Systematic Review and Meta-Analysis on Human African Trypanocide Resistance

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Abstract: Background Human African trypanocide resistance (HATr) is a challenge for the eradication of Human African Trypanosomiasis (HAT) following the widespread emergence of increased monotherapy drug treatment failures against Trypanosoma brucei gambiense and T. b. rhodesiense that are associated with changes in pathogen receptors. Methods: Electronic searches of 12 databases and 3 Google search websites for human African trypanocide resistance were performed using a keyword search criterion applied to both laboratory and clinical studies. Fifty-one publications were identified and included in this study using the PRISMA checklist. Data were analyzed using RevMan and random effect sizes were computed for the statistics at the 95% confidence interval. Results: Pentamidine/melarsoprol/nifurtimox cross-resistance is associated with loss of the T. brucei adenosine transporter 1/purine 2 gene (TbAT1/P2), aquaglyceroporins (TbAQP) 2 and 3, followed by the high affinity pentamidine melarsoprol transporter (HAPT) 1. In addition, the loss of the amino acid transporter (AAT) 6 is associated with eflornithine resistance. Nifurtimox/eflornithine combination therapy resistance is associated with AAT6 and nitroreductase loss, and high resistance and parasite regrowth is responsible for treatment relapse. In clinical studies, the TbAT1 proportion of total random effects was 68% (95% CI: 38.0–91.6); I² = 96.99% (95% CI: 94.6–98.3). Treatment failure rates were highest with melarsoprol followed by eflornithine at 41.49% (95% CI: 24.94–59.09) and 6.56% (3.06–11.25) respectively. HATr-resistant phenotypes used in most laboratory experiments demonstrated significantly higher pentamidine resistance than other trypanocides. Conclusion: The emergence of drug resistance across the spectrum of trypanocidal agents that are used to treat HAT is a major threat to the global WHO target to eliminate HAT by 2030. T. brucei strains were largely resistant to diamidines and the use of high trypanocide concentrations in clinical studies have proved fatal in humans. Studies to develop novel chemotherapeutical agents and identify alternative protein targets could help to reduce the emergence and spread of HATr.

Keywords: human African trypanosomiasis; trypanosomes; drug resistance; pentamidines; nifurtimox/eflornithine combination therapy; fexinidazole; NECT; TbAT1; amino-aquapurine transporters; amino acid transporters; trypanosoma brucei rhodesiense; trypanosoma brucei gambiense; neglected tropical diseases

1. Introduction

The World Health Organization (WHO) has set 2030 as a target for the elimination of human African trypanosomiasis (HAT) [1]; however, the development of drug-resistant phenotypes (see [2] on trypanocide resistance) in resource-poor countries affected by HAT presents a major challenge for HAT control. In Africa, HAT is caused by Trypanosoma brucei gambiense (TGB, chronic variant) and T. b. rhodesiense (TBR, acute variant), also referred to as gHAT and rHAT, respectively. Uganda has the misfortune to harbor both sub-species
within its borders [3]. Trypanosomes are able to evade host immune defenses through a process of antigenic variation (while each genome contains over $10^3$ distinct variable surface glycoprotein (VSG) genes, every trypanosome typically expresses a single VSG that rapidly switches [4], compromising any efforts to develop vaccines).

Globally, six major drugs are available for treatment of HAT depending on the stage of the infection: pentamidine, suramin, melarsoprol, eflornithine, nifurtimox/eflornithine combination therapy (NECT), and fexinidazole (see [2] on HAT pharmaceutics and limitations of current approved therapies). The emergence of human African trypanocidal resistance (HATr) has undermined the use of monotherapy for HAT treatment.

Pentamidine was the first antiprotozoal diamidine to be routinely used for HAT treatment, its mode of action serving to disrupt the AATT-rich portions of Trypanosoma DNA and suppress mitochondrial activity [5]. Pentamidine resistance has been linked to changes in the transmembrane transport of the drug i.e., Trypanosoma brucei adenosine transporter 1/purine 2 (TbAT1/P2) and high-affinity pentamidine transporter 1 (HAPT1) [5–7]. Melarsoprol is an arsenical with which resistance has been associated with aquaglyceroporin transporters 2/3 (AQP2/3) in trypanosomes [8]. Resistance has led to the adoption of combination nifurtimox/eflornithine combination therapy (NECT), which has low toxicity and a shortened therapeutic period, although weekly intravenous infusions have proved challenging in resource-limited settings [9]. In early stages, pentamidine (TBG) and suramin (TBR) are used while melarsoprol (TBRG and TBR) and eflornithine (TBRG) are recommended for late-stage HAT [3]. New drugs, including pafuramidine maleate for early HAT, have failed, while acoziborole for stage 1 and 2 HAT is under continuous review [10], demonstrating the need to develop novel therapies; for example, fexinidazole is easier to administer since it is an oral medication [11,12]. The objective of the current study was to identify major parasitic markers in HATr associated with clinical studies (field surveys and clinical trials) and experimental (laboratory-based) studies.

