First molecular identification of *Entamoeba moshkovskii* from diarrhoeic stools in Kaduna State, Nigeria: a short report

Dawah I.S*, Inabo H.I, Abdullahi I.O and Machido D.A

Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

*Corresponding author: dawailiyah@yahoo.com

Received: 11.12.16; Accepted: 12.06.17; Published: 19.06.17

INTRODUCTION

*Entamoeba moshkovskii* cysts and trophozoites are not easily distinguished from those of the nonpathogenic *E. dispar* and the disease-causing *E. histolytica* and can therefore confound interpretation of stool microscopy.[1] *Entamoeba moshkovskii* is primarily described

ABSTRACT

Background: *Entamoeba moshkovskii* is morphologically identical, but different to *Entamoeba histolytica* and *Entamoeba dispar* biochemically and genetically. There are paucity of data on the prevalence of these commensal infectious organisms from Africa. **Aim:** The present study was carried out to identify and differentiate *Entamoeba moshkovskii* from *Entamoeba histolytica* and *Entamoeba dispar* genetically using PCR and DNA sequencing. **Methods:** Genomic DNA was extracted from microscopically positive stool samples and amplified using the Nested Multiplex Polymerase Chain Reaction (NM-PCR). The PCR products of 16S-like rRNA genes of *E. histolytica*, *E. dispar* and *E. moshkovskii* were sequenced on ABI3730XL sequencer (Macrogen, Seoul, South Korea) using species specific primers. **Results:** Of the 46 microscopically positive stool samples, only 16 (34.8%) successfully amplified *Entamoeba* species DNA. Out of the 16 PCR positive results, 3 (18.8%) contained *Entamoeba moshkovskii*, of which co-infection with *Entamoeba dispar* and *Entamoeba moshkovskii* was found in 2 (12.5%) samples, while 1 (6.3%) sample had only *Entamoeba moshkovskii* mono infection. **Conclusion:** This study reports the first intestinal infections with *Entamoeba moshkovskii* in diarrhoeic patients in Kaduna State, Nigeria. The recovery of *E moshkovskii* from diarrhoeic stools supports the concept that it could be a potential pathogen for humans as reported by many authors from different parts of the world. Therefore, the microscopic detection of *Entamoeba* needs further confirmation using molecular techniques in order to avoid misdiagnosis and unnecessary treatment.

Key words: *Entamoeba moshkovskii*, *Entamoeba moshkovskii*, *Entamoeba dispar*. Polymerase Chain Reaction, DNA sequencing, diarrhoeal disease
as a free-living amoeba that rarely infects humans. A high incidence of *E. moshkovskii* infections was reported in humans from Bangladesh, India, Turkey, Australia and Cameroon, but to the best of our knowledge, the infection has not been reported in Kaduna State, Nigeria.

The morphological similarity of *E. moshkovskii* and *E. dispar* to disease-causing *E. histolytica* makes it important to differentiate the three species by polymerase chain reaction (PCR). In the clinical setting, this may lead to a misdiagnosis and unnecessary treatment with anti-amoebic chemotherapy.

This study reports the first molecular identification of *E. moshkovskii* in Kaduna State, Nigeria. We used microscopy, polymerase chain reaction (PCR) and DNA sequencing to detect and differentiate *E. moshkovskii*, *E. dispar* and *E. histolytica* in stool samples from diarrhoeic patients in Kaduna State, Nigeria.

**METHODOLOGY**

Stool specimens for this study were obtained from diarrhoeic patients attending six hospitals in Kaduna State, Nigeria. Two hospitals from each of the three Senatorial Districts in Kaduna State, Nigeria, were selected namely: North, Central and Southern Senatorial Districts. The study was approved by the Department of Microbiology, Ahmadu Bello University, Zaria and the Ethical Committee of the Ministry of Health, Kaduna State, Nigeria (MOH/ADM/744/Vol 1).

Eighty eight (88) stool samples were collected from each of the six selected hospitals, totaling 528 stool samples collected from January, 2013 to December 2015. Iodine wet mounts of fresh unpreserved faecal samples were examined microscopically for demonstrating cysts and trophozoites of *Entamoeba* species complex as described by Cheesbrough. Briefly, a small fraction of feces was mixed with a small drop of Lugols iodine (diluted 1: 5 with water) on a microscope slide, and observed under microscope after placing a cover slip over the preparation.

The DNA extraction of all microscopy-positive samples was carried out using the MagNa Pure DNA isolation kit (Roche Applied Sciences) according to the manufacturer’s instruction. Primary PCR for the detection of *Entamoeba* genus used forward primer E-1 (5’-TAAGATGGA GAGCGAAA-3’) and reverse primer E-2 (5’-GTACAAAGGGGAGGGACGTA-3’) and secondary nested set Mos-1 (5’-GAA ACC AAG AGT TTC ACA AC-3’) and Mos-2 (5’-CAA TAT AAG GCT TGG ATG AT-3’) to detect *E. moshkovskii* (553 bp) was carried out as described by Khaimar and Parija. *Entamoeba* species genomic DNA (positive control) was included in each PCR run. “The PCR products were also sequenced using an ABI Prism 377 DNA sequencer (Applied Bio system)”.

The 16S rRNA gene sequences obtained were compared with those available in the GenBank database with the BLASTn program run on the http:// www.ncbi.nlm.nih.gov/BLAST.

**RESULTS**

Out of the 46 microscopy positive samples, 16 (34.8%) successfully amplified *Entamoeba* species DNA. Of the 16 PCR positive results, *Entamoeba dispar* infection 11 (68.8%) was the most prevalent, followed by *Entamoeba histolytica* 6 (37.5%) and *Entamoeba moshkovskii* 3 (18.8%). Mixed infection with *Entamoeba moshkovskii* and *Entamoeba dispar* was seen in 2 (12.5%) samples, while only 1 (6.3%) sample had only *Entamoeba moshkovskii* mono infection. The sequences of the three identified *E. moshkovskii* isolates in Figure 1 showed 98% similarity to seven *E. moshkovskii* sequences deposited in the GenBank (for example, KP722600.1).

**DISCUSSION**

The findings of the present study revealed for the first time the presence of *E. moshkovskii* in Kaduna State, Nigeria. Similar cases of humans infected with *Entamoeba moshkovskii* have been reported sporadically in different parts of the world including Bangladesh, India, Turkey, Australia and Cameroon. In clinical practice, the distinction of such infectious protozoan from the morphologically similar *E. histolytica* and *E. dispar* would avoid a misdiagnosis and unnecessary management with anti-amoebic drugs.
The observed symptoms of diarrhoea or dysentery in the individuals infected with *E. moshkovskii* in this study, suggest that it could be a potential pathogen for humans as reported by many authors.\[^{5,11}\] The co-infection of *E. moshkovskii* and *E. dispers* also agreed with previous reports from different parts of the world.\[^{3,4,12}\] The co-infections could be explained by the identical patterns of human contamination of these infectious intestinal amoebae.\[^{13}\]

The high degree in similarity (99%) of *Entamoeba moshkovskii* ribosomal RNA gene sequenced to the *Entamoeba moshkovskii* sequences in GenBank (such as accession no. KP722605.1) confirmed the DNAs amplified as being of *Entamoeba moshkovskii* origin. We could not able to use PCR to amplify genomic DNA from some samples that were positive for *Entamoeba* complex by microscopy with the primer used despite the absence of inhibitors. These results can potentially be explained by the presence of another *Entamoeba* species, genetically distinct but identical in microscopic appearance to the small cysts of *E. coli* or the large cysts of *E. Hartman*,\[^{11}\] or it could be due to complexities associated with faecal samples for direct PCR testing because of the presence of PCR inhibitors, such as heme, bilirubins, bile salts, and complex carbohydrates, which are often correlated along with pathogen DNA.\[^{5}\]

**CONCLUSION**

To the best of our knowledge, this is the first report of *E. moshkovskii* infection from Kaduna State, Nigeria. Although most reports support the commensal nature of *E. moshkovskii*, this report however, agrees with Fotedar and co-workers,\[^{5}\] that it may be pathogenic. We recommend the use of molecular techniques in the diagnosis of amoebiasis and further studies should be carried out to establish the causal link between the presence of *E. moshkovskii* and the symptoms of the disease.

**ACKNOWLEDGEMENT**

We thank Dr C. Graham Clark from the London School of Hygiene and Tropical Medicine for providing us with the lyophilized DNA of standard strains of *E. moshkovskii* Laredo.
are also grateful to the staff of The DNA Laboratory in Nigeria, for their technical assistance.

REFERENCES


Submit your valuable manuscripts to Michael Joanna Publications for:

• User-friendly online submission
• Rigorous, constructive and unbiased peer-review
• No space constraints or colour figure charges
• Immediate publication on acceptance
• Unlimited readership
• Inclusion in AJOL, CAS, DOAJ, and Google Scholar

Submit your manuscript at
www.michaeljoanna.com/journals.php

doi: http://dx.doi.org/10.14194/ijitd.4.1.5


Conflict of Interest: None declared
Submit your next manuscript to any of our journals that is the best fit for your research

International Journal of Medicine and Biomedical Research
Scope: IJMBR publishes cutting edge studies in medical sciences
Editor-in-Chief: Sofola A. Olusoga, MBBS, PhD, FAS
Deputy Editor: Lehr J. Eric, MD, PhD, FRCSC
URL: www.ijmbr.com
E-mail: editor@ijmbr.com
Pissn: 2277-0941, eISSN: 2315-5019

International Journal of Ethnomedicine and Pharmacognosy
Scope: IEP publishes novel findings on the use of complementary and alternative medicine in the management of diseases
Editor-in-Chief: Dickson A. Rita, B.Pharm, GCAP, PhD, MPSGh, MCPA
Deputy Editor: Kuete V., PhD
URL: www.ijepharm.com
E-mail: editor@ijepharm.com
Pissn: 2437-1262, eISSN: 2437-1254

International Journal of Infectious and Tropical Diseases
Scope: IJITD publishes interesting findings on infectious and tropical diseases of public health importance
Editor-in-Chief: Yang Z., PhD
Deputy Editor: Liping L.P., MD, PhD
URL: www.ijitd.com
E-mail: editor@ijitd.com
Pissn: 2384-6607, eISSN: 2384-6585

Reasons to publish your manuscript with Michael Joanna Publications:
• User-friendly online submission • Rigorous, constructive and unbiased peer-review • No space constraints or coloured figure charges • Immediate publication on acceptance • Authors retain copyright • Inclusion in AJOL, CAS, CNKI, DOAJ, EBSCO, Google Scholar, and J-Gate • Unlimited and wide readership • Member of COPE and CrossRef

Editorial Director
Professor Sofola A. Olusoga,
Department of Physiology,
University of Lagos,
Nigeria.
Tel: +234(0) 7093848134
Email: enquiry@michaeljoanna.com
www.michaeljoanna.com

Open Access