

**Molecular epidemiology of *Blastocystis*
sp.**

By
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(Science) at the University of Technology, Sydney

Certificate of Original Authorship

This study was carried out in the Microbiology Department, St. Vincent's Hospital, Sydney under the supervision of Professor John Ellis and Dr Damien Stark. I certify that this thesis has not been submitted previously as part of any course or degree other than in fulfilment of the requirements of a PhD degree at the University of Technology, Sydney. I certify that this thesis has been written by me and the vast majority of work described was completed by me. All other contributors have been acknowledged throughout this thesis as necessary.

I hereby certify that the above statements are true and correct:

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Date:

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Chapter 4:

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Abbreviations

AIDS	Acquire immunodeficiency syndrome
ART	Antiretroviral therapy
bp	base pairs
CDC	Centre for Disease Control
CPS	Cat protection society
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbant assay
HIV	Human immunodeficiency virus
HM	Haematological malignancies
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IFN	Interferon
IgA	Immunoglobulin alpha
IgG	Immunoglobulin gamma
IL	Interleukin
kb	Kilobases
kDa	Kilo Dalton
MALDI-TOF	Matrix-assisted laser desorption/ionisation time-of-flight
MBD	Modified Boeck and Drbohlav's
Mb	Megabase
mg	milligram
MIC	Minimum inhibitory concentration
MLC	Minimum lethal concentration

ml	millilitre/s
MLO	Mitochondria like organelle
MLST	Multilocus sequence typing
NCBI	National center for Biotechnology Information
NJ	Neighbour joining
NSW	New South Wales
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Peace corps volunteers
rDNA	ribosomal Deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
rRNA	ribosomal Ribonucleic acid
qPCR	Quantitative polymerase chain reaction
SAF	Sodium acetate acetic acid formalin
SNPs	Single nucleotide polymorphisms
SSU	Small subunit
ST	Subtype
SUPAMAC	Sydney University Prince Alfred Macromolecular Analysis Centre
TMP-SMX	trimethoprim-sulfamethoxazole
TNF	Tumor necrosis factor
TYGM-9	Tryptose, yeast extract, glucose, methionine 9
µl	microlitre
µg	microgram
w/v	weight per volume

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Abstract

Blastocystis sp. is the most common enteric protist of the human gastrointestinal tract. There has been continual controversy over the role *Blastocystis* plays in causing gastrointestinal disease in humans. It has been suggested to be a pathogen or an opportunistic commensal and it has also been suggested that pathogenicity could be related to subtype (ST) determined by molecular methods. Until recently there was little known about this protist in terms of epidemiology, pathogenicity and treatment. Clinical diagnosis has traditionally been based on microscopy of wet preparations or permanent stains but there has recently been a push towards more sensitive techniques such as culture and polymerase chain reaction (PCR). The correct diagnosis of *Blastocystis* is necessary for epidemiological and clinical studies which will aid in determining the actual role of this parasite in the gut and in producing disease. Due to the lack of knowledge on the pathogenicity of this parasite, research into treatment options is limited. Metronidazole is a commonly used anti-parasitic drug that has frequently been used for *Blastocystis* treatment. There is evidence that this drug may not actually have much efficacy at all on *Blastocystis* and therefore be the incorrect treatment option.

This project was designed to address some of the shortcomings in the literature surrounding this parasite. The overall aim of the project was to describe the molecular epidemiology of *Blastocystis* sp. from Australia and comment on the pathogenicity of *Blastocystis* in humans. To be able to determine the molecular epidemiology, it was necessary to use the correct diagnostic method and therefore the first aim of this study was to determine the best diagnostic technique used for the detection of *Blastocystis* (aim 1 of this study). Five different techniques were tested for their sensitivity for detecting *Blastocystis* and it was found that microscopy of a permanent stain was the least sensitive at detecting *Blastocystis* and that PCR was the most sensitive technique. Once the most sensitive diagnostic technique was established it was then possible to determine the prevalence of *Blastocystis* within the Sydney population from clinical samples (aim 2 of this study). It was found that there was a 19% incidence of *Blastocystis* in this population and seven subtypes (STs) were identified by sequencing- ST1, ST2, ST3, ST4, ST6, ST7 and ST8. ST3 was found to be the most common ST in this population.

This study then investigated the prevalence of *Blastocystis* in animals and determined the STs present (aim 3 of this study). There were 38 different species of animal from seven different locations investigated for the presence of *Blastocystis* using PCR. There were 80 (18%) positive isolates from 18 species, and nine different STs were identified- ST1, ST2, ST3, ST4, ST5, ST7, ST11, ST12 and ST13. This is the first report of *Blastocystis* from the eastern grey kangaroo, red kangaroo, wallaroo, snow leopard and ostrich. This study has expanded current knowledge on the host range of *Blastocystis*.

Blastocystis is associated with symptoms in humans similar to irritable bowel syndrome (IBS) such as bloating, diarrhoea and abdominal pain and therefore this study aimed to look at the relationship between *Blastocystis* and IBS (aim 4 of this study). This study showed that though there was not a significantly higher percentage of *Blastocystis* seen in the IBS group compared to the control group, there was a difference in the STs present with ST4 only present in the IBS group. This study also highlighted the need for full microbiological work-up before a diagnosis of IBS can be given as *Blastocystis*, along with other microbes, may actually be a contributor to the disease process.

The final part of this study was to look at treatment options for *Blastocystis*. Due to the lack of knowledge on the pathogenicity of *Blastocystis* there have only been a few studies on treatment options and much more information is needed (aim 5 of this study). This study followed 18 patients with chronic *Blastocystis* infection who were treated with a variety of antimicrobials. It was seen that the most common drug treatment of choice, metronidazole, was not effective for the clearance of *Blastocystis*. This study also highlighted the chronic nature of *Blastocystis* infection in the absence of any other infectious agents. This study also carried out *in vitro* testing for four common human *Blastocystis* STs (ST1, ST3, ST4 and ST8) against 12 commonly used antimicrobials- metronidazole, paromomycin, ornidazole, albendazole, ivermectin, trimethoprim- sulfamethoxazole (TMP-SMX), furazolidone, nitazoxonide, secnidazole, fluconazole, nystatin and itraconazole. Cultures were maintained in media that was determined the best for *Blastocystis* growth from aim 1 of this study. From this *in vitro* study the lack of efficacy of commonly used antimicrobials for the treatment of *Blastocystis* was shown in particular metronidazole, paromomycin and a triple therapy combination of furazolidone,

nitazoxanide and secnidazole. This study did show the efficacy of two drugs- TMP-SMX and ivermectin and suggested the use of these treatments instead of metronidazole.

Each of these studies aims has furthered the knowledge on *Blastocystis* epidemiology, pathogenicity and treatment options. This is the largest molecular epidemiological study to be completed in Australia and also the largest animal study to be undertaken thus far. Overall, this PhD project has contributed significantly by enhancing and extending current knowledge on *Blastocystis* and will hopefully encourage future research on this fascinating protist.