مجلة روافد المعرفة

تصدر عن كلية العلوم
جامعة الزيتونة

الرقم الدولي الموحد

ISSN: 2709-0345

ISSN : 2709-0345
Linking ISSN (ISSN-L): 2709-0345
Key-title: Rawāfid al-ma'rifat
Key-title in original characters: روافد المعرفة

العدد العاشر

2024 - 12

ترسل البحوث وجميع المراسلات المتعلقة بالمجلة إلى العنوان التالي:

كلية العلـــوم – جامعــة الزيتونــة – ترهـــونة

هـ: 0926825815 _0913253199

rwafedalmarefa@gmail.com

الكلمة الافتتاحية،،

السلام عليكم ورحمة الله وبركاته،،

عليه نتوكل، وبه نستعين، نحمده سبحانه على كل عمل.

أعزائي القراء والمهتمين بالمعرفة والعلم،

بحمد الله وتوفيقه تم صدور العدد العاشر من مجلة "روافد المعرفة"، الصادرة عن كلية العلوم بجامعة الزيتونة. إن هذا الإصدار الذي نقدمه لكم يعكس التفاني والتميز الذي يتميز به فريق العمل والباحثين الذين ساهموا في إثراء هذا العدد بمقالاتهم وأبحاثهم الرائعة.

مجلة "روافد المعرفة" تعد نافذةً مهمة لنشر العلم والبحث العلمي، وهي تسعى جاهدة لتعزيز التواصل العلمي وتبادل المعرفة بين الباحثين والمهتمين بالمجالات العلمية المختلفة. إن تنوع المواضيع المطروحة في هذا العدد يعكس الاهتمام الكبير بمجالات العلوم الطبيعية والتطبيقية، ويعزز الوعي والفهم العلمي للقراء.

في هذا العدد العاشر، ستجدون مقالات متنوعة تتناول العديد من المواضيع المميزة والمفيدة في مجالات العلوم الطبيعية والتطبيقية. ولذلك، نحن واثقون من أن هذا العدد سيثري ثقافتكم ويوسّع آفاق المعرفة لديكم.

في ختام كلمتنا، أود أن نعرب عن امتناننا العميق للفريق الذي عمل بجد واجتهاد لجعل هذا العدد حقيقةً، وأشكر جميع الباحثين الذين شاركوا معنا معرفتهم وخبراتهم. وأتمنى أن يكون هذا العدد بمثابة نقطة انطلاق لمزيد من النجاح والتألق في المستقبل.

نتمنى لكم قراءة ممتعة ومفيدة، ونحن في انتظار ملاحظاتكم وآرائكم القيّمة.

شكراً لثقتكم ودعمكم المستمر.

دمتم بخير وعلم نافع.

هيئة التحرير

اشتراطات النشر في مجلة روافد المعرفة

1- أن يكون البحث أصيلاً ومبتكراً ولم يسبق نشره في أي جهة أخرى، وتتوفر فيه شروط البحث العلمي المعتمدة على الأصول العلمية والمنهجية المتعارف عليها في كتابة البحوث الاكاديمية.

-2- أن يكون البحث مكتوباً بلغة سليمة، ومراعياً لقواعد الضبط ودقة الرسوم والاشكال – إن وجدت و مطبوعاً بخط Microsoft Word (Simplified Arabic) ببنط (14) للغة العربية، وخط (Times New Roman) ببنط (12) للغة الإنجليزية، وألا تزيد صفحات البحث عن (35) صفحة متضمنة المراجع والملاحق (إن وجدت).

-3- يجب أن يشتمل البحث على العناصر التالية - عنوان البحث باللغتين العربية والإنجليزية - - ملخص تنفيذي باللغتين العربية والإنجليزية في نحو 100 - 125 كلمة والكلمات المفتاحية (keywords) بعد كل ملخص .

-4 يتم توثيق الهوامش وفق طريقة الجمعية الأمريكية السيكولوجية (APA) بإصدارتها المختلفة.

-5- يُفضل أن تكون الجداول والاشكال مدرجة في أماكنها الصحيحة، وأن تشمل العناوين والبيانات الإيضاحية الضرورية، ويراعى ألا تتجاوز أبعاد الاشكال والجداول حجم حيز الكتابة في صفحة.

-6- أن يكون البحث ملتزماً بدقة التوثيق، استخدام المصادر والمراجع، وأن تثبت مصادر ومراجع البحث في نهاية البحث.

7- تحتفظ المجلة بحقها في اخراج البحث وإبراز عناوينه بما يتناسب واسلوبها في النشر.

-8- - ترحب المجلة بنشر ما يصلها من ملخصات الرسائل الجامعية التي تمت مناقشتها وإجازتها على أن يكون الملخص من إعداد صاحب الرسالة نفسه.

9 - تُرسل نسخة من البحث مطبوعة على ورق بحجم (A4) إلى مقر المجلة، ونسخة إلكترونية إلى إيميل المجلة : wafedalmarefa@gmail.com او على رقم الواتساب 0921253199 على أن يدون على صفحة الغلاف اسم الباحث لقبه العلمي، مكان عمله، تخصصه، رقم هاتفه وبريده الإلكتروني.

10- يخطر الباحث بقرار صلاحية بحثه للنشر من عدمها خلال مدة شهرين من تاريخ استلام البحث.

11- في حالة ورود ملاحظات وتعديلات على البحث من المحكم ترسل تلك الملاحظات إلى الباحث لإجراء التعديلات اللازمة بموجبها على أن تعاد للمجلة خلال مدة أقصاها شهر واحد.

-12- الأبحاث التي لم تتم الموافقة على نشرها لا تعاد إلى الباحثين.

