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Short Communication

First Report of Vannellidae Amoebae (*Vannella* Spp.) Isolated From Biofilm Source

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ABSTRACT

Background: Members of the Vannellidae family are free-living amoebae (FLA) distributed mainly in water and soil sources. The present study reports the first isolation of this genus in the biofilm source from hospital environment in Tehran, Iran.

Methods: Biofilm samples were collected from hospital environment. Cultivation was performed in non-nutrient agar covered with a heat-killed *Escherichia coli*. Cloning of the suspected amoebae was done. PCR amplification and Homology analysis using the Basic Local Alignment Search Tool (BLASTn) was performed to search for the most similar reference sequences.

Results: Microscopic examination showed numerous fan-shaped amoebae and peculiar cysts different to the usual shape of typical FLA. Sequence analysis of the PCR-product revealed that the suspected amoebae are highly homologous with *Vannella* spp. gene (99% identity and 100% query coverage) available in the gene bank database.

Conclusion: Although *Vannella* spp. is not proved to be pathogenic itself, but they are capable of harboring pathogenic intracellular organisms such as *Microsporidian* parasites. Thus, identification of such amoebae can be of clinical importance, as they could lead to transmission of other pathogens to human.

Keywords: *Vannella*, Biofilm, Iran

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Introduction

Free-living amoebae (FLA) include various taxa with high distribution in the environmental sources such as fresh water, hot springs, sea water, mineral water, soil, dust, clay and moist habitats such as biofilms (1-3). The ubiquitous nature of these organisms leads to the common exposure of human to FLA (4). However, various genera are known as potentially pathogenic organism including *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri* and *Sappinia pedata* (1, 5). To date, 17 different genotypes of *Acanthamoeba* has been identified according to the diagnostic fragment 3 (DF3) of the 18S rRNA gene (6) and most of these genotypes have been proved to be pathogenic in human being (2). Recently, other genera of FLA have been identified as pathogens of cornea and Central Nervous System (CNS) (7, 8). Indeed, recent reports of mixed corneal infection due to *Acanthamoeba* with *Vahlkampfia* and *Hartmannella* and also brain involvement due to *Paravahlkampfia francinae* lead to the more attention to other FLA, worldwide (7-9). Other families within FLA include Vannellidae amoebae (Vannellids) which were previously introduced as the family belonged to the Thecamoebidae. However, to date Vannellidae is a totally different family from Thecamoebidae (10). Vannellidae contains several genera such as *Vannella*, *platyamoeba* and *Pessonella* (10). The genus *Vannella* was first described by Bovee, 1965 (10, 11) and occur mainly in water sources (marine and fresh water). This genus contains approximately 40 described species identified in marine and fresh water sources (10, 12, 13). The trophozoite form is rather fan-shaped and some of them are capable to form cysts (12, 14). Few researches reported the presence of these amoebae in soil samples (12).

It should be mentioned that amoebae belong to this genera could act as a trojan horse for microbial world and they could support their endosymbiont proliferation (15). This property makes non-pathogenic FLA as relevant organisms for human being. Indeed, the carrier property of *Vannella* for intracellular microorganism such as pathogenic *Microsporidian* parasites is of utmost clinical importance (15, 16).

The present study is the first report regarding the isolation of Vannellidae amoebae (Vannellids) in the biofilm sources in Iran using both morphological tests and molecular approaches.

Material and Methods

Sample Processing

Biofilm samples were collected using sterile swab from hospital environment in our previous study (17). Briefly, biofilms were dissolved in distilled sterile water, incubated for an hour and filtrated using membrane filters (Pore size 1.2 μ). Filters were then placed upside down on to non-nutrient agar covered with a heat-killed *Escherichia coli*. Incubation was performed at 30 °C. Plates were then monitored for the presence of FLA using both light and inverted microscope.

Microscopic examination

One plate showed numerous fan-shaped amoebae and peculiar cysts different to the usual shape of typical FLA and thus fresh smear of the suspected culture-plate was examined under inverted microscope. Suspected amoebae were then submitted to cloning to obtain a single line cell and to eliminate bacterial and fungi contamination of plates for subsequent evaluation according to our previous studies (17, 18).

