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Promising Drug Repurposing Candidates Targeting Free-Living Amoebae: A Systematic and Critical Review of Laboratory-Based Evidence

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Beni Jequicene Mussengue Chaúque[1,2], Luiza Bernardes Chagas[3], Thaisla Cristiane Borella da Silva[4], Denise Leal dos Santos[1], Luciano Palmeiro Rodrigues[1,5], Lucile da Silva Lins Baia[6], Manoella Kessler Gomes Rodrigues[7], Guilherme Brittes Benitez[8], Thais Lemos Mendes[9], Hellen Kempfer Philippsen[10], Luciana Dalla Rosa[9], Fabrício Souza Campos[11,12], Marilise Brittes Rott[4], Régis Adriel Zanette[6], José Roberto Goldim[1], Beni Chauque[1,2]

[1]Master's Program in Clinical Research, Hospital de Clínicas de Porto Alegre, Rio Grande do Sul, Porto Alegre, Brazil.;
[2]Center of Studies in Science and Technology (NECET), Biology Course, Universidade Rovuma, Niassa, Lichinga, Mozambique.;
[3]Faculty of Pharmacy, Federal University of Rio Grande do Sul, RS, Porto Alegre, Brazil.;
[4]Protozoology Laboratory, Microbiology Immunology and Parasitology Department, Basic Health Sciences Institute, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.;
[5]Physiotherapy Course, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.;
[6]Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.;
[7]School of Medicine, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil.;
[8]Industrial and Systems Engineering Graduate Program, Polytechnic School, Pontifical Catholic University of Parana (PUCPR), Brazil.;
[9]Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil.;
[10]Socio-Environmental and Water Resources Institute, Federal Rural University of the Amazon (UFRA), Belém, 66077-830, Pará, Brazil.;
[11]Laboratório de Bioinformática 26 Biotecnologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre 90010-150, RS, Brazil.;
[12]Department of Public 26 Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Beni Chaúque

Beni Chauque

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We use this protocol and it's working

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Abstract

Free-living amoebae (FLA) are opportunistic protozoan pathogens capable of causing severe and frequently fatal infections in humans and animals, including primary amoebic meningoencephalitis, granulomatous amoebic encephalitis, and Acanthamoeba keratitis. Current therapeutic options remain limited, largely empirical, and often associated with high toxicity and inconsistent efficacy. Drug repurposing represents a promising strategy for accelerating the identification of effective anti-FLA therapies by leveraging compounds with established pharmacological and toxicological profiles. This systematic review aims to identify approved drugs evaluated for antiamoebic activity against FLA species in laboratory models and to critically assess their potential for therapeutic repurposing. The review will synthesize evidence from in vitro and in vivo studies, focusing on trophocidal and cysticidal activity, potency, and toxicity parameters under defined ADMET conditions.

Objectives

1 To systematically identify approved drugs with anti-FLA activity evaluated in laboratory models.

1.1 To compare trophocidal and cysticidal efficacy across compounds.

1.2 To evaluate potency based on IC_50 values (≤ 20 µM threshold).

1.3 To assess cytotoxicity in mammalian cell models.

1.4 To identify candidates suitable for prioritization in translational research.

Eligibility Criteria

2 Studies will be included if they report full-text articles available online; are written in English, Portuguese, Spanish, or translatable languages; evaluate approved drugs; present in vitro and/or in vivo antiamoebic activity; investigate at least one FLA genus: Acanthamoeba, Naegleria, Balamuthia, Vermamoeba, Sappinia.

3 Studies will be excluded if they are review articles; are preprints or non–peer-reviewed publications; evaluate experimental or non-approved compounds; lack experimental laboratory data.

Information Sources

4 Searches will be conducted in the following databases:
Web of Science
EMBASE
PubMed
ProQuest
Reference lists of included studies will also be screened.

Search Strategy

5 A broad search strategy will be applied: *Acanthamoeba* OR *Naegleria* OR *Balamuthia* OR *Vermamoeba_*.

No publication date restrictions will be applied.
Final search date: June 11, 2024.
Search consistency across databases will be independently verified by two reviewers.

Study Selection Process

6 Screening conducted independently by at least two reviewers.
Steps:
Duplicate removal
Title/abstract screening
Full-text assessment
Disagreements resolved through consensus with a third reviewer.
Workflow documented using PRISMA 2020 flow diagram.

Data Extraction

7 Data extracted will include:
Study reference
Author nationality
Drug name and pharmacological class
Approved indication
FLA species
Life stage (trophozoite/cyst)
Amoebae density
Drug concentration (µM)
Exposure time
Mortality rate
IC_50
CC_50 (mammalian cells)
Cytotoxicity rate
Mammalian cell line
Extraction performed by one reviewer and validated by two additional reviewers.

Outcomes

8 Primary Outcome
Amoebicidal potency (IC_50 ≤ 20 µM).
Secondary Outcomes
Cysticidal activity
Exposure-response relationship
Cytotoxicity profiles
Selectivity indicators (IC_50 vs CC_50)

Data Analysis Plan

9 Statistical analyses will be performed using R software (R Foundation for Statistical Computing, Vienna, Austria).
For each compound:
Mean
Standard deviation
Minimum and maximum values
Exposure time
Effective concentrations
Mortality parameters
Standard deviation will be calculated only when ≥2 observations exist.
Data will be curated and analyzed using a customized script to ensure reproducibility.
Figures will be generated using GraphPad Prism v5.

Risk of Bias and Quality Assessment

10 Although laboratory studies lack standardized clinical bias tools, methodological consistency will be assessed considering:
Experimental reproducibility
Controls used
Replication reporting
Cytotoxicity evaluation
Clarity of outcome reporting

Acknowledgements

Data Management and Transparency

All datasets will undergo cleaning, curation, and documentation. Analytical scripts and procedures will be described to ensure reproducibility and transparency.

Expected Contributions

This review aims to:

- Identify high-priority drug repurposing candidates;
- Support translational antiamoebic research;
- Inform development of improved therapeutic strategies against neglected FLA infections.

Protocol Version

Version 1.0 — Initial registration.