2. Methods

2.1. Study Design

Multiple electronic databases were searched using the Ovid interface: AMED (Allied and Complementary Medicine) 1985 to March 2022, CAB Abstracts 1973 to 2022 Week 13, APA PsycInfo 1806 to March Week 4 2022, Books@Ovid 28 March 2022, Journals@Ovid Full Text 01 April 2022, Your Journals@Ovid, APA PsycArticles Full Text, CAB Abstracts 1910 to 1989, Embase Classic+Embase 1947 to 01 April 2022, Global Health 1910 to 2022 Week 13, Ovid MEDLINE(R), and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions 1946 to 01 April 2022. These searches generated a total of 172 publications and the Web of Science generated 111 publications using the following keywords as shown in Supplementary File S1.

ALL = ((african trypanosomiasis)) OR ALL = (trypanosoma brucei)) OR ALL = (‘tsetse fly-borne diseases’ OR ‘HAT’ or ‘human African trypanosomiasis’)) AND ALL = (((trypanosoma brucei gambiensis) or (trypanosoma brucei rhodesiensis)) AND ALL = ((suramin OR melarsoprol OR eflornithine OR nifurtimox OR pentamidine OR (NECT or (Nifurtimox Eflornithine Combination Therapy)))))) AND ALL = (trypanocides resistance or drug resistance).

Grey literature was searched on Google using the WHO and CDC websites, and citations in systematic reviews were also searched for papers that might have been missed (Figure 1).

2.2. Article Screening on Inclusion and Exclusion

Data files were exported to EndNote 2020 and all papers were merged (N = 283). The SR depublicator removed 79 duplicates. A total of 204 papers were then exported to Covidence, which removed 3 additional duplicates, and these were confirmed by authors. Title and abstract screening removed 121 papers and only 80 papers were subjected to full-text review. After removing review articles, a total of 46 papers were acquired from
the search and an additional 5 papers were added (i.e., 1 from Google search and 4 from review searches), as shown in Figure 1.

Figure 1. PRISMA checklist showing studies from database, Google search, and citation review.

2.3. Statistical Analysis

Data were exported into MS Excel and categorized into laboratory studies (cell culture and rodents) and clinical studies (involving humans). These data were descriptively presented in tables while quantitative data were analyzed using RevMan® for meta-analysis using proportions and random effect sizes at 95% confidence interval. Data on the impact of resistance were analyzed using GraphPad Prism version 6 and posthoc Tukey’s tests were conducted and represented with different superscripts (a,b) being used to indicate significant differences ($p < 0.05$).

3. Results and Discussion

3.1. Description of Human African Trypanocide Resistance in the Study

Pentamidine/melarsoprol cross-resistance (PMXR) is associated with loss of and mutations in $Tb$AT1/P2 genes [13], followed by mitochondrial, post translational activator XAC1, and flagellar genes [14]. The loss of $Tb$AT1 alone does not effectively prevent endocytosis of pentamidine and melarsoprol (see [15], melarsoprol has other entry targets); however, a loss of aquaglyceroprotein ($Tb$AQP, see [16], AQPs are important for viability and osmoregulation) has been associated with complete loss of trypanocide endocytosis [17]. In particular, the loss of $Tb$AQP2/3 following point mutations in $Tb$AT1 leads to a loss of the high-affinity pentamidine/melarsoprol transporter (HAPMT) [18,19]. In pentamidine diamidine resistance, HAPT1 and low-affinity pentamidine transporter 1 (LAPT1) are responsible for the residual uptake of melaninophenyl arsenic [20]. The presence of multiple resistance genes in single parasites contributes to a markedly resistant phenotype. For example, $Tb$AT1 and T. brucei multidrug-resistant pentamidine-associated gene ($Tb$MRPA) further complicate melarsoprol resistance [21] (Table 1).
These findings are important since pentamidine and melarsoprol have been the major trypanocides involved in HAT chemotherapy for over 60 years [22]. Cross-resistance between melarsoprol-pentamidine, diminazene aceturate, isometamidium chloride has been reported [23]. Although drugs used in HAT are different from those used for animal African trypanosomiasis, pentamidines/diamidines with similar pathogen targets (TbAT1/P2) contribute to this selection pressure [24]. This is important since TBR and TBG have been isolated from livestock species that have been recognized as maintenance hosts (see [25] in pigs, [26] in small ruminants, and [27] in cattle) and sources of re-infection and introduction of resistant phenotypes in humans [28].