- 13- تؤول جميع حقوق النشر للمجلة.

ملاحظة.

البحوث المنشورة في هذه المجلة تعبر عن رأي أصحابها ولا تعبر بالضرورة عن رأي المجلة أو الكلية أو الجامعة.

المحتويات

Single nucleotide polymorphisms in Leishmania resistant to fluorinated pyrimidines

Juma Ahmed Mohmed Ali

Zoology Department, Faculty of Science, Gharyan University

jumamali1972@gmail.com

الملخص

داء الليشمانيات هو مرض تسببه انواع من مختلفة من طفيل الليشمانيا، حيث يشكل المرض عبئاً عالمياً كبيراً. على الرغم من الجهود المستمرة فان العلاج المتاح لداء الليشمانيات لا يزال حتى الان غير مرضي. ان غياب الادوية واللقاحات الفعّالة ضد طفيليات الليشمانيا يستلزم استكشاف استراتيجيات علاجية جديدة. عليه يهدف هذا البحث الى دراسة العلاقة بين تعدد الاشكال المفرد في أنواع من طفيليات الليشمانيا المقاومة للفلوروبيريميدينات، تركز هذه الدراسة على طفيل *Leishmania mexicana* و *Leishmania major*. بالنسبة لطفيل *Leishmania mexicana* تم تحليل أربع مجموعات مرتبطة بالمقاومة المتوسطة لمركب 5 فلوروبوراسيل وثلاث مجموعات مرتبطة بالمقاومة النهائية لنفس المركب. أما بالنسبة لطفيل *Leishmania major* تم فحص مجموعة واحدة تتعلق بالمقاومة النهائية لمركب 5- فلوروبوراسيل ومجموعة أخرى مرتبطة بالمقاومة لمركب 5-فلورو-2'-ديوكسيوريدين. من خلال دراسة هذه المجموعات تم تحديد تعدد الأشكال المفرد الذي قد يساهم في تطوير مقاومة الفلوروبيريميدينات في انواع من طفيليات الليشمانيا. فقد تم تحديد تتابع كامل الجينات لكل سلالة ولوحظ وجود الآلاف من تعدد الاشكال المفرد في السلالات المقاومة للادوية مقارنة بقرينها من النوع الجامح. النتائج تتضمن قوائم شاملة تشير الى مواضع الجينات التي يتوجد بها تعدد الاشكال المفرد بدرجة عالية الثقة في كل مجموعة. ختاماً من الضروري اجراء بحوث اضافية لتوضيح الاهمية الوظيفية لتعدد الاشكال المفرد ودوره المحتمل في منح المقاومة لمركبات الفلوروبيريميدين في طفيليات الليشمانيا. ومن خلال التعمق في الآليات الجزيئية الكامنة وراء مقاومة الادوية، يمكننا تعزيز فهمنا لهذه الظاهرة المعقدة وربما تحديد اهداف جديدة للتدخل العلاجي.

الكلمات المفتاحية: الليشمانيا، تعدد الأشكال المفرد، الفلوروبيريميدينات، الجينات.

Abstract

Leishmaniasis, caused by Leishmania parasites, is a significant global health issue with limited treatment options. This study focuses on investigating the association between single nucleotide polymorphisms (SNPs) and resistance to fluorinated pyrimidines in *Leishmania mexicana* and *Leishmania major*. Multiple datasets were analyzed for each species, assessing resistance to specific drugs. The aim is to identify SNPs that contribute to drug resistance in Leishmania. Through full genome sequencing, numerous SNPs were detected in resistant cell lines compared to wild-type strains. The dataset provides comprehensive lists of high-confidence SNPs and their genomic positions. Further research is necessary to understand the functional implications of these SNPs and their role in conferring resistance to fluorinated pyrimidines. By exploring the molecular mechanisms of drug resistance, we can gain insights and potentially uncover new therapeutic targets for leishmaniasis.

Keywords: Leishmania, SNPs, Fluorinated pyrimidines, Genome.

1-Introduction

Cutaneous leishmaniasis exhibits a broad geographical distribution, posing a significant risk to millions of individuals worldwide, with an estimated one million new cases annually (WHO, 2022). The disease has become prevalent and widely spread throughout various regions of Libya, especially in the northwestern area (Amro et al., 2012; El-Badry et al., 2017). Historically, antimonial compounds have been the preferred treatment for kinetoplastid infections. However, reports indicate that leishmania parasites have developed resistance to these drugs (Ponte-Sucre et al., 2017). Consequently, there is an urgent need to develop new, effective treatments for leishmaniasis. Resistance of kinetoplastids to drugs can occur through various mechanisms. One possible mechanism involves the loss of specific transport functions in the parasite's cell membrane (De Koning, 2001). Additionally, the drug can be inactivated, excreted, or modified within the cytoplasm of the parasite (Borst and Ouellette, 1995). In a study conducted by Ali et al. (2013), it was demonstrated that pyrimidine analogues possess antimetabolite activity against kinetoplastids. Furthermore, research has been carried out to investigate the potential mechanisms of resistance to these drugs (Alzahrani et al., 2017).

In a previous study conducted by Verma et al. (2017), a comparison was made between leishmania cell lines resistant to paromomycin and their parental wildtype strains. This study revealed changes in the expression of several genes associated with drug resistance. Recent advancements in technology have made it possible to sequence entire genomes, providing a valuable approach to investigate drug resistance. Ghosh et al. (2020) highlighted the significance of whole genome sequencing in studying drug resistance mechanisms.

