomonas maltophilia*, *Gloeotinia*

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1. Introduction

SARS-CoV-2 causes COVID-19, which may infect individuals with mild to severe symptoms (1, 2). Patients with severe coronavirus disease are susceptible to a variety of infections, especially bacterial infections (1). COVID-19 patients with bacterial coinfection or secondary infection have a higher in-hospital mortality rate (3). The predominant agent detected, Staphylococcus aureus, accounted for about half of the early-onset bacterial etiologies (4). However, with the increase in the number of COVID-19 patients, the possibility of bacterial, fungal, and even parasitic co-infections may exist. On the other hand, exposure to opportunistic microorganisms and a compromised immune system may result in lifethreatening diseases (5). In this unique case, we would like to introduce a patient with chronic obstructive pulmonary disease (COPD) who infected COVID-19 and was interestingly infected with Stenotrophomonas bacterium, Glutinaea fungus, and Acanthamoeba parasite.

2. Case report

A 70-year-old male was admitted to the Razi hospital in northern Iran with complaints of having breathing difficulties and a loss of appetite. The patient has also had several respiratory infections in the past few years due to his history of smoking and COPD. The decision was reached to admit the patient and carry out further inquiry based on the patient's condition, laboratory results, and positive COVID-19 test result. Considering the disorder caused respiratory by the Acanthamoeba parasite, samples were taken from his nasal and pharyngeal. Then it was cultured in non-nutrient agar (NNA) medium and underwent daily surveys to ascertain the existence of parasites. On the third day, Acanthamoeba was detected (Fig. 1).

To confirm the pathogenicity assessment, a polymerase chain reaction (PCR) was done, and it was found that the genotype of the isolate is T4, which is the most pathogenic type of *Acanthamoeba*.

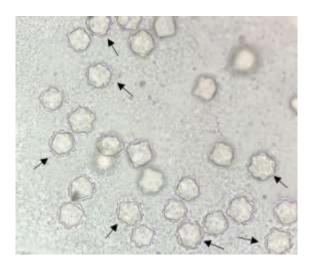


Fig 1. Cysts of *Acanthamoeba* (arrows) in NNA medium culture

Also, to further evaluate the pathogenicity, osmo-tolerance. thermo-tolerance, and cytopathic effect assays (CPE) were done. The isolate was able to grow even at an osmolarity of 1% and a temperature of 37 and even 42 degrees Celsius. It also had the killing power of all Vero cells in the CPE test, which showed its high pathogenicity. Also, due to the ability Acanthamoeba of to carry different microorganisms, endosymbiont fungi and bacteria were investigated through PCR and relevant universal primers and sequencing in the Gene Bank. Stenotrophomonas maltophilia (S. maltophilia), a significant nosocomial and resistant to most antibiotics, has been detected. For the first time in the world, the fungus was Gloeotinia found as an of endosymbiont Acanthamoeba. Pathogenicity assessment and genotyping of Acanthamoeba and its endosymbionts are shown in Table 1. Prescribed Tab: cotrimoxazole 960 mg and ciprofloxacin 500 every 12 hours. Also, for remdesivir, the initial dose was 200 mg on the first day, followed by a dosage of 100 mg for the next four days.

	Accession numbers	Pathogenicity assessment						Endosymbiont			Accession
Acanthamoeba Genotype		Osmo- tolerance		Thermo- tolerance			СРЕ	Bacterium	Accession numbers	Endosymbiont Fungus	numbers
		0.5 M	1 M	30 °	37 °	42 °			numbers	rungus	
T4	OQ804428	+	+	+	+	+	+	Stenotrophomonas maltophilia	OR230259	<i>Gloeotinia</i> sp	OQ822822

Table 1. Genotyping and pathogenicity assessment of *Acanthamoeba* and endosymbionts in a COPD patient in northern Iran

He was discharged from the hospital in relatively good condition. The follow-up of the patient in relation to these four microorganisms continued for two months until he completely recovered.

Discussion:

This is the first report of a simultaneous infection involving four different types of microorganisms in COPD patients. Individuals with a susceptibility to opportunistic infections should be concerned about the rising number of S. maltophilia infections because of the high fatality/case ratio linked to this bacterial killer. Although this bacterium can infect a variety of organs and tissues, respiratory tract infections are the most prevalent site of infection (1). The presence of Acanthamoeba in dust and biofilm samples collected from hospitals and clinical environments across the world gives rise to significant health apprehensions, especially for individuals who are highly susceptible to infections (2, 3). This prevalent and widely spread free-living amoeba is commonly found in various ecological settings, such as soil, water, air, dust, and sewage samples (4). It is considered a nosocomial infection and can serve as a reservoir for some pathogenic microorganisms (5, 6).

The relationship between *Acanthamoeba* and SARS-CoV-2 is currently not well understood, and much more study is needed (7). *Gloeotinia* is an unexplored group of plant pathogenic fungi that exhibit necrotrophic and

opportunistic behavior. It decomposes tissues of diverse plant species (8), and probably this person had been exposed to soil that was contaminated by it and also Acanthamoeba. It is very interesting that Acanthamoeba, a nosocomial infection (6), has been discovered to harbor another nosocomial infection (S. maltophilia) in a patient with COVID-19. In some studies, evidence has been provided that endosymbionts play a role in increasing the pathogenicity of Acanthamoeba. A study done by Fritsche et al. showed that endosymbionts could enhance CPE in cell culture (9). Also, Hajialilo found that Acanthamoeba lacking endosymbionts exhibited mild pathogenicity, whereas those with endosymbionts were highly pathogenic and caused the destruction of vero cells in the CPE test (6). We recommend that related assessments be taken for Acanthamoeba and its endosymbionts in respiratory patients because the treatment of those can be other respiratory disorder challenges, such as COVID-19.

Conflict of interest

None declared.

Author contribution

Eissa Soleymani and Amir Hossein Maghsood were involved in the collection of samples and data. Shadi Shayesteh Azar, Keihan Shabankhani, and Seyed Reza Mirbadie were involved in the interpretation, writing, and editing of the manuscript. Eissa Soleymani prepared the draft and final version of the manuscript and also performed the PCR. Mahdi Fakhar judgmentally revised the entire manuscript. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The data that supports the findings of this study is available from the corresponding authors upon reasonable request.

Ethics statement

The authors declared that appropriate written informed consent was obtained from the patient for the publication of this manuscript.

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