

# Narrow-spectrum drug repurposing: targeting *Gardnerella vaginalis* biofilms associated with bacterial vaginosis

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## Abstract

**Aim** Bacterial vaginosis (BV) is the most common vaginal disorder in women of reproductive age. Current therapies are limited by poor activity against biofilms and high recurrence rates (> 50%), demonstrating that new antimicrobials are required. Drug repurposing is an attractive approach for the discovery of new antimicrobials, so we aimed to screen repurposed libraries for activity against the key BV pathobiont *Gardnerella vaginalis*.

**Methods and results** Two drug libraries from Medicines for Malaria Venture comprising 640 compounds were screened against *G. vaginalis* and various *Lactobacilli* species. Initial screening identified 16 *G. vaginalis*-selective compounds, of which 10 showed  $\geq 90\%$  inhibition of planktonic growth while sparing *Lactobacillus crispatus*. Subsequent assays revealed that three candidates displayed activity against pre-formed *G. vaginalis* biofilms; MMV1634360 (an antiproliferative compound with reported anticancer and antifungal activity), MMV1582487 (originally developed as an *Escherichia coli* aminopeptidase N inhibitor), and MMV1582497 (a thymidylate kinase inhibitor developed for *Mycobacterium tuberculosis*). All three produced >2-log reduction in viable cell counts at 10  $\mu\text{M}$  ( $P < 0.05$  for all compounds). Further cytotoxicity testing in VK2/E6E7 vaginal epithelial cells excluded MMV1634360 and MMV1582497 due to off-target effects, leaving MMV1582487 as a leading candidate. MMV1582487 demonstrated further activity against a high biofilm-forming *G. vaginalis* clinical isolate with >4log<sub>10</sub> CFU/ml reduction in viable cell counts at 10  $\mu\text{M}$  ( $P < 0.001$ ), and synergy with existing antibiotic therapy.

**Conclusions** We demonstrate that MMV1582487 is a selective, non-cytotoxic, anti-biofilm candidate against *G. vaginalis*, supporting its potential as a novel therapeutic option for BV.

## Impact statement

Our study identified drug repurposing as a valuable discovery tool for selective and anti-biofilm drug candidates against microbiome-based diseases such as BV, warranting future mechanistic based studies.

**Keywords** biofilm, *Gardnerella vaginalis*, *Lactobacillus*, bacterial vaginosis, treatment, new drug

## Introduction

Bacterial vaginosis (BV) is the most common vaginal disorder among women of childbearing age, with global estimates suggesting a prevalence of 23%–29% (Peebles et al. 2019). It is characterized as a dysbiosis of the vaginal microbiota (VMB), where the typi-

cally dominant, protective *Lactobacillus* spp., are depleted and replaced by increased numbers of facultative and obligate anaerobic bacteria, such as *Gardnerella vaginalis*, which forms recalcitrant biofilms on the vaginal epithelium (Muzny et al. 2020, Saraf et al. 2021). An optimal VMB is dominated by *Lactobacillus* spp., which helps maintain a state of eubiosis through the production of lactic

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acid, preserving the low vaginal pH of 3.5–4.5 (Saraf et al. 2021). Different *Lactobacillus* species offer varying degrees of protection, with *L. crispatus* most strongly associated with stability, whereas *L. iners* is linked to increased community fluctuation (Carter et al. 2023).

Although BV presents asymptotically in ~50%–84% of cases (Koumans et al. 2007), symptomatic clinical manifestations include abnormal vaginal discharge, unpleasant fishy odour, vaginal pH above 4.5, and the presence of clue cells (Bradshaw et al. 2025). Notably, symptomatic and asymptomatic BV are associated with an increased risk of acquiring sexually transmitted infections including HIV, and of developing genital inflammation (McKinnon et al. 2019). Furthermore, it can lead to adverse outcomes in pregnancy such as preterm labour and miscarriage (Leitich and Kiss 2007).