However, the exploration of single nucleotide polymorphisms (SNPs) in Leishmania strains resistant to pyrimidine analogues remains limited. Therefore, the objective of this current study is to identify SNPs that are specifically associated with resistance to fluorinated pyrimidines in both *L. mexicana* and *L. major*. By analyzing these SNPs, we aim to enhance our understanding of the genetic basis of drug resistance in Leishmania and potentially identify new targets for effective treatment strategies.

2. Materials and Methods

The methods employed to generate Leishmania strains resistant to fluorinated pyrimidines were outlined in the study conducted by Alzahrani et al. (2017). Specifically, the resistance to fluorinated pyrimidines in *L. mexicana* was investigated, resulting in the generation of four datasets associated with intermediate resistance to 5-fluorouracil (Lmex-Int-5FURes) and three datasets associated with final resistance to 5-fluorouracil (Lmex-Fin-5FURes). Additionally, for *L. major*, one dataset was generated for resistance to 5-fluorouracil (Lmaj-5FURes), and another dataset was generated for resistance to 5-fluoro-2'-deoxyuridine (Lmaj-5F2'dURes).

In this study, the objective was to identify genetic variations known as single nucleotide polymorphisms (SNPs) in a specific strain of

Leishmania. The focus was on comparing resistant strains to their respective parental wild-type strains (*L. mexicana* strain M379 and *L. major* strain Friedlin) to uncover SNPs associated with resistance to fluorinated pyrimidines in Leishmania species.

To achieve this, the study employed a set of specific criteria for the identification and analysis of SNPs. These criteria included minimum thresholds for high-quality base calls, coverage depth, quality scores, mapping quality, and second-best genotype likelihood values. Additionally, the study considered the maximum fraction of conflicting base calls for homozygous genotypes and the minimum percentage of base calls mapping to the forward or reverse strand to address potential biases.

By applying these rigorous criteria, the researcher successfully identified and analyzed SNPs in the Leishmania strains. This approach enabled the detection of genetic variations associated with resistance to fluorinated pyrimidines, contributing to a better understanding of drug response mechanisms and the development of more effective treatment strategies for Leishmania infections. By applying these stringent criteria, the study ensured the selection of high-quality SNPs associated with resistance to fluorinated pyrimidines in Leishmania species.

In the study, the researcher conducted searches for amino acid and nucleotide sequences using the Gene DB and Tri Tryp DB websites. These databases provide comprehensive collections of genetic information for various organisms, including trypanosomes and related parasites. The researcher likely utilized these databases to access and retrieve relevant sequences for their analysis. By searching these databases, they could compare and analyze the sequences of interest, potentially identifying specific genes or genetic variations associated with the study's focus on pyrimidine metabolism or biosynthesis.

The TMHMM server at http://www.cbs.dtu.dk/services/TMHMM/ was utilized in the study to estimate the number of transmembrane domains (TMDs) in the specified genes. This information is crucial as the study focused on identifying single nucleotide polymorphisms (SNPs) that occur within TMDs. Such SNPs may potentially be associated with alterations in pyrimidine transport. Additionally, the researcher employed the Basic Local Alignment Search Tool (BLAST) available on Gene DB or Tri Tryp DB websites to compare gene sequences. This facilitated the identification of sequence similarities and differences. Furthermore, the CLC workbench software was utilized for sequence alignments, aiding in the analysis and visualization of sequence data. The study specifically targeted SNPs within genes known to be involved in pyrimidine metabolism or biosynthesis.

3. Results and Discussion

In the study, a substantial number of single nucleotide polymorphisms (SNPs) were observed in each resistant strain. However, the researcher focused on including SNPs that met certain criteria to ensure high confidence and functional relevance. Specifically, they considered SNPs that resulted in non-synonymous changes in the coding sequences, indicating potential impact on protein function. The collected data included information about each SNP, such as the genomic position where the SNP was identified and its associated chromosome name and position. Additionally, the predicted number of transmembrane domains (TMDs)

for each SNP was recorded, as this information was relevant to the study's focus on SNPs within TMDs. The style of mapping sequence reads to the reference genome was also noted, indicating the approach used for alignment and analysis.

To ensure the reliability and significance of the identified SNPs, only those deemed high confidence were included in the analysis. These high-confidence SNPs likely underwent stringent quality control measures and met specific criteria for selection, ensuring their reliability and relevance to the study's objectives. For each strain analyzed in the study, the researcher provided information on the genotype of the reference sequence obtained from GeneDB, as well as the inferred diploid genotypes of both the parental wild-type strain and the drug-resistant strain. Additionally, details regarding the indicated strain and its associated GeneID were included.

Regarding the SNPs, the following information was provided:

1. Position of open reading frame (ORF): The genomic position of the ORF where the SNP was located.

2. Affected triplet in the reference sequence: The specific nucleotide triplet (codon) in the reference sequence that was altered by the SNP.

3. Changed amino acid between wild-type and resistant line: The amino acid substitution resulting from the SNP between the wild-type and drug-resistant strains.

4. Filtering status: Whether the SNP call passed the applied filtering criteria (it should always be indicated as "OK").

5. Genotype differences: Whether there were differences in the genotypes between the wild-type and drug-resistant strains (it should always provide informative data).

6. Chromosomal location: For non-Leishmania organisms, it would indicate whether the SNP was located in the subtelomere ("end") or the chromosome "cores." However, this information may not be applicable for Leishmania.

7. Quality control indicators: Information related to the quality control measures applied during the mapping of sequence reads to the reference genome.

8. SNPs in protein coding sequences: Details about SNPs occurring within protein-coding sequences.

9. SNPs in 5'UTRs: Information on SNPs located within the 5' untranslated regions (UTRs) of genes, including the length of the 5'UTR as defined by George Cross' data or a default length of 115 bp.

