Subtyping identification of *Blastocystis sp.* isolated from symptomatic and asymptomatic individuals in Makkah, KSA.

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Introduction

Blastocystis is one of the most understood protozoan, anaerobic and zoonotic parasites that live in the gastrointestinal tract of humans and an extensive variety of animal hosts including no less than 17genetically specific small subunit ribosomal RNA subtypes)STs), nine of which have been found in humans. The geographic distribution of *Blastocystis* subtypes is very variable. Until now the subtypes present in Saudi Arabia were unknown.

Methods

Sample collection: From March 2014to July 2015, a total of 130 fecal samples from those who had been re- ferred to King Abdellah medical city and Al Nour Hospital, Makkah were collected. Six- ty nine (%53)had gastrointestinal symptoms and sixty one (%47) with no gastrointestinal symptoms were tested

Microscopic identification and stool culture: Blastocystis sp. And other intestinal protozoan parasites were recognized in the stool sam- ples using direct smear method, The fresh stool samples were cultured in Dulbecco's modified Eagle medium)DMEM))Gibco) con- taining 12mg/ml ampicillin and 4mg/ml strep- tomycin supplemented with % 20inactivated horse serum (Gibco(.Cultured samples were incubated in ° 37C in anaerobic gas pack)BD gas pack-Becton, Dickinson, USA) for 72 h and examined with direct slide smear.

DNA extraction: Genomic DNA was extract from positive stool samples with ZR fecal DNA minprep (Zymo Research, USA), while positive culture was extracted with DNA extraction kit, according to manufacturer's direction)QIAmp, QIAGEN Inc, Germany). The concentration of each DNA was measured.

PCR with the STS primers: To identify genotypes of Blastocystis sp., a PCR was carried out using ten pairs of STS primers)SB82, SB83, SB340, SB227, SB228, SB229, SB337,SB336, SB332 and SB155) as shown in table 1. using ready to use AmpliTaq Gold 360master mix.

Results

*Fig :1*Microscopic examination of *Blastocystis sp.* parasites cultures showing different morphological stages: granular, vacuolar and avacuolar.

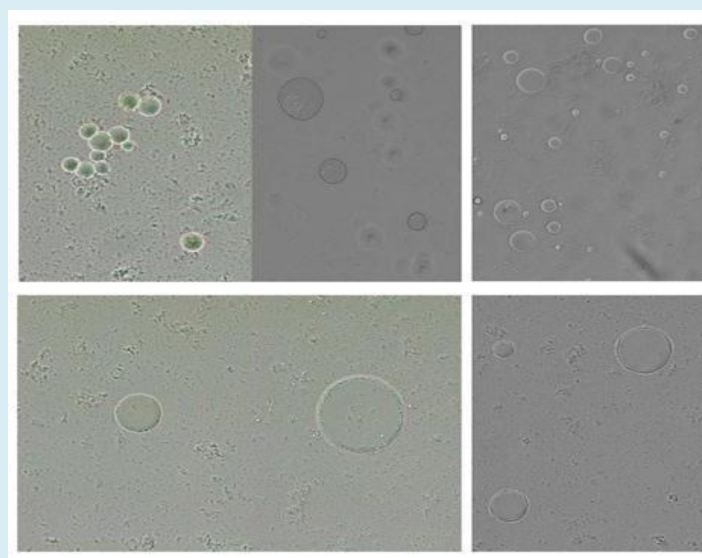
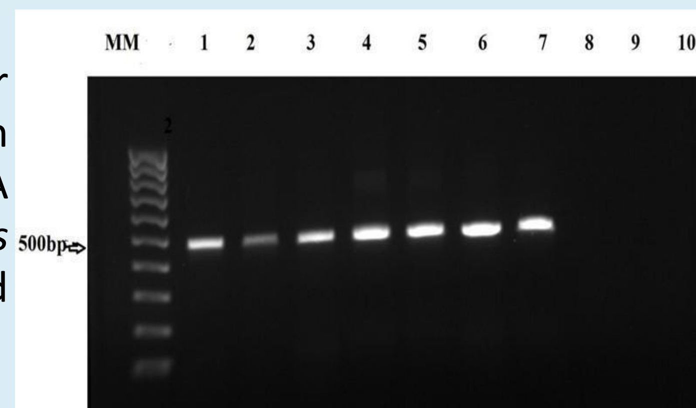


*Fig :2*Specific Diagnostic PCR for detection of *Blastocystis sp.* in stool samples. lane 1: 100bp DNA ladder, lane :8-2*Blastocystis* positive samples, and lane 9 and :10negative control samples.



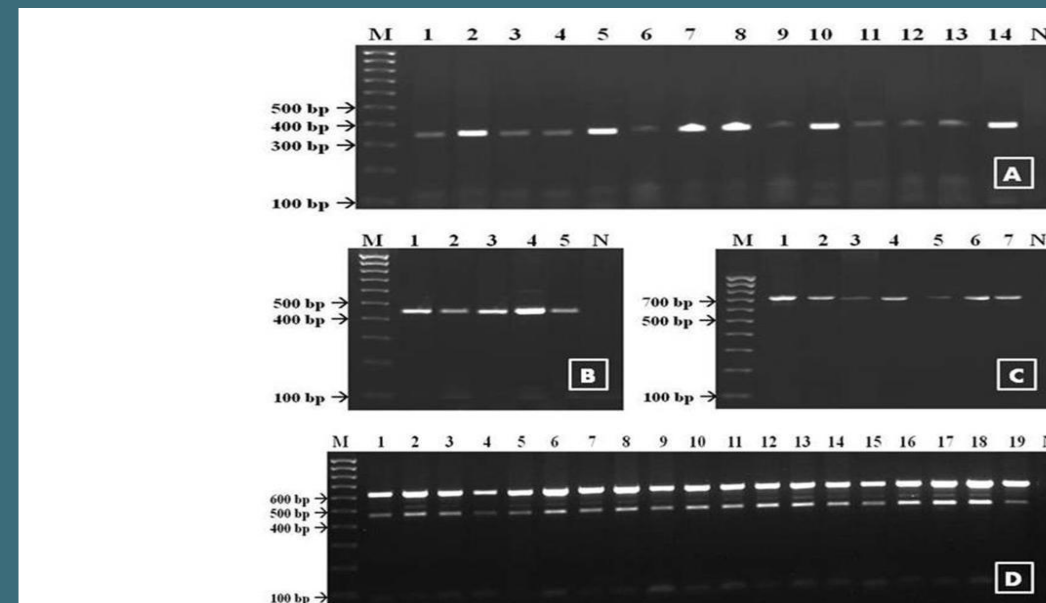


FIG. 1: STS primer-based PCR analysis of *Blastocystis sp.* genotypes using SB83 (ST1-351bp) (A), SB82 (ST1 variant-462bp) (B), SB340 (ST2-704bp) (C), and SB227 (ST3-526bp), SB228 (ST3-473bp), and SB229 (ST3-631bp) in a multiplex reaction (D). Panel A: isolates genotyped as ST1 (lanes 1-8 from symptomatic patients and 9-14 from asymptomatic individuals. Panel B: five isolates, all from asymptomatic individuals, genotyped as ST1variant. Panel C: isolates genotyped as ST2 (lanes 1- 3 from symptomatic patients and 4-7 from asymptomatic individuals). Panel D: representative isolates out of 102 genotyped as ST3 (lanes 1-10 from symptomatic patients and 11-19 from asymptomatic individuals). Lanes N: PCR reactions negative controls. Lanes M 100bp molecular size marker.

Discussion

Nine subtypes of *Blastocystis sp.* (ST1 to ST9) were identified in humans (1). To our Knowledge, this is the first report exploring the subtypes of *Blastocystis* in Makkah city, KSA. We detected, by using STS-PCR technique the four subtypes; ST1, ST1variant, ST2 and ST3 among 133 positive cases from the city. The high predominance of ST3 (77%) in our study population, is reliable with different reports in the literature; ST3 was found as the most dominant subtype; varying from 41% to 92% in a comparative study in between Japan, Bangladesh, Pakistan and Germany states (2), and of 78%, 75.9%, 54.5%, 53.5% and 33.3% in Singapore, Turkey, Egypt, France and Lebanon, respectively (3.4).

Conclusion

The outcome of this study provides the first run-through information on *Blastocystis sp.* epidemiology in Makkah city, revealing a relatively moderate prevalence (10.5%) as well as the presence of four subtypes: ST3 being the most predominant in particular among symptomatic patients, ST1, ST2, and ST1 variant which were detected in asymptomatic individuals only. This study underlines the advantage of SSU rRNA gene STS-PCR as a significant technique for *Blastocystis sp.* subtyping in epidemiological study.

Acknowledgments

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