Typically, only symptomatic BV is treated, initially with oral metronidazole which may be followed by topical application of metronidazole or clindamycin gel if the initial treatment was not tolerated or if the condition recurs (Bagnall and Rizzolo 2017). Despite these treatment options, BV recurrence rates remain high at  $\geq 50\%$  within 12 months of initial treatment (Bradshaw et al. 2006, Cohen et al. 2020). This persistence is likely due to the protective and recalcitrant nature of biofilms dominated by *G. vaginalis*, which form on the vaginal epithelium and confer tolerance to conventional antibiotics (Machado and Cerca 2015, Machado et al. 2016). Unsurprisingly, *Gardnerella* spp., the putative microbial drivers of BV, are often resistant to the limited antibiotics available (Zhang et al. 2022, Rashidifar et al. 2023). Collectively, these factors contribute to an estimated global annual cost of \$4.8 billion for BV treatments, more than half of which is due to recurrent cases (Peebles et al. 2019).

The high rate of recurrence in BV, combined with the limited number of available treatments, highlights the pressing need for new therapeutic options. Whilst there is ongoing research into novel approaches (Landlinger et al. 2021, Johnston et al. 2023), these candidates face time- and resource-intensive challenges of developing entirely new compounds. Therefore, the repurposing of existing compounds represents a more pragmatic and potentially faster alternative (Wall and Lopez-Ribot 2020).

An organization which embodies this drug repurposing strategy is Medicines for Malaria Venture (MMV), a Swiss-based not-for-profit organization focused on expanding the applications of existing antimalarials and related compounds by identifying new targets and developing innovative uses for these drugs. The Global Health Priority Box (GHPB) (Adam et al. 2024) and the Pandemic Response Box (PRB) (Samby et al. 2022) represent a collection of 640 structurally diverse compounds with confirmed activity against infectious pathogens and disease vectors. In this study, we screened these compounds against *G. vaginalis*, focusing on their ability to inhibit planktonic growth and disrupt pre-formed biofilms, while excluding off-target effects on commensal vaginal bacteria and host epithelial cells.

## Materials and methods

### Microbial culture and standardization

For this study, *G. vaginalis* (ATCC 14018) was cultured as required on Columbia agar (Merck KGaA, Germany) containing 5% defibri-

nated horse blood (E&O laboratories, UK). For broth culture, New York City III (NYCIII) broth supplemented with 10% (v/v) inactivated horse serum was used, as per previous studies (Rosca et al. 2020, Johnston et al. 2023, Johnston and Kean 2025). Liquid cultures were prepared by inoculating *G. vaginalis* colonies into NYCIII and incubating for 16–24 h at 37°C in an anaerobic incubator (Don Whitley Scientific MACS MG-500), with anaerobic gas influx (5% CO<sub>2</sub>, 10% H<sub>2</sub>, and 85% N<sub>2</sub>). For experimental use, *G. vaginalis* was standardized to  $\sim 1 \times 10^8$  CFU/ml as previously described (Johnston et al. 2023).

For selective antimicrobial testing, three *G. vaginalis* clinical isolates, and three vaginally derived lactobacilli isolates (*L. crispatus* CCUG 42898, *L. gasseri* CCUG 44059, and *L. jensenii* DSMZ 20557) were used. Clinical *G. vaginalis* isolates were provided by Sandyford Sexual Health clinic (Glasgow, UK) and grown as described above. Lactobacilli isolates were propagated on De Man–Ragosa–Sharpe (MRS) agar (Merck). For experiments utilizing Lactobacilli spp., isolates were cultured anaerobically in NYCIII as above to maintain consistency with *G. vaginalis* conditions.

### Compound library preparation

The GHPB (Adam et al. 2024) and PRB (Samby et al. 2022) were supplied by MMV (Geneva, Switzerland). Libraries were provided in 96 well plates, and a total of 640 compounds were screened in this study. Specifically, the GHPB comprised 80 compounds with confirmed activity against drug-resistant malaria, 80 compounds intended for screening against neglected and zoonotic diseases or diseases at risk of developing drug resistance, and 80 compounds tested for activity against vector species. The PRB comprised 201 antibacterials, 153 antivirals, and 46 antifungal compounds. The two libraries were provided as 10 mM stock solutions in 100% dimethyl sulfoxide (DMSO) prepared externally by Evotec (PRB) or in-house upon arrival (GHPB). The 10 mM master plates were further diluted 1:10 in DMSO to generate 1 mM working plates and stored at –20°C until use.