10. SNPs in 3'UTRs: Details on SNPs found within the 3'UTRs of genes, including the length of the 3'UTR as defined by George Cross' data or a default length of 480 bp.

Finally, by providing this comprehensive information, the study aimed to present a detailed analysis of the SNPs identified, their locations, and their potential implications for the wild-type and drug-resistant strains.

The summary tables provided in the study contained seven columns with the following headings:

1. Strains: This column listed the cell line or strain name associated with the SNP.

2. GeneID: The column denoted the gene identification, allowing for easy identification of the specific gene associated with the SNP.

3. TM (Transmembrane domains): This column provided information on the number

of predicted transmembrane domains associated with the gene.

4. ORF (Open Reading Frame) position: The column indicated the position of the open reading frame where the SNP was located within the gene.

5. GeneDB: This column specified whether the indicated nucleotide information was obtained from GeneDB.

6. WT (Wild Type): The column indicated the nucleotide associated with the wild-type strain for the given SNP.

7. Resis (Resistant Line): This column provided the nucleotide associated with the drug-resistant strain for the given SNP.

Additionally, the following columns were included to describe the amino acid mutation and codon changes:

- Amino acid mutation: This column detailed the variation between the wild-type and resistant line, with a focus on non-synonymous (non-syn) codon changes.

- Codon: The genetic code of the codon was represented in this column. The capital letter indicated the changed nucleotide in the parental wild-type strain based on the GeneDB code. When the nucleotide was the same, it indicated that it was located on the regular strand, while when it was complementary, it indicated that it was located on the reverse strand.

- Change: This column assigned a numerical value to the type of change based on the resulting amino acid. Each type of change was assigned a specific number, ranging from 0 to 9, or denoted with "S" for changes involving stop codons.

Therefore, by organizing the SNP information in this tabular format, the study aimed to provide a clear and structured overview of the SNPs, their associated genes, and the specific changes observed between wild-type and drug-resistant strains. The additional information on amino acid changes and codon variations further enhanced the understanding of the functional impact of these SNPs.

In Table 1 of the study, the researcher performed pairwise BLAST comparisons to identify similar genes that contained SNPs in both *L. mexicana* and *L. major*. However, due to the large number of hits obtained in each species, only the closest homologues were listed in the table. The selection criteria for inclusion in the table were based on genes that had transmembrane domains (TMDs) and/or were associated with nucleotide metabolism. Among the interesting findings, the study highlighted some hypothetical proteins from both *L. mexicana* and *L. major*. These hypothetical proteins likely lacked functional annotations or were poorly characterized. However, the researcher noted that the only near-identical match found was a 5 TMD-protein with the gene ID LmxM.03.0370 in *L. mexicana* and its counterpart LmjF.03.0370 in *L. major*. This suggests a high degree of similarity between the two species in terms of this specific gene.

By focusing on genes with TMDs and those associated with nucleotide metabolism, the study aimed to identify potential candidate genes that could be linked to the observed SNPs and their impact on pyrimidine transport. The inclusion of hypothetical proteins and the discovery of near-identical matches between *L. mexicana* and *L. major* provided valuable insights into potentially important genes for further investigation in the study. Additionally, it was observed that

the gene mentioned (LmxM.03.0370 in *L. mexicana* and its counterpart LmjF.03.0370 in *L. major*) was mutated in two of the final-adapted *L. mexicana* lines, specifically the Fin2-Lmex-5FURes and Fin3-Lmex-5FURes lines. However, none of the intermediate lines showed mutations in this gene. This finding suggests that the mutation in this particular gene may be associated with the development of resistance to 5-fluorouracil (5-FU) in *L. mexicana*. To further investigate high-confidence SNPs that led to non-synonymous changes in coding sequences with five or more transmembrane (TM) domains, the study summarized the relevant information in tables 2-5. These tables likely provided details on the genomic positions, affected triplets, amino acid mutations, codon changes, and other relevant information for the identified SNPs in genes with five or more TM domains.

Furthermore, the study also summarized high-confidence SNPs that led to non-synonymous changes in coding sequences but had no TM domains in tables 6 and 7. These tables likely included information on the genomic positions, affected triplets, amino acid mutations, codon changes, and other relevant details for the identified SNPs in genes without TM domains.

In General, the generation of genome sequences for Leishmania species resistant to fluorinated pyrimidines, as described in the study, revealed a significant number of changes in each resistant strain. In comparison, a previous study conducted by Ghosh et al. in 2020 identified 240 single nucleotide polymorphisms (SNPs) in artesunate-resistant Leishmania donovani parasites when compared to their sensitive wild-type counterparts. However, in the current study, the number of SNPs found in the resistant Leishmania strains was considerably higher. This difference in the number of SNPs between the two studies suggests that the mechanisms of resistance and the genomic changes associated with resistance can vary depending on the specific drug and the Leishmania species being studied. The fluorinated pyrimidines used in the current study likely induced a broader range of genomic alterations in the resistant strains compared to artesunate.

The larger number of SNPs identified in the current study emphasizes the complexity and diversity of genetic changes involved in drug resistance mechanisms in Leishmania. Understanding these genomic alterations can provide valuable insights into the development and spread of drug resistance, aiding in the identification of potential targets for therapeutic interventions.