Nifurtimox is metabolized rapidly and its metabolites are not effective against T. brucei s.l., thus reducing its therapeutical effect [29]. Similar results have been reported for TbAT1, where the loss of P2 favors trypanosome survival [30]. The loss of amino acid transporter (AAT) 6 leads to increased eflornithine resistance [24,25] while NECT resistance is associated with multiple loss of function of AAT6 and nitroreductase (NTR) [26,27]. Resistance to arsenicals and diamidines is associated with HAPT loss [31] while TbAT1 loss disrupts the uptake of diamidines [32]. In addition, cross-resistance in arsenical and suramin [33], arsenical/melarsoprol/pentamidine and diminazene aceturate [15] as well as isometamidium and diminazene aceturate, which are mainly used in livestock [34], raises major public health concerns since pentamidines and diminazene aceturate are used in both humans and animals [24]. Furthermore, the presence of putative nascent polypeptide associated complex (NAC) isoforms [35] presents a dilemma with opportunities for drug targets and challenges to address HATr (Table 1). Nifurtimox resistance has also been associated with TbAT1/P2 (contrary to previous assumptions [36] in which no relationship was made); however, AAT6 loss has been associated with eflornithine resistance. In NECT resistance, multiple loss of AAT6 and NTR is the hallmark of drug resistance. Nifurtimox–fexinidazole resistance is associated with the rapid metabolism of the sulfoxide and sulfone forms of this compound [22]. Since nifurtimox was first developed for use against American trypanosomiasis [37], it is apparent that further research exploring combination therapy could yield more efficient trypanocides.

Resistance to suramin (developed in 1916) has been associated with switching of VSG expression [31,38]. Furthermore, other mechanisms associated with ATP production, metabolism, cell cycle, and genome segregation [39] will continue to offer opportunities for continued research (Table 1). Although the cellular pathways involved in suramin resistance remain to be discovered (see [40] where resistance was postulated to develop after changes in the drug target by expression of drug extrusion mechanisms), since each parasite contains over 1000 VSG genes, expression switching to one particular VSGSur implies that it is close to impossible to eliminate suramin resistance in a population [41].

Table 1. Characterization of pathogens, interventions used, and gene targets in human African trypanosome resistance in laboratory studies.