### Initial compound screening

All 640 compounds were initially screened against *G. vaginalis* and *L. crispatus* for their capacity to inhibit planktonic growth at a final concentration of 10  $\mu$ M, based on recommendations from the supplier. These screens were performed by diluting both organisms to  $\sim 1 \times 10^6$  CFU/ml in NYC III medium. A total of 198  $\mu$ l of diluted bacterial suspensions was added to a sterile 96-well round bottom microtiter plate, followed by 2  $\mu$ l of each compound (1 mM) to achieve a final concentration of 10  $\mu$ M. DMSO-only controls were included within the screening process to ensure no off-target effect on microbial growth. Plates were then incubated for 24-h anaerobically at 37°C. Following incubation, bacterial pellets were resuspended and optical density at 550 nm (OD<sub>550</sub>) was measured using a FLUOStar Omega microplate reader (BMG labtech, Germany). Results are presented as a percentage inhibition relative to untreated controls.

### Planktonic efficacy testing

Compounds showing  $\geq 90\%$  inhibition of *G. vaginalis*, and  $\leq 10\%$  inhibition of *L. crispatus*, proceeded to further testing ( $n = 10$ ). These compounds were supplied as fresh powder stocks from

Evotec and reconstituted at 10 mM in DMSO. Planktonic minimum inhibitory concentration (pMIC) assays were then performed against *G. vaginalis*, *L. crispatus*, *L. gasseri*, and *L. jensenii*, to confirm selective cytotoxicity.

These experiments followed the Clinical and Laboratory Standards Institute (CLSI) M11-A8 broth microdilution method in NYC III with some minor modifications (Johnston et al. 2023). Briefly, the 10 compounds and antibiotics were serially diluted 1:2 in NYC III in round-bottom microtiter plates at a concentration range of 0.004–10  $\mu\text{M}$  for *G. vaginalis* and 0.31–160  $\mu\text{M}$  for Lactobacilli spp. Untreated controls (bacteria only) and negative controls (media only) were included in each assay.

Plates were incubated for 24 h at 37°C. For all plate-based assays, organisms were incubated in an anaerobic incubator (Don Whitley Scientific MACS MG-500), with anaerobic gas influx (5% CO<sub>2</sub>, 10% H<sub>2</sub>, and 85% N<sub>2</sub>). Following growth, bacterial pellets were resuspended and optical density at 550 nm (OD<sub>550</sub>) was measured using a microplate reader (BMG Labtech, Germany). Results are presented as a percentage inhibition in comparison to untreated controls, with the MIC<sub>90</sub> defined as the lowest concentration resulting in  $\geq 90\%$  inhibition.

## Biofilm efficacy testing

Following pMIC testing, compounds anti-biofilm activity was investigated against *G. vaginalis*. For these experiments, *G. vaginalis* biofilms were grown by adding 200  $\mu\text{l}$  of  $\sim 1 \times 10^7$  CFU/ml in NYC III into flat bottomed 96-well microtiter plates (Johnston et al. 2023). Plates were then incubated anaerobically for 24 h at 37°C for biofilm development.

Following growth, supernatants were removed, and biofilms washed once with sterile phosphate buffered saline (PBS) to remove non-adherent cells. Serially diluted compounds were then applied as above, followed by a further 24-h anaerobic incubation. Afterwards, supernatants were removed again, biofilms were washed in PBS and 200  $\mu\text{l}$  0.001% resazurin (Sigma-Aldrich, Gillingham, UK) was applied to each well as to measure biofilm metabolic activity (Turovskiy et al. 2012). Following 1 h incubation in 5% CO<sub>2</sub> at 37°C, fluorescence was measured at 544ex/590em using a FLUOStar Optima microplate reader, with results are presented as a percentage metabolic activity relative to untreated controls.