By categorizing and presenting the high-confidence SNPs in separate tables based on the presence or absence of TM domains, the study aimed to highlight the potential functional implications of these SNPs and their association with drug resistance in *L. mexicana*. In the study, four intermediate strains of *L. mexicana* resistant to 5-fluorouracil (5-FU) were analyzed. The number of single nucleotide polymorphisms (SNPs) observed in these strains were as follows:

- Lmex-5FURes-Int-1: 849 SNPs
- Lmex-5FURes-Int-2: 868 SNPs
- Lmex-5FURes-Int-3: 618 SNPs
- Lmex-5FURes-Int-4: 1018 SNPs

Out of these SNPs, only a subset was considered high-confidence and led to non-synonymous changes in coding sequences.

The numbers of such high-confidence SNPs for the intermediate strains were:

- Lmex-5FURes-Int-1: 204 SNPs
- Lmex-5FURes-Int-2: 197 SNPs
- Lmex-5FURes-Int-3: 138 SNPs
- Lmex-5FURes-Int-4: 208 SNPs

Similarly, three final strains of *L. mexicana* with high levels of resistance to 5-FU (Lmex-5FURes-Fin 1, 2, and 3) were examined. The number of SNPs observed in these final strains were:

- Lmex-5FURes-Fin-1: 1748 SNPs
- Lmex-5FURes-Fin-2: 953 SNPs
- Lmex-5FURes-Fin-3: 926 SNPs

Among these SNPs, the high-confidence SNPs that led to non-synonymous changes in coding sequences were:

- Lmex-5FURes-Fin-1: 447 SNPs
- Lmex-5FURes-Fin-2: 210 SNPs
- Lmex-5FURes-Fin-3: 208 SNPs

These findings indicate that as the resistance to 5-FU increased from intermediate to final strains, the number of SNPs and high-confidence SNPs leading to non-synonymous changes in coding sequences also increased. The accumulation of these genomic alterations may play a role in the development of high-level resistance to 5-FU in *L. mexicana* strains. In addition to *L. mexicana*, SNPs changes were also observed in the genome of *L. major* strains resistant to fluorinated pyrimidines. Specifically, the number of SNPs identified in the Lmaj-5FURes strain was 212, while in the Lmaj-5F2'dURes strain, it was 466. However, when considering only high-confidence SNPs that resulted in non-synonymous changes in coding sequences, the numbers were 74 and 116, respectively.

Based on these findings, the study recommends further investigations to identify the specific genetic changes that are associated with resistance to fluorinated pyrimidines. The high-confidence SNPs identified in multiple resistant lines can serve as starting points for these investigations. By studying these SNPs in more detail and understanding their functional implications, researchers can gain insights into the mechanisms underlying resistance to fluorinated pyrimidines in Leishmania species, particularly *L. major*. In this frame, Identifying the specific genetic changes responsible for resistance is crucial for developing effective strategies to overcome drug resistance and improve treatment outcomes. Further investigations and in-depth analysis of the observed SNPs can provide valuable information for targeted interventions and the development of novel therapeutics against Leishmania infections.

Acknowledgements

I would like to thank Harry De Koning and Bernardo Foth for their full cooperation in this work.

Table 1: Pair-wise blast comparisons among *Leishmania* to identify the closest homologues of genes of particular interest, with TM domain(s) or associated with pyrimidine biosynthesis.

GeneID- *L. major*	GeneID-*L. mexicana*	Lmaj TMd	Lmex TMd	Identity %
LmjF.03.0370	LmxM.03.0370	5	5	79.04
LmjF.03.0370	LmxM.03.0380	5	6	27.98
LmjF.32.0810	LmxM.08_29.2570	3	0	27.49

Table 2: SNPs with ≥5 TM domains and known function genes, which are divided into groups based on the gene product; the blank gray line separates groups. This table lists the SNPs in identical (Gene ID) and/or highly similar genes. NB: in the change column change is listed for both alleles of this gene.

Strains	Gene ID	TM	ORF	GeneDB	WT	Resis.	Amino acid mutation	Codon	Change
Lmex-Int1-5FURes	LmxM.27.0970	14	4151	G	G	AG	nonsyn_Thr1384Met/Thr	aCg	9/0
Lmex-Int3-5FURes	LmxM.11.1290	13	3463	T	T	GT	nonsyn_Phe1155Phe/Val	Ttt	0/9
Lmex-Int4-5FURes	LmxM.33.0990	9	53	G	AG	G	nonsyn_Glu18Ala/Glu	gAg	3/0
Lmex-Int1-5FURes	LmxM.36.4480	11	1309	G	G	GT	nonsyn_Ala437Ala/Ser	Gcc	0/9
Lmex-Int2-5FURes		11	1309	G	G	GT	nonsyn_Ala437Ala/Ser	Gcc	0/9
Lmex-Int3-5FURes		11	1309	G	G	GT	nonsyn_Ala437Ala/Ser	Gcc	0/9
Lmex-Int4-5FURes		11	5089	G	G	GT	nonsyn_Leu/Val1697Leu	Ctg	9/0