DNA extraction, PCR amplification and sequencing analysis

Cloned plate containing suspected amoebae were then scraped using pH 7 PBS. Pellet of amoebae were then obtained using 3000 rpm centrifuge and DNA extraction were done using Chelex kit (Instagen matrix, Biorad). PCR were performed by NA primers which amplify a partial 18S rRNA gene of some FLA such as *Vannella* and *Hartmannella*. The primers sequences were: NA1: 5'-GCT CCA ATA GCG TAT ATT

AA-3' and NA2: 5'-AGA AAG AGC TAT CAATCT GT-3' (17). PCR reactions were performed in 30 µl Ampliqone (Taq DNA Polymerase Master Mix RED, Denmark) as a ready-made mixture. Briefly, 25 µl of Taq Master mix were used with 10 ng template DNA, 0.1 µM of each primer and distilled water. PCR cycling conditions included initial denaturing step of 94°C for 1 min, followed by 35 repetition cycles at 94°C for 35 s, annealing at 58°C for 45 s, and at 72°C for 1 min. PCR products were electrophoresed using 2% agarose gel stained with a solution of ethidium bromide and visualized under UV light.

PCR product was submitted to sequencing using the ABI 3130X automatic sequencer in the Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran. Homology analysis using the Basic Local Alignment Search Tool (BLASTn) was performed to search for the most similar reference sequences.

Results and Discussion

Plate culture revealed fan-shape trophozoites and unusual cysts after one week of incubation. *Thecamoeba* were detected according to ovoid and flat shape of their trophozoites and therefore cloning have been done to rescue our targeted amoebae from *Thecamoeba*

and to obtain free-bacteria and fungi plate. Flabellate shaped trophozoites measured about 20 µ and cysts were detected in clump manner (Fig. 1). Microscopic examination revealed our suspected amoebae as *Vannellidae*. Sequence analysis of the PCR product (Fig. 2) confirmed that the suspected amoebae are highly homologous with *Vannella* spp. gene (99% identity and 100% query coverage) available in the gene bank database (Accession number: **AY929909.1**; strain 4362V/II 18S small subunit ribosomal RNA gene) and this confirms our microscopic observation. The new gene have been deposited in the gene bank database under accession number: **JN009668**.

This is the first report of *Vannella* spp. isolated from biofilm sources in Iran. Our previous study showed that biofilm could harbor various free-living amoebae such as *Acanthamoeba* T4 and T5 genotype and *Vahlkampfia* (17). The present study proved that *Vannella* also could proliferate in such habitats. Indeed, microorganism within biofilms could be a great source of food for FLA and therefore biofilm sources introduced as serious hazard in hospital environment (15). Improved disinfection measures are recommended in health-care settings to avoid aggregation of biofilms. Previous researches isolated *Vannella* from marine and fresh water environments (10, 12). Few studies also report the isolation of *Vannella* from soil samples (12). Although, there is no report of human infection due to *Vannella*, but it is worthy to mention that this genus could be a carrier of other pathogens such as *Microsporidian*- like protozoa (15, 16). In support of this, Hoffman et al. (1998) researches revealed that *Vannella* could be infected by *Microsporidian* organisms. The sources of *Vannella* containing *Microsporidian* organisms in Hoffman et al. study were a domestic tap- water supply (16). This is important since *Vannella* could protect their endosymbiont from chlorine and harsh envi-

ronment and this can lead to multiplying of pathogenic organism inside the host amoebae. Indeed, infected amoebae can act as a vehicle for transmission of microorganisms to cornea. Subsequently, Scheid (2007) reported a case of keratitis in a contact lens wearer without a history of corneal trauma (19). *Pseudomonas aeruginosa* was identified as the etiological agent for the mentioned patient; however, *Vannella* was also detected in the material of the patient eye and intracellular organism detected in this amoebae resembling *Microsporidian* organism (19).

In conclusion, although *Vannella* is not proved to be pathogenic yet, but they are capable of harboring pathogenic organisms such as *Microsporidian* parasites and thus identification of this genus could lead to further studies regarding bacteria and amoebae relations. The present study gave information regarding the presence of *Vannella* in biofilm sources in hospital environment in Iran. More Hygienic measures and improved disinfection methods is recommended to health authorities to prevent FLA- related disease.