From the above assay, three compounds showed significant reductions in metabolic activity when applied to pre-formed biofilms. The bactericidal activity of these compounds was then confirmed using colony counting (Miles et al. 1938) at 1 and 10  $\mu\text{M}$ . Biofilms were grown for 24 h before being treated for a further 24 h as described above. Following treatment, supernatant was removed and biofilms washed in sterile PBS. Biofilms were then resuspended in PBS, serially diluted, and spot plated onto Columbia blood agar, then incubated anaerobically at 37°C for 48 h. The number of colonies following incubation was then used to calculate the CFU/ml concentration of each resuspended biofilm sample.

## Cytotoxicity testing

Following biofilm efficacy testing, the cytotoxicity of promising candidates was evaluated against vaginal VK2/E6E7 cells. These cells were grown in Keratinocyte serum free media (KSFM) sup-

plemented with 0.1 ng/ml human recombinant EGF, 0.05 mg/ml bovine pituitary extract, and calcium chloride at 44.1 mg/L. Cells were grown in 75 cm<sup>3</sup> flasks in 5% CO<sub>2</sub> at 37°C until 80%–90% confluent. Cells were then seeded in 96-well plates at  $1 \times 10^4$  cells/well for 24 h in 5% CO<sub>2</sub>. After 24 h, compounds were applied (1.25–80  $\mu\text{M}$ ). Following treatment, supernatants were harvested for lactate dehydrogenase (LDH) quantification, and the metabolic activity of the remaining cells was assessed using alamarBlue™.

For LDH assays, the Invitrogen™ CyQUANT™ LDH cytotoxicity assay was used on fresh cell supernatants following manufacturer's instructions. Briefly, 50  $\mu\text{l}$  of substrate solution was mixed with 50  $\mu\text{l}$  cell culture supernatant in 96 well round bottom microtiter plates. Plates were incubated at room temperature in the dark for 30 min, before 50  $\mu\text{l}$  of stop solution was applied. The absorbance was then measured at OD<sub>490nm</sub> and OD<sub>680nm</sub> using a FLUOstar Omega microplate reader.

In parallel, cell metabolic activity was assessed using the Invitrogen™ alamarBlue™ cell viability assay. AlamarBlue™ reagent was diluted 1:10 in KSFM, added to cells at 200  $\mu\text{l}$  per well, followed by incubation for 2 h in 5% CO<sub>2</sub> at 37°C. Fluorescence was then measured as described above.

## Synergy testing

Having identified a leading repurposing candidate, checkerboard assays were conducted with metronidazole to evaluate potential synergy/antagonism. These assays were performed on planktonic *G. vaginalis* inoculated on 96 well round bottom plates. The process involved serial dilutions of both the repurposed compound and metronidazole, as well as varying concentrations of both drugs in combination as previously described (Sun et al. 2009). Once plates were inoculated, they were incubated anaerobically for 24 h at 37°C. Following the pMIC protocol above, bacterial pellets were then resuspended, and absorbance (OD<sub>550</sub>) was measured using a FLUOstar Omega BMG Labtech, results presented as a percentage of the untreated positive control. Drug interactions were calculated using the Bliss scoring model using SynergyFinder+ (Zheng et al. 2022).