Lmex-Fin1-5FURes		11	1309	G	G	GT	nonsyn_Ala437Ala/Ser	Gcc	0/9
Lmex-Fin2-5FURes		11	1309	G	G	GT	nonsyn_Ala437Ala/Ser	Gcc	0/9
Lmex-Fin3-5FURes		11	1309	G	G	GT	nonsyn_Ala437Ala/Ser	Gcc	0/9
Lmex-Int1-5FURes	LmxM.30.0350	11	1421	G	G	CG	nonsyn_Thr474Ser/Thr	aCt	9/0
Lmex-Int4-5FURes	LmxM.30.0350	11	628	G	G	CG	nonsyn_His/Tyr210Tyr	Cat	9/0
Lmex-Fin1-5FURes	LmxM.30.0350	11	1421	G	G	CG	nonsyn_Thr474Ser/Thr	aCt	9/0
Lmex-Fin1-5FURes	LmxM.22.0230	10	557	T	T	AT	nonsyn_Asn186Ile/Asn	aAc	9/0
Lmex-Int2-5FURes	LmxM.27.1580	10	800	A	A	AG	nonsyn_Asn267Asn/Ser	aAc	0/9
Lmex-Int3-5FURes	LmxM.27.1580	10	800	A	A	AG	nonsyn_Asn267Asn/Ser	aAc	0/9
Lmex-Int4-5FURes	LmxM.27.1580	10	1198	A	A	AG	nonsyn_Ile/Val400Ile	Gtc	0/9
Lmex-Int1-5FURes		6	934	T	CT	C	nonsyn_Phe/Leu312Leu	Ttc	9/0
Lmex-Int2-5FURes		6	934	T	CT	C	nonsyn_Phe/Leu312Leu	Ttc	9/0
Lmex-Int3-5FURes		6	934	T	CT	C	nonsyn_Phe/Leu312Leu	Ttc	9/0
Lmex-Int4-5FURes	LmxM.32.3200	6	686	T	CT	C	nonsyn_Glu229Ala/Glu	gAg	3/0
Lmex-Fin1-5FURes		6	934	T	CT	C	nonsyn_Phe/Leu312Leu	Ttc	9/0
Lmex-Fin2-5FURes		6	934	T	CT	C	nonsyn_Phe/Leu312Leu	Ttc	9/0
Lmex-Fin3-5FURes		6	934	T	CT	C	nonsyn_Phe/Leu312Leu	Ttc	9/0

Table 3: SNPs in genes of known function with ≥5 TM domains that occurred in only one strain (unique mutations).

Table 3: SNPs in genes of known function with ≥5 TM domains that occurred in only one strain (unique mutations).

Strains	Gene ID	TM	ORF	GeneDB	WT	Resis.	Amino acid mutation	Codon	Change
Lmaj-5F2dURes	LmjF.17.0600	6	3142	T	T	GT	nonsyn_Thr1048Pro/Thr	Act	9/0
Lmex-Int1-5FURes	LmxM.21.1810	7	536	A	A	AC	nonsyn_Lys179Lys/Thr	aAg	0/4
Lmex-Int1-5FURes	LmxM.13.1210	11	493	A	A	AC	nonsyn_Phe165Phe/Val	Ttt	0/9
Lmex-Int2-5FURes	LmxM.18.0040	11	103	A	A	AG	nonsyn_Trp35Arg/Trp	Tgg	6/0
Lmex-Int4-5FURes	LmxM.06.0080	6	1011	T	T	GT	nonsyn_STOP/Tyr337Tyr	taC	3S/0
Lmex-Fin1-5FURes	LmxM.24.1090	10	1402	G	AG	G	nonsyn_Ile/Val468Val	Gtc	9/0
Lmex-Fin2-5FURes	LmxM.23.0830	12	2248	A	A	AC	nonsyn_Phe750Phe/Val	Ttt	0/9

Table 4: SNPs in genes of unknown function with ≥5 TM domains that are found in multiple resistant strains; mutations are divided into groups based on gene ID.

Strains	Gene ID	TM	ORF	GeneDB	WT	Resis.	Amino acid mutation	Codon	Change
Lmex-Int1-5FURes	LmxM.10.0380	12	1455	C	CG	C	nonsyn_Ile/Met485Met	atG	9/0
Lmex-Fin2-5FURes		12	1455	C	CG	C	nonsyn_Ile/Met485Met	atG	9/0
Lmex-Fin3-5FURes		12	1455	C	CG	C	nonsyn_Ile/Met485Met	atG	9/0
Lmex-Int1-5FURes	LmxM.17.1440	20	479	T	CT	T	nonsyn_Ala/Val160Val	gTc	9/0
Lmex-Int2-5FURes		20	479	T	CT	T	nonsyn_Ala/Val160Val	gTc	9/0
Lmex-Int3-5FURes		20	479	T	CT	T	nonsyn_Ala/Val160Val	gTc	9/0

Lmex-Int4-5FURes		20	1028	T	CT	T	nonsyn_Leu343Leu/Pro	cTg	0/9
Lmex-Fin1-5FURes		20	479	T	CT	T	nonsyn_Ala/Val160Val	gTc	9/0
Lmex-Fin2-5FURes		20	479	T	CT	T	nonsyn_Ala/Val160Val	gTc	9/0
Lmex-Fin3-5FURes		20	479	T	CT	T	nonsyn_Ala/Val160Val	gTc	9/0
Lmex-Int1-5FURes	LmxM.30.0030	14	857	C	T	CT	nonsyn_Asp286Asp/Gly	gGc	0/3
Lmex-Int4-5FURes		14	3745	C	T	CT	nonsyn_Ile/Leu1249Leu	Tta	9/0
Lmex-Fin1-5FURes		14	857	C	T	CT	nonsyn_Asp286Asp/Gly	gGc	0/3
Lmex-Int1-5FURes	LmxM.32.0710	10	730	T	T	CT	nonsyn_Trp244Arg/Trp	Tgg	6/0
Lmex-Fin2-5FURes		10	191	T	CT	C	nonsyn_Ala/Val64Ala	gTt	0/9
Lmex-Int1-5FURes	LmxM.32.1940	9	2438	C	AC	C	nonsyn_STOP/Ser813Ser	tCg	3S/0
Lmex-Int2-5FURes		9	2438	C	AC	C	nonsyn_STOP/Ser813Ser	tCg	3S/0
Lmex-Int3-5FURes		9	2438	C	AC	C	nonsyn_STOP/Ser813Ser	tCg	3S/0
Lmex-Int4-5FURes		9	3028	C	AC	C	nonsyn_His/Asn1010Asn	Cac	9/0
Lmex-Fin1-5FURes		9	2438	C	AC	C	nonsyn_STOP/Ser813Ser	tCg	3S/0
Lmex-Fin2-5FURes		9	2438	C	AC	C	nonsyn_STOP/Ser813Ser	tCg	3S/0
Lmex-Fin3-5FURes		9	2438	C	AC	C	nonsyn_STOP/Ser813Ser	tCg	3S/0
Lmex-Int2-5FURes	LmxM.30.0800	5	1457	C	CT	C	nonsyn_Asn/Ser486Ser	aGc	9/0