| Study          | Study Population | Source of Pathogen | Intervention/Drugs Used | Gene Targets for Resistance |
|----------------|------------------|--------------------|-------------------------|----------------------------|
| Bernhard 2007  | Mice             | TBR                | Pentamidine-melarsoprol | TbAT1 loss indicated cross-resistance on both compounds |
| Carter 2020    | T. brucei ORFeome| Parasite library   | Melarsoprol             | Genes encoding trypanothione Mitochondrial and flagellar gene expression (post translational activator XAC1). |
| Study          | Ref         | Study Population | Source of Pathogen | Intervention/Drugs Used                                                                 | Gene Targets for Resistance                                                                 |
|---------------|-------------|------------------|--------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Scott 1997    | [15]        | Procyclics       | TBB                | Cross resistance with arsenical-melarsoprol-pentamidine and diminazene aceturate         | Melarsoprol can enter parasite through another route than TbAT1                            |
| Jeacock 2017  | [16]        | Mice             | TBB/TBG            | AQP2 disrupts glycerol transport                                                         | AQP's important for viability and osmoregulation                                          |
| Graf 2013     | [17]        | Procyclics       | TBR and TBG isolates from 7 African countries | Pentamidine and melarsoprol                                                              | TbAT1 loss leads to a loss of transporter activity Aquaglyceroprotein (TbAQP2)             |
| Graf 2016     | [18]        | Procyclics       | TBR from male patient in Tanzania             | Pentamidine and melarsoprol                                                              | Loss of transporter genes TbAQP2/3 Point mutation renders TbAT1 useless (lacks HAPMT = high affinity pentamidine-melarsoprol transporter) |
| Graf 2015     | [19]        | Procyclics       | TBG                | Pentamidine and melarsoprol                                                              | TbAQP2 reintroduced reversed resistance                                                   |
| Matovu 2003   | [20]        | Procyclics/mice  | TBB                | Loss of TbAT1/P2 in pentamidine and diamidine uptake                                     | HAPT1 and LAPT1 responsible for residual uptake of melaminophenyl arsenical               |
| Lusher 2006   | [21]        | Procyclics       | TBB                | Melarsoprol resistance                                                                  | TbAT1 and TMRPA when both present lead to significant decrease in drug influx             |
| Sokolova 2010 | [29]        | Procyclics       | TB strain *        | Nifurtimox-resistant cell lines                                                          | Nifurtimox metabolized fast and metabolites not effective on pathogen                     |
| Geiser 2005   | [30]        | Procyclics       | TBB strain BS 221  | Adenosine metabolites                                                                    | P1/P2 TbAT1 loss. Conditions other than drugs themselves may favor loss of P1 to increase pathogen survival in bloodstream form of the parasite. |
| Burkard 2011  | [42]        | RNAi induction   | RNAi library       | NA                                                                                      | Loss of TbAT1 leads to melarsoprol resistance. Loss of AAT6 leads to increased eflornithine resistance. |
| Vincent 2010  | [43]        | Procyclics       | T. brucei strain 427 wildtype                   | Eflornithine resistance                                                                  | Ornithine decarboxylase unaltered in parasite. Deletion of TbAAT6                         |
| Study | Ref | Study Population | Source of Pathogen | Intervention/Drugs Used | Gene Targets for Resistance |
|-------|-----|------------------|--------------------|------------------------|---------------------------|
| Baker 2011 | [44] | Procyclics | TBR | NECT | Loss of amino acid transporter (AAT6) and nitroreductase (NTR) induces resistance |
| Wyllie 2016 | [38] | Procyclic/mice | Non-specific trypanosome used | Nifurtimox | NTR resistance determinants |
| Bridges 2007 | [31] | Rats | TBG | Arsenical and diamidine | High-affinity pentamidine transporter (HAPT) loss for cross-resistance |
| Lanteri 2006 | [32] | Procyclics/mice | TBB | 2,5-BIS(4-amidinophenyl)furan (DB75) (diamidine) | Loss of T Annotations leads to loss in uptake of DB75. |
| Scott 1996 | [33] | Mice | TBB from Tanzania TBG from man in Ivory coast | Cross-resistance to MelCy and suramin | Differences in in vivo and in vitro results indicated alteration in surface adenosine transporters. |
| Matovu 1997 | [34] | Humans and livestock | TBR in Uganda | Resistant to ISM, DA | Cross-species resistance |
| Foucher 2006 | [35] | Procyclics | TBG clones | Cymelarsan | Putative NAC isoform loss. Alterations in the activity of the enzyme that generates protein translation modifiers. |
| Wiedemar 2019 | [45] | Procyclics | TBB | VSG expression has impact on suramin sensitivity and uptake | Decrease specific receptor-mediated endocytosis |
| Zeelen 2021 | [41] | Procyclics | TBR | VSG-suramin binding interactions | Resistance phenotype dependent on suramin binding with VSG
head |
| Worthen 2010 | [39] | Mice | Modeling HATr resistance | Pentamidine, prostaglandin D2, quercetin, etoposide, camptothecin, tetrahydroquinoline | Defects in mitochondrial activity, ROS, cell cycle, and genome segregation. |
| Bacchi 1994 | [46] | Mice | TBR from Kenya | Combination of DFMO, eflornithine, and ornidyl | Cure rate in days |
| Bacchi 1993 | [47] | Mice | TBR from Kenya | DFMO resistance | S-adenosylmethionine metabolism increases resistance |
| Pati 2014 | [48] | Humans | TBG in DRC | Melarsoprol | Relapse following mutations in AQP2/3 |
| Matovu 2001 | [49] | Procyclics | TBG from northwestern Uganda | Melarsoprol | Elevated MIC |
Table 1. Cont.