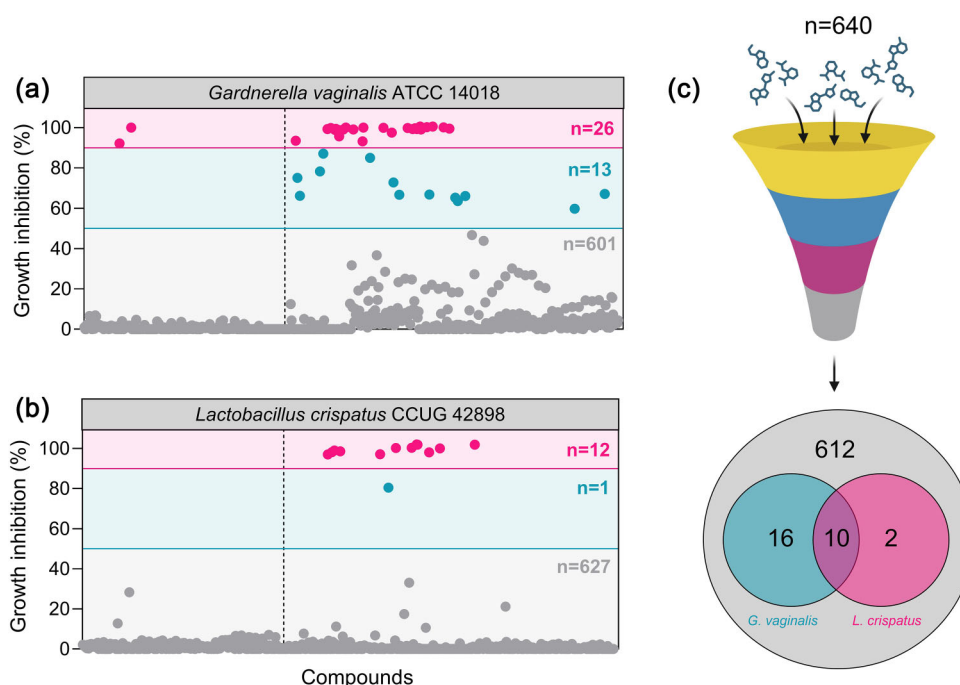
## Data analysis

Raw data was analysed and processed using both Microsoft Excel (Microsoft 365, version 2407) and GraphPad PRISM (version 10.0.2). Statistical analysis was carried out after normality testing, and appropriate parametric and non-parametric tests performed. Exact statistical tests are highlighted in the legend of each figure. All experiments involved three independent biological replicates ( $n = 3$ ).

## Results

### Screening compound libraries

To identify compounds with selective antibacterial activity relevant to BV, we conducted a broad screen of two drug libraries. The MMV GHPB and PRB were initially screened against *G. vaginalis*, the putative microbial driver of BV. Screening was performed at 10  $\mu\text{M}$  to assess inhibition of planktonic growth as an ini-



**Figure 1** Screening of the MMV GHPB and PRB against (a) *G. vaginalis* ATCC 14018 and (b) *L. crispatus* CCUG 42898 at 10  $\mu$ M. Graphs show the mean percentage growth inhibition of each organism compared to a positive control. The dashed line separates GHPB compounds (left) from PRB compounds (right). In total, 640 compounds were screened: of these, 16 selectively inhibited *G. vaginalis*, 2 selectively inhibited *L. crispatus*, and 10 inhibited both organisms by  $\geq 90\%$  at this concentration (c). The top half of figure C was created in Biorender. Means plotted from  $N = 3$  screens.

tial marker of efficacy. Across three independent experiments, compounds producing a mean growth inhibition of  $\geq 90\%$  relative to untreated controls were classified as ‘hits’, while those with  $\geq 50\%$  but  $< 90\%$  inhibition were designated as ‘near-misses’. As shown in Fig. 1a, of the 640 compounds screened, 26 inhibited *G. vaginalis* growth ( $\geq 90\%$ ), with an additional 13 identified as near-misses. Notably, 24 of these hits originated from the PRB, and only 2 from the GHPB. Screening the same libraries against *L. crispatus*, a commensal vaginal species associated with health, identified 12 hits and 1 near-miss compound, all from the PRB (Fig. 1b). Comparing the effects across both organisms (Fig. 1c) revealed 10 compounds that inhibited both at 10  $\mu$ M, 16 that selectively inhibited *G. vaginalis*, and 2 that selectively inhibited *L. crispatus*. Full data for each compound against both organisms are provided (Supplementary File 1). Together, this initial screen demonstrated that some of these compounds exhibit selective toxicity towards *G. vaginalis* while sparing *L. crispatus* at this concentration.