Lmex-Int2-5FURes		5	892	A	A	AC	nonsyn_Phe298Phe/Val	Ttt	0/9
Lmex-Int2-5FURes		5	125	C	CT	C	nonsyn_Glu/Gly42Gly	gGg	3/0
Lmex-Int3-5FURes		5	892	A	A	AC	nonsyn_Phe298Phe/Val	Ttt	0/9
Lmex-Int3-5FURes		5	125	C	CT	C	nonsyn_Glu/Gly42Gly	gGg	3/0
Lmex-Int1-5FURes	LmxM.34.3190	5	521	T	T	CT	nonsyn_Glu174Glu/Gly	gAg	0/3
Lmex-Int4-5FURes		5	1343	A	AG	G	nonsyn_Ala/Gly448Ala	gGt	9/0
Lmex-Int4-5FURes		5	1334	C	AC	A	nonsyn_Ala/Gly445Gly	gCg	9/0
Lmex-Fin1-5FURes		5	2588	A	AG	G	nonsyn_Leu/Ser863Ser	tTg	9/0
Lmex-Fin1-5FURes		5	2438	C	AC	A	nonsyn_Gly/Val813Val	gGc	9/0
Lmex-Int4-5FURes	LmxM.33.1070	9	935	G	CG	G	nonsyn_Gln312Gln/Arg	cAg	0/6
Lmex-Int4-5FURes		9	437	T	CT	T	nonsyn_Gln146Leu/Gln	cAg	9/0
Lmex-Int4-5FURes		9	1265	C	AC	C	nonsyn_Cys/Tyr422Cys	tAc	0/9
Lmex-Fin1-5FURes	LmxM.24.1451	11	2480	C	AC	A	nonsyn_Ala/Glu827Glu	gCa	5/0
Lmex-Fin1-5FURes		11	3187	A	AG	G	nonsyn_Ala/Thr1063Ala	Aca	0/9
Lmex-Fin1-5FURes		11	4384	G	AG	G	nonsyn_Gly/Ser1462Gly	Ggt	9/0
Lmex-Fin1-5FURes		11	5806	G	GT	G	nonsyn_Cys/Gly1936Gly	Ggt	9/0
Lmex-Fin1-5FURes	LmxM.26.2590	9	1562	G	AG	A	nonsyn_His/Arg521His	cGt	0/4
Lmex-Fin3-5FURes		9	1562	G	AG	A	nonsyn_His/Arg521His	cGt	0/4
Lmex-Fin1-5FURes	LmxM.30.0300	12	3030	T	CT	T	nonsyn_Ile/Met1010Ile	atA	0/9

Lmex-Fin1-5FURes		12	3026	C	CG	C	nonsyn_Ala/Gly1009Gly	gGg	9/0
Lmex-Fin1-5FURes		12	2531	G	GT	G	nonsyn_Ala/Glu844Ala	gCa	0/3
Lmex-Fin1-5FURes		12	818	C	CT	C	nonsyn_Asn/Ser273Ser	aGc	9/0
Lmex-Fin1-5FURes		12	454	C	CT	C	nonsyn_Gly/Ser152Gly	Ggc	0/9
Lmex-Fin1-5FURes	LmxM.30.0310	12	3032	T	CT	T	nonsyn_Asp/Gly1011Asp	gAc	0/5
Lmex-Fin1-5FURes		12	1792	C	CT	C	nonsyn_Ile/Val598Val	Gtt	9/0
Lmex-Fin1-5FURes		12	1171	T	CT	T	nonsyn_Gly/Ser391Ser	Agt	9/0
Lmex-Fin2-5FURes	LmxM.03.0370	5	665	C	C	A	nonsyn_Thr222Asn	aCt	9
Lmex-Fin3-5FURes		5	665	C	C	A	nonsyn_Thr222Asn	aCt	9
Lmex-Fin2-5FURes	LmxM.03.0380	6	692	C	CT	T	nonsyn_Phe/Ser231Phe	tCc	0/9
Lmex-Fin3-5FURes		6	692	C	CT	T	nonsyn_Phe/Ser231Phe	tCc	0/9

Table 5: SNPs with ≥5 TM domains in genes of unknown function; these mutations are unique for the indicated strain only.

