

Original Article

Free Living Amoeba Belonging to *Vannella* Spp. Isolated from a Hotspring in Amol City, Northern Iran

Maryam Niyiyati*, Alireza Latifi

Department of Medical Parasitology & Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Abstract

Background: Free-living amoebae have various genera that are found in several environmental niches such as soil, freshwater, dust, seawater and hot springs. Most of Free-living amoebae are normally harmless to humans. However, some amoeba such as *Acanthamoeba* and also *Naegleria fowleri*, *Balamuthia manderillaris* and *Sappinia* are identified as opportunistic and pathogenic amoebae that can cause eye diseases, encephalitis, and meningoencephalitis in human. Vannellidae are a family of free-living amoebae and exist mainly in soil, freshwater, and marine habitats. This amoeba is nonpathogenic for human, but can act as a Trojan horse for other pathogens such as Microsporidia. The present study reports the occurrence of *Vannella* spp. in a hot spring of Amol city.

Materials and Methods: 22 samples were taken from hot springs of Mazandaran province during our previous study. The plates were checked for the presence of *Vannella* spp. according to the specific morphological criteria. DNA extraction, PCR and sequencing was performed on the positive isolate.

Results: The result showed that one plate contained fan-shaped amoebae suspected to *Vannella* spp. PCR analysis and sequencing was confirmed the occurrence of *Vannella* spp. in one sample of a hot spring of Amol, northern Iran.

Conclusion: The result confirmed the presence of *Vannella* amoebae in the hot spring of Amol city. More studies are needed to clarify the real distribution of *Vannella* spp. in environmental niches and its pathogenic potential in Iran and worldwide.

Keywords: Free-living amoeba, *Vannella amoeba*, Hot springs, Amol city

***Corresponding Author:** Dr. Maryam Niyiyati, Department of Medical Parasitology & Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: maryamniyati@yahoo.com

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Introduction

Free-living amoebae widely exist in soil, fresh water, dust, sea water, hot springs, marine habitats, and wet environments such as biofilms^{1,12}. These protozoans consist of pathogenic amoeba such as *Naegleria fowleri*, *Balamuthia manderillaris*, *Sappinia* and some genotypes of *Acanthamoeba* can cause severe eye disease such as Amoebic Keratitis,

skin disease, meningoencephalitis and Amoebic encephalitis². *Vannella* spp. amoebae are of the genus of free-living amoebae. In the past, this amoeba was classified as Thecamoebidae family, but nowadays it is in Vannellidae amoeba (Vannellid) family with various genera such as *Vannella*, *Platyamoeba* and *Pessonella*³. This amoeba was first introduced by Bovee in 1965⁴. Currently, almost 40 species of this amoeba are identified in sources such as water, soil,

dust, biofilms, surfaces of leaf, marine settlements, and fish gills^{5,6}. The trophozoite of this amoeba is flat and fan-shaped. Some of these trophozoites are flat and long and has a prominent anterior margin and are about 10-40 microns and just some of the *Vannella* amoebas are able to form the cysts⁷. It is worthy to mention that *Vannella* cannot be considered as the pathogenic amoebae, but it can be a Trojan horse for other pathogenic microorganisms such as *Microsporidia*^{8,9}.

As there are few studies reporting *Vannella* spp. in Iran, the main aim of the present study was to check the culture plates of the collected samples from hot springs in Mazandaran province, Iran. Morphological and Molecular-based tests revealed the presence of *Vannella* in one sample. To the best of our knowledge, this is the first report of the occurrence of *Vannella* spp. in hot spring source in Iran.

Methods

Sample Collection: Twenty two samples were taken from hot springs of Mazandaran province during our previous study¹⁰. The plates were checked for the presence of *Vannella* spp. according to morphological criteria.

Cloning of the targeted Amoebae: In the process of checking the plates, in one of them the amoebae were observed with the flat and fan-shaped forms close to the cyst and trophozoites of *Vannella* (Figure 1). In order to isolate this amoeba from the other similar amoebae such as *Thecamoeba* and also fungal and bacteria, the isolated trophozoites were transferred to the other plate. After a few days the isolated plate had many flat and fan-shaped trophozoites that were suspiciously similar to the family of Vannellidae amoebae. In order to predicate the amoebae and clean it from fungal and bacterial contamination for molecular testing, the trophozoites of amoebae were isolated from the other amoebae and were transferred

to other plates. This was done for several times. Eventually, there was no sign of bacterial and fungal contamination.

Extraction of DNA, PCR Amplification and Analysis of Sequences: At this stage, small amount of buffer PBS with pH \approx 7 was added to the plates that contained the amoebae, and after 5-10 minutes the scraping was done. Modified phenol and chloroform methods were done for DNA extraction according to our previous study¹¹. The PCR reaction was performed using NA primers. These primers could amplify the sequences of some free-living amoebae such as *Vannella* and *Vermamoebae*. The sequence of this primer is: FNA1: 5'-GCT CCA ATA GCG TAT ATT AA-3' and RNA2: 5'-AGA AAG AGC TAT CAATCT GT-3'¹². The PCR reaction volumes contained 30 microliters that is included of 25 microliters Ampliqone (consisting of Taq DNA polymerase Master Mix RED, Denmark) and utilized as ready solution. In addition, 0.1 ng DNA, 0.1 micromol of each of the forward and reversed primers along with distilled water were used. The PCR products were electrophoresed by the usage of 2% agarose gel and ethidium bromide. Finally the PCR product band was observed by duct gel and UV light. PCR-products were then submitted for sequencing. Blast analysis of the sequence with other genes available in the Genebank data was performed using BLAST software from the National Center of Biotechnology Information (NCBI) site. The maximum homology was the base of genera identification. The sequences were submitted to gene bank under following accession numbers: KX371576.

Results

The result showed that one plate contained fan-shaped amoebae suspected to *Vannella*. PCR analysis and sequencing was confirmed the occurrence of *Vannella* spp. in one sample of a hot spring of Amol, northern Iran.

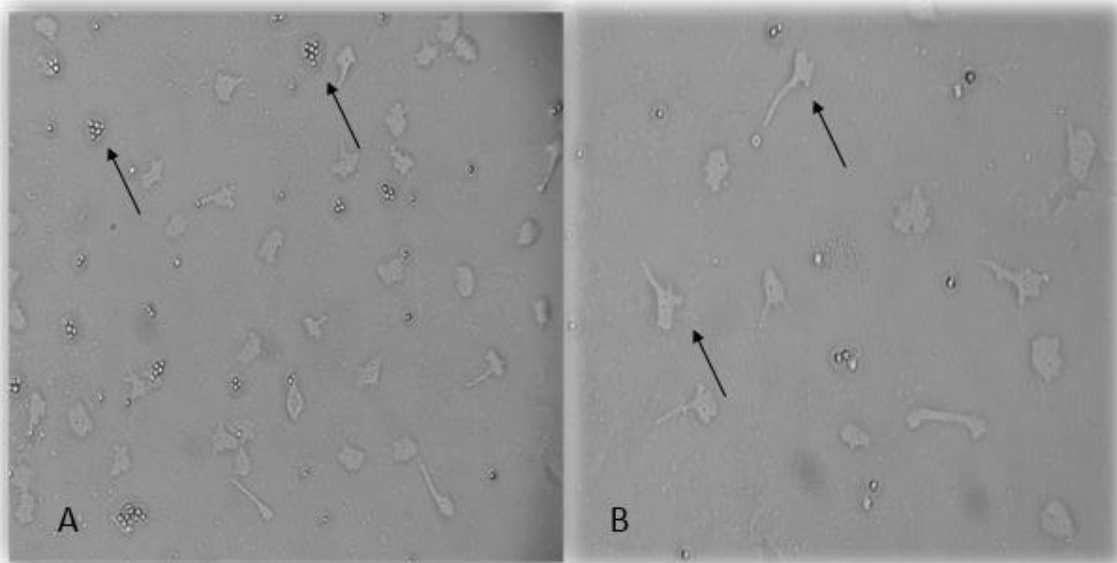


Figure 1. Fan-shaped trophozoites of *Vannella* spp isolated from hot spring in Amol city ($\times 400$).

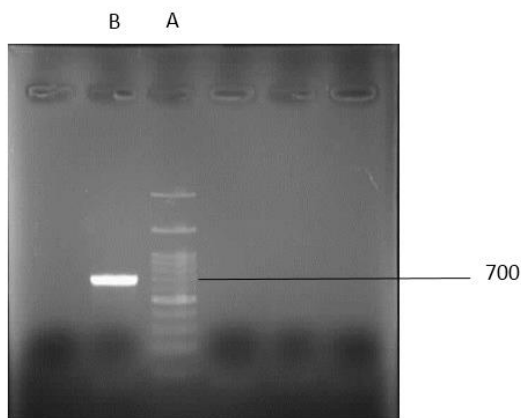


Figure 2. Gel electrophoresis of the 800 bp PCR-product of *Vannella* spp. isolated from Mazandaran Hot Springs, Northern Iran (A:marker-B:sample).

Discussion

The result of the current study showed that one of the plates contained amoebae with flat and fan-shaped forms and highlighted margin trophozoites in the frontal part with the size of 10-40 micron (Figure 1). Sequencing analysis revealed that suspicious amoeba is homologues to the family of Vannelidae and amoebae *Vannella* (Figure 2). This amoebae were isolated from "Abe Ask hotspring" in Amol city. Although *Vannella* spp. is not be accounted as a

pathogen for human, but it could be a Trojan horse for other pathogens such as *Microsporidia*. Previous studies in Iran revealed the presence of *Vannella* spp. from water and biofilm sources^{12,13,14}. However, this is the first report of isolation of *Vannella* spp. from hotsprings in Iran. Lasjerdi *et al*, (2011), for the first time isolated *Vannella* amoebae from the biofilm samples by sterile swab from a hospital environment¹². In another study conducted by Todd and colleagues in 2015, the species of *Vannella* by the name of *Vannella epipetala* was isolated from the sediments of freshwater of a river in Jamaica¹⁵. Similarly, Kuroki and colleagues in 1998 conducted a study by checking of the basins of a bathroom in Japan and they isolated the *Vannella* that sheltered *legionella*, this gram negative bacteria is accounted as a pathogen for the human¹⁶. Scheid in 2007, in a study regarding patients with keratitis, isolated *Vannella* spp.. He found that amoeba *Vannella* was not the agent of amoeba keratitis, but it was a Trojan horse for *Microsporidia* which caused keratitis in the patients¹⁷. Gianinazzi and colleagues (2009) isolated *Vannella* from the samples in order to isolate the free-living amoebae by screening of ponds, rivers and swimming pools of Switzerland¹⁸. Buse HY and colleagues in 2013 carried a similar study in order to identify the variety of eukaryotes in the drinking water and sanitary water by isolating *Vannella* as the

host of transporting of the potential microbiologic pathogens¹⁹. Smirnov and colleagues in 2016 isolated a species of *Vannella* by the name of *Vannella croatica* from the hot springs. Although *Vannella* is not pathogenic to human, it can act as a Trojan horse for other pathogens such as *Microsporidia* and other pathogenic microorganisms²⁰.

Conclusion

The results of the present study gave shed light upon the presence of *Vannella* in hot springs sources. More studies are needed to clarify the real distribution of *Vannella* in environmental niches.

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