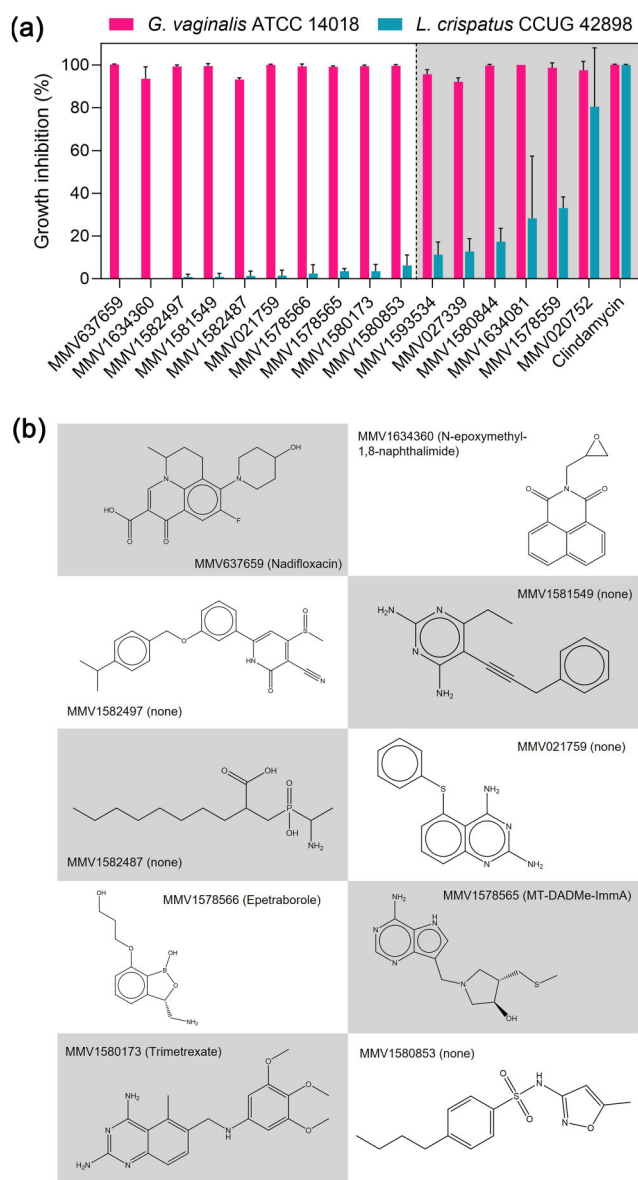
## Streamlining candidate compounds

Having established a pool of ‘selective’ compounds, we next sought to streamline these based on a more stringent selection criterion. Leading compounds were chosen based on their selective inhibition of *G. vaginalis* relative to *L. crispatus* (Fig. 2a). When growth inhibition of the two organisms was compared, 10 compounds demonstrated  $\geq 90\%$  inhibition of *G. vaginalis* and  $\leq 10\%$  inhibition of *L. crispatus* at 10  $\mu$ M. These compounds were considered the leading candidates and progressed to subsequent testing (Fig. 2b). This panel included both approved drugs, such as Nadi-

floxacin (MMV637659), a broad-spectrum quinolone used for topical acne vulgaris, and trimetrexate (MMV1580173), a dihydrofolate reductase inhibitor structurally related to methotrexate. In addition, several experimental and preclinical candidates were identified. Notably, all 10 compounds displayed a greater degree of selective toxicity towards *G. vaginalis* at this set concentration than clindamycin, a key BV therapeutic, which is included in the MMV PRB as a reference antimicrobial.

## Planktonic MIC testing

Having identified promising candidates, we next determined the pMICs against *G. vaginalis* and representative commensal lactobacilli: *L. crispatus*, *L. gasseri*, and *L. jensenii*, which are dominant members of the ‘protective’ community state types (CST) I, II, and V (Ravel et al. 2011). Across all compounds, the pMICs for *G. vaginalis* were generally lower than for any of the lactobacilli (Fig. 3), confirming selective activity to varying degrees. Notably, MMV637659 (Nadifloxacin) showed higher activity against *L. jensenii* than against the other lactobacilli, while MMV1580853 inhibited *L. gasseri* at levels similarly observed for *G. vaginalis*. This reinforces the importance of including multiple lactobacilli species to more robustly evaluate potential off-target effects on the healthy VMB. Encouragingly, several compounds such as MMV1581549, MMV1578565 (MT-DADMe-ImMA), and MMV1580173 (Trimetrexate), demonstrated potent inhibition of *G. vaginalis* with pMIC values  $< 1 \mu$ M, while exhibiting minimal activity against the commensal lactobacilli—a key characteristic of novel BV therapeutics.