Strains	Gene ID	TM	ORF	GeneDB	WT	Resis.	Amino acid mutation	Codon	Change
Lmaj-5FURes	LmjF.03.0370	5	859	A	A	C	nonsyn_Thr287Pro	Acc	9
	LmjF.32.2310	9	790	A	A	AC	nonsyn_Ser264Ala/Ser	Tcg	9/0
Lmaj-5F2dURes	LmjF.08.0190	13	2116	G	G	AG	nonsyn_Val706Ile/Val	Gtt	9/0
	LmjF.25.1160	9	1438	T	T	GT	nonsyn_Ser480Arg/Ser	Agc	6/0
	LmjF.26.0520	5	2335	T	T	CT	nonsyn_Lys779Glu/Lys	Aag	2/0
	LmxM.01.0440	10	421	C	CT	C	nonsyn_Phe/Leu141Leu	Ctc	9/0

Lmex-Int1-5FURes	LmxM.27.1510	5	1376	T	T	GT	nonsyn_Val459Gly/Val	gTg	9/0
Lmex-Int4-5FURes	LmxM.14.0640	6	904	T	AC	C	nonsyn_Pro/Ser302Pro	Ccg	0/9
	LmxM.14.0730	7	427	C	CT	C	nonsyn_Pro/Ser143Pro	Ccg	0/9
	LmxM.33.1270	13	4196	T	CT	T	nonsyn_Asp/Gly1399Asp	gGc	0/5
	LmxM.34.3320	5	2423	T	CT	T	nonsyn_Ala/Glu808Glu	gCg	5/0
Fin2-Lmex-5FURes	LmxM.34.2810b	11	530	T	T	CT	nonsyn_Tyr177Cys/Tyr	tAt	9/0
	LmxM.25.0790	6	3503	A	AG	A	nonsyn_Asp/Gly1168Asp	gAc	0/5

Table 6: SNPs that are associated with the pyrimidine biosynthesis and salvage pathways.

Strains	**Gene ID**	**ORF**	**GDB**	**WT**	**Resist**	**A.A. mutation**	**Codon**	**Type change**
Lmex-Int3-5FURes	LmxM.28.0890	1918	C	C	AC	nonsyn_Arg640Arg/Ser	Cgc	0/4
Lmex-Fin2-5FURes								
Lmex-Fin3-5FURes								

Continued **Table 6** SNPs that are associated with the pyrimidine biosynthesis and salvage pathways; the gray line divides the groups based on gene function

Strains	Gene ID	ORF	GDB	WT	Resis.	A.A. mutation	Codon	Type change
Lmaj-5F2dURes	LmjF.30.1960	1522	T	T	GT	nonsyn_STOP508STOP/Glu	Tag	0/1S
Lmex-Fin1-5FURes	LmxM.16.0550	434	T	T	GT	nonsyn_Gln145Pro/Gln	cAg	9/0
Lmex-Fin3-5FURes	LmxM.10.1010	601	T	T	CT	nonsyn_Thr201Ala/Thr	Acc	9/0

Table 7: SNPs in genes, without TMDs, involved in nucleotide metabolism, with unique mutation for the indicated strain only.

Strains	Gene ID	ORF	GDB	WT	Resist	A.A. mutation	Codon	Type change
Lmaj-5F2dURes	LmjF.23.1590	788	C	C	CT	nonsyn_Ser263Leu/ Ser	tCa	9/0
Lmex-Fin1-5FURes	LmxM.36.0330	497	T	T	GT	nonsyn_Glu166Ala/ Glu	gAg	3/0
Lmex-Fin1-5FURes	LmxM.34.4800	281	G	G	AG	nonsyn_Arg94His/ Arg	cGc	4/0

References

1. *Ali, J. A., Creek, D.J., Burgess, K., Allison, H. C., Field, M. C., Maser, P. and de Koning, H. P. (2013). Pyrimidine salvage in Trypanosoma brucei bloodstream forms and the trypanocidal action of halogenated pyrimidines. Molecular Pharmacology, 83, 439-453.*
2. *Alzahrani, K. J., Ali, J. A., Eze, A. A., Looi, W. L., Tagoe, D. N., Creek, D. J., Barrett, M. P. and de Koning, H. P. (2017). Functional and genetic evidence that nucleoside transport is highly conserved in Leishmania species: Implications for pyrimidine-based chemotherapy. Int J Parasitol Drugs Drug Resist, 7(2):206-226.*
3. *Amro, A., Gashout, A., Al-Dwibe, H., Zahangir Alam, M. and Annajar, B. (2012). First Molecular Epidemiological Study of Cutaneous Leishmaniasis in Libya. PLoS Negl Trop Dis, 6(6), e1700.*
4. *Borst, P. and Ouellette, M. (1995). New mechanisms of drug resistance in parasitic protozoa. Annu Rev Microbiol, 49, 427-460.*
5. *De Koning, H. P. (2001). Uptake of pentamidine in Trypanosoma brucei brucei is mediated by three distinct transporters: implications for cross-resistance with arsenicals. Molecular Pharmacology, 59, 586-592.*
6. *El-Badry, A. A., El-Dwibe, H., Basyoni, M., Al-Antably, A. and Al-Bashier, W. A. (2017). Molecular prevalence and estimated risk of cutaneous leishmaniasis in Libya. Journal of microbiology, immunology, and infection, 50(6), 805–810.*
7. *Ghosh, S., Aditya V., Vinay K., Dibyabhaba P., Angamuthu S., Poonam S. and Ruchi S. (2020). Genomic and Transcriptomic Analysis for Identification of Genes and Interlinked Pathways Mediating Artemisinin Resistance in Leishmania donovani. Genes 11(11), 1362.*
8. *Ponte-Sucre A, Gamarro F, Dujardin J., Barrett, M. P., et al. (2017). Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Neglected Tropical Diseases, 11, e0006052.*
9. *Verma, A., Bhandari, V., Deep, D.K., Sundar, S., Dujardin, J. C., Singh, R. and Salotra, P. (2017) Transcriptome profiling identifies genes/pathways associated with experimental resistance to paromomycin in Leishmania donovani. Int. J. Parasitol. Drugs Drug Resist. 7, 370–377.*
10. *WHO. (2022). Fact Sheet. ed. World Health Organization.*