| Study           | Ref   | Study Population                  | Source of Pathogen                                                                 | Intervention/Drugs Used | Gene Targets for Resistance                  |
|-----------------|-------|------------------------------------|-------------------------------------------------------------------------------------|-------------------------|---------------------------------------------|
| Brun 2001       | [50]  | Humans and then mice               | TBR KETRI and EATRO trypanosome isolates from Kenya STIB 241 and STIB 704 from Uganda | Melarsoprol             | Cure rate                                   |
| Hawking 1941    | [51]  | Mice inoculated with patient blood/CSF | TBR                                                                                 | Melarsoprol-pentamidine cross resistance (MPXR) | Relapse                                     |
| Kagira 2007     | [52]  | Mice                               | TBR in patients from Uganda and Kenya                                              | Melarsoprol             | High minimum inhibition concentrations (MIC) and IC50 |
| Kibona 2006     | [53]  | Mice                               | TBR from Tanzania                                                                  | Melarsoprol-pentamidine cross resistance (MPXR) | Relapse                                     |
| Maina 2007      | [54]  | Humans/mice TBG in South Sudan     | Melarsoprol resistance                                                             | TBAT1/P2 loss           |                                             |
| Mpiia 2002      | [55]  | Humans TBG                         | Combination of eflornithine and melarsoprol                                        | Cleared infection though toxicity concerns raised. |                                             |
| Munday 2014     | [56]  | ProcyclicsTB                        | TBB                                                                                 | TbAQP2 is HAPAT and source of resistance |                                             |
| Munday 2015     | [57]  | ProcyclicsTB                       | TBB                                                                                | TBAT1                   | Residues F19, D140, and F316 interact with the Tbat1 substrate. |
| Mutuku 2021     | [58]  | Mice TBG in Busoga, Uganda         | Suramin resistance                                                                 | Differential pathogenicity in TBR strains |                                             |
| Nerima 2007     | [59]  | Mice TBG northwest Uganda          | Detection of mutant P2/TBAT1                                                      | Allele-specific PCR is cheaper than SfajN1 RFLP for screening of TBAT1 |                                             |
| Nnadi 2019      | [60]  | ProcyclicsT. congo                  | Holarrhetine                                                                       | TBAT1, AQP1-3           |                                             |
| Sanderson 2009  | [61]  | Mice TBB                           | Pentamidine                                                                        | Blood-brain barrier via P-glycoprotein and multiple drug resistance-associated protein transporters. |                                             |

Key: Superscript (*) = strain genus not defined in the article. NA = not applicable.

3.2. Human African Trypanocide Resistance in Clinical Studies

In clinical studies, relapse/cure rates have been used to study HATr (see [46] on using DL-α-difluoromethylornithine (DFMO, also referred to as eflornithine in most studies) with suramin against arsenical refractory HAT in mice). This is important since DFMO alone has been associated with resistance [47]. Combination of metronidazole and suramin were used to address arsenical resistance in HATr in Zambia [62], while high doses of nifurtimox have also been explored in the Democratic Republic of the Congo (DRC) [63,64]. Melarsoprol resistance in DRC has been associated with mutations in the AQP2/3 gene [48] and parasite regrowth [65]. Human samples from Tanzania and Ivory Coast showed cross-resistance to melarsoprol and suramin due to alterations in TbAT1 [33] or other transport mechanisms [15] (Table 2).

Melarsoprol resistance in Uganda and Tanzania has also been associated with mutations in the TbAT1 gene [66], and high minimum inhibition concentration (MIC) titers (see [49]) in Uganda. In DRC, melarsoprol alone is no longer used in late HAT [67] and
eflornithine is now used at this stage [68]. The increasing levels of HATr has led to the promotion of combination therapy [69], as well as capital to invest in the discovery of more potent drugs better than the current drugs on the market.