**Figure 2** Streamlining candidates based on selective toxicity towards *G. vaginalis*. Graph displays the growth inhibition percentage in comparison to positive control of the 16 leading compounds from the initial screen, against *G. vaginalis* and *L. crispatus* (a). Of these, 10 compounds displayed  $\leq 10\%$  inhibition of *L. crispatus* and were selected for further testing. The chemical structures of these compounds are shown (b), provided by MMV. Graph displays mean and standard deviation,  $N = 3$ .

## Biofilm efficacy testing

Given the key role of biofilm formation in the pathophysiology of BV (Machado et al. 2016, Muzny et al. 2020), we next assessed the anti-biofilm activity of compounds against *G. vaginalis*. An initial screen was conducted across a concentration range of 1.25–80  $\mu\text{M}$ , measuring biofilm metabolic activity using a resazurin assay (Fig. 4a). Compared with planktonic efficacy, compounds generally exhibited reduced activity against established biofilms, consistent with the known increased tolerance of biofilm-associated bacteria. Nonetheless, three compounds demonstrated a notable reduction in biofilm metabolic activity following applica-

tion. These compounds were MMV1634360 (N-epoxymethyl-1,8-naphthalimide), an antiproliferative compound with reported anti-cancer and antifungal activity (Krishnan et al. 2007); MMV1582487, an inhibitor of bacterial aminopeptidase N (PepN) originally developed against *Escherichia coli* (Yang et al. 2005); and MMV1582497, a thymidylate kinase inhibitor developed for *Mycobacterium tuberculosis* (Naik et al. 2015). Most notable being MMV1582487, which reduced biofilm metabolic activity by around 80% across all tested concentrations, and as low as 1.25  $\mu\text{M}$ .

To confirm these findings, the bactericidal activity of these three compounds was further evaluated by enumerating biofilm CFU/ml at 1 and 10  $\mu\text{M}$  (Fig. 4b). As expected, no significant reduction in viable counts was observed at 1  $\mu\text{M}$ , although MMV1582487 showed a non-significant quantitative decrease of  $\sim 2\log_{10}$  CFU/ml. In contrast, all three compounds significantly reduced biofilm CFU/ml at 10  $\mu\text{M}$  (MMV1634360  $P < 0.001$ , MMV1582487  $P = 0.038$ , MMV1582497  $P = 0.009$ , Kruskal-Wallis with Dunn's post-hoc vs. untreated). Interestingly, despite producing the largest reduction in biofilm metabolic activity, MMV1582487 appeared the least bactericidal when evaluated using colony counting, suggesting a potential effect on biofilm metabolism even at sub-inhibitory doses. Collectively, this data demonstrates that three compounds were both selective for *G. vaginalis* over lactobacilli and retained efficacy against biofilm-embedded bacteria. This counteracts the heightened tolerance of *G. vaginalis* biofilms to conventional antibiotics (metronidazole and clindamycin) at concentrations far exceeding the pMIC (Rosca et al. 2022b, Johnston et al. 2023).

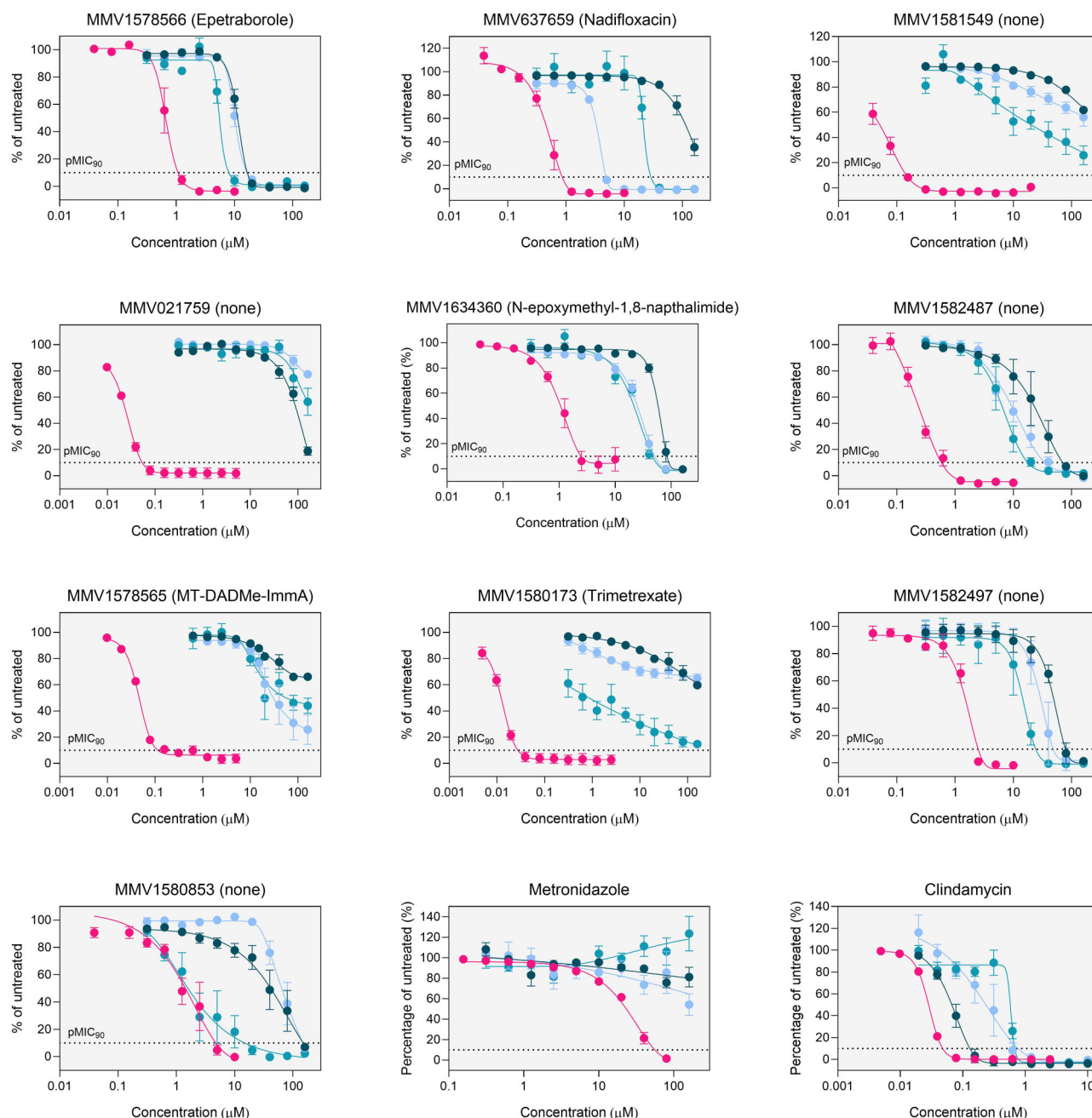
## Cytotoxicity of candidate compounds

Having identified selective compounds with anti-biofilm activity against *G. vaginalis*, we next evaluated their potential off-target cytotoxic effects on vaginal-derived VK2/E6E7 cells. Assessment of cell metabolic activity revealed significant reductions following treatment with MMV1634360 at concentrations between 20 and 80  $\mu\text{M}$ , and with MMV1582497 at concentrations as low as 5  $\mu\text{M}$  (Fig. 5a). Furthermore, LDH release (a marker of cell membrane integrity) was significantly elevated at concentrations  $\geq 2.5$   $\mu\text{M}$  for MMV1582497 (Fig. 5b). Interestingly, although MMV1634360 impaired metabolic activity, it did not significantly increase LDH release, suggesting a differential mode of cytotoxicity. Importantly, MMV1582487 did not exhibit any detectable adverse effects on mammalian cells in either assay, even at the highest tested concentration (80  $\mu\text{M}$ ), which far exceeds the levels required for both planktonic and biofilm inhibition of *G. vaginalis*.

## MMV1582487 as a novel therapeutic candidate in BV

To further assess the potential of repurposing MMV1582487 for the treatment of BV, we evaluated its interaction with metronidazole against *G. vaginalis* ATCC 14018 (Supplementary Fig. 1). Across tested combinations, the mean Bliss score of 12.25 suggested mild synergy between the two agents.

Finally, we examined the activity of MMV1582487 against a broader panel of clinical *G. vaginalis* isolates