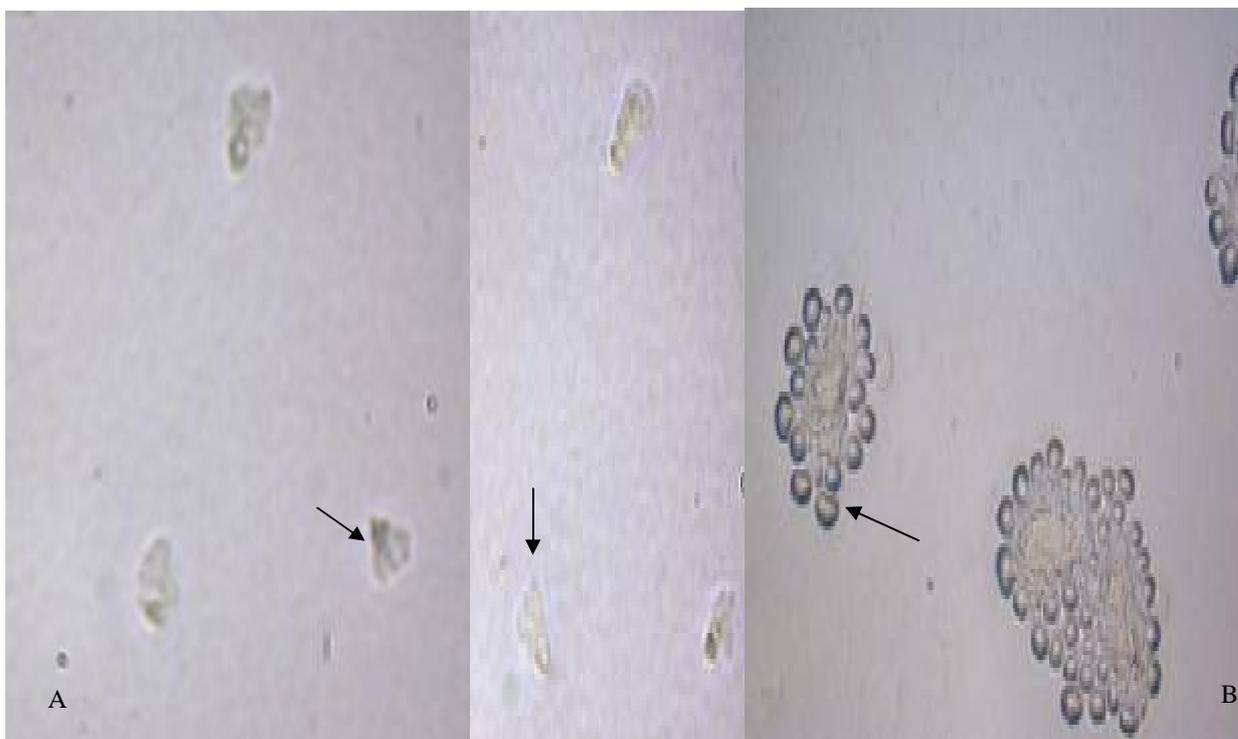


Fig. 1: A: Fan-shape trophozoit of *Vannella* spp X400; B Cyst of *Vannella* spp X100 isolated from biofilm source in Tehran, Iran

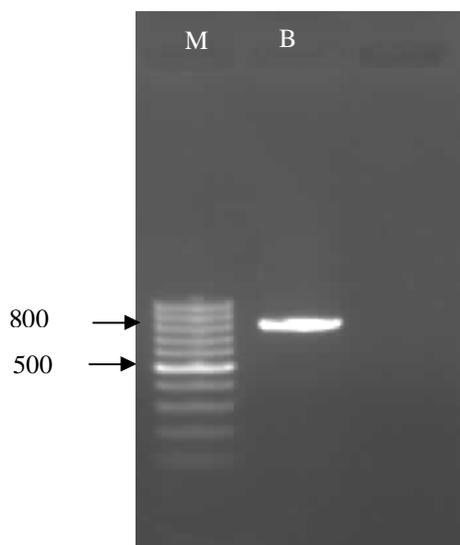


Fig. 2: Gel electrophoresis of the 800 bp PCR-product of *Vannella* spp isolated from biofilm in Tehran, Iran (M= Marker, B: Biofilm sample)

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