**Table 2.** Human African trypanosome resistance in human populations with location and drugs used during interventions.

| Study             | Ref       | Study Population | Source of Pathogen | Intervention/Drugs Used                              | Marker for Resistance                      |
|-------------------|-----------|------------------|--------------------|-----------------------------------------------------|-------------------------------------------|
| Foulkes 1996      | [62]      | Human            | TBR in Zambia      | Melarsoprol resistance Then given suramin           | Melarsoprol refractory period/relapse      |
| Pepin 1989        | [63]      | Human            | TBG in DRC         | Nifurtimox for arsena-resistance                   | No relapse                                |
| Pepin 1992        | [64]      | Human            | TBG in DRC         | Arsenic resistance                                 | High-dose nifurtimox                      |
| Richardson 2016   | [65]      | Human            | TBG in DRC         | Parasite regrowth leads to relapse not reinfection |                                           |
| Matovu 2001       | [66]      | Human            | TBR in Uganda      | Melarsoprol                                        | Mutated TbAT1                             |
| Burri 2001        | [70]      | Human            | TBG in M’banza     | Melarsoprol                                        | Cure rate in patients                      |
| Kazibwe 2009      | [71]      | Human            | TBG from northwestern Uganda | Melarsoprol withdrawal                        | TbAT1/P2 present in pathogen              |
| Pyana 2015        | [72]      | Human            | TBG in DRC         | Pentamidine melarsoprol resistance                 |                                           |
| Balasegaram 2006  | [73]      | Human            | TBG in DRC         | Pentamidine                                        | Relapse rate measured                     |
| Balasegaram 2006  | [67]      | Human            | TBG in DRC         | Melarsoprol and eflornithine                       | In late HAT, more patients died with melarsoprol alone than eflornithine alone. |
| Pepin 2000        | [68]      | Human            | TBG in DRC         | Eflornithine given to relapsing patients           | 7-day treatment reduced relapse           |
| WHO 2001          | [69]      | Human            | HAT                | HATr                                               |                                           |

3.3. Evidence of Human African Trypanocide Resistance of TbAT1 in Clinical Studies

Here, we provide evidence with a total random effect proportion of 68.0% (95% CI: 38.0–91.6) being reported from 2001–2014 (Table 3). Test for heterogeneity: Q (df) = 99.7 (3), p < 0.0001. $I^2 = 96.99\%$ (95% CI: 94.6–98.3). The high $I^2$ value indicates great variation, which could be associated with the different study designs, time scope, and geographical locations for these studies. Egger’s test (Intercept = −16.5, 95% CI: −30.8 to −2.1, $p = 0.04$), Begg’s test (Tau = −1.0, $p = 0.04$) showed publication bias. Pati [48] reported a further proportion of 4/6 (TbAT1) having mutations associated with the AQP2/3 genes.
3.4. Human African Trypanocide Treatment Relapse Rates

Treatment failure rates were highest with melarsoprol, followed by eflornithine, 41.49% (95% CI: 24.94–59.09) and 6.56% (3.06–11.25), respectively; however, the reliability of these findings may be biased due to the high $I^2$ value associated with melarsoprol studies (Table 4). Furthermore, a high level of consistency was associated with nifurtimox studies ($I^2 = 0.0\%$) since this showed a low publication bias.

### Table 4. Human African trypanocide relapses following pentamidine, nifurtimox, eflornithine, and melarsoprol therapy in humans.

| Study | Ref | Pentamidine | Nifurtimox | Eflornithine | Melarsoprol | Combination Melarso-prol/Eflornithine |
|-------|-----|-------------|------------|--------------|-------------|-------------------------------------|
|       |     | Relapse | Total | Relapse | Total | Relapse | Total | Relapse | Total | Relapse | Total |
| Balasegaram 2006 | [73] | 33 | 692 | | | | | | | |
| Balasegaram 2006 | [67] | 11 | 136 | 36 | 258 | | | | | |
| Brun 2001 | [50] | 8 | 36 | | | | | | | |
| Burri 2001 | [70] | 7 | 16 | | | | | | | |
| Kagira 2007 | [52] | 5 | 6 | | | | | | | |
| Kazibwe 2009 | [71] | 9 | 101 | | | | | | | |
| Matovu 2001 | [66] | 43 | 65 | | | | | | | |
| Mopia 2002 | [55] | 19 | 42 | | | | | | | |
| Pati 2014 | [48] | 12 | 19 | | | | | | | |
| Pepin 1989 | [63] | 0 | 7 | | | | | | | |
| Pepin 1989 | [63] | 0 | 9 | | | | | | | |
| Pepin 1992 | [64] | 0 | 33 | 0 | 33 | | | | | |
| Pepin 2000 in Côte d’Ivoire | [68] | 7 | 140 | | | | | | | |
| Pepin 2000 in DRC | [68] | | | | | | | | | |
| Pepin 2000 in Uganda | [68] | 13 | 116 | | | | | | | |
| Total | | | | | | | | | | |
| Total random effects | | | | | | | | | | |
| Test for heterogeneity, $Q(df), p$ value | | | | | | | | | | |
| $I^2$ (inconsistency), 95% CI | | | | | | | | | | |
| Publication bias: Egger’s test intercept (95% CI, $p$ value); Begg’s test Kendall’s Tau, $p$ value | | | | | | | | | | |

Superscript: (*) denotes toxic observations; 8 patients developed neurological conditions and 1 died under high nifurtimox dosage. #: Observations taken within a 2-year period. NA = Not applicable for meta-analysis of a low number of studies ($n = 1$).
Treatment relapse rates have been used as indicators of resistance and combination therapies using DFMO-suramin against arsine/meralsoprol resistance have been explored since eflornithone alone is ineffective [74]. This has continued as an alternative combination for use in late HAT cases because of melarsoprol resistance [75]. To overcome arsenical resistance, combination therapies of metronidazole and suramin have also been used since these are associated with mild side effects when compared with suramin monotherapy (in Zambia), while high doses of nifurtimox have also been used in DRC. Melarsoprol resistance in DRC has been associated with mutations in the AQP2/3 gene [35] and parasite regrowth [56]. Human samples from Tanzania and Ivory Coast have shown cross resistance to melarsoprol and suramin due to alterations in \( T_b \)AT1 [30] or other transport mechanisms [15].

Melarsoprol resistance in Uganda and Tanzania has also been associated with mutations in the \( T_b \)AT1 gene [57]. In DRC, melarsoprol alone is no longer used in late-stage HAT [58] and has been substituted with eflornithine for this stage [59]. The increasing levels of HATr have led to the promotion of combination therapy [60], as well as increased capital to invest in the discovery of more potent drugs superior those currently on the market. Furthermore, melarsoprol toxicity and decreasing efficacy has led to phasing out the drug as a frontline treatment against \( T_b \) gambiense; this is now possible with the emergence of potent, safe combination chemotherapies, such as NECT, showing that eflornithine will continue to be around for decades to come. The \( T_b \)AT1 genotype was the most prevalent marker, although few studies have been conducted in humans on the African continent exploring the genome diversity of HATr. This is important since melarsoprol has the highest treatment failure rates [66,73].

3.5. Drug Sensitivity Profiles on HATr Using Resistance Profiling

Some experimental laboratory studies have proved to be unreliable due to low reproducibility, especially when conducting clinical (field-based) studies [76]. This has subsequently given rise to speculative interpretations that make it hard to inform policy [77]. Here, we investigated the level of resistance induced in experimental studies for HATr. The resistance factor was calculated by dividing the IC50 of the resistant population by the IC50 of the wild-type in these studies [13,18,20,29,40]. The mean ± SEM for resistance factors of pentamidine, melarsoprol, suramin, and DB 75 were 84.3 ± 20.12, 12.5 ± 3.0, 8.2 ± 2.0, and 10.8 ± 3.5, respectively (Figure 2). Furthermore, most laboratory studies have continued to produce strains that are significantly resistant to pentamidine, demonstrating a shift in research direction for the next novel trypanocides.

Figure 2. Resistance profiles of laboratory-developed HATr phenotypes. Generally, much emphasis has been placed on development of strains heavily expressing resistance to pentamidine compared with all other trypanocides. Different subscripts in figure 2 are introduced under statistical analysis (i.e., different superscripts (a,b) signify significant differences).
4. Conclusions

Cross-resistance across trypanocides is a major threat to the development of novel monotherapy due to the targeting of similar molecules in the pathogen. TbAT1/P2 are the leading pathogenic transporter targets; however, total resistance is associated with the loss of TbAQ2/3, HAPT1, and LAPlT1 in melarsoprol-pentamidine resistance, while AAT6 and NTRs are common in nifurtimox–eflornithine resistance. High treatment failure rates in humans have led to the implementation of high doses, which have proved fatal to patients, highlighting the desperate situation created by HATr in endemic communities.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pathogens11101100/s1, File S1: keyword search criteria on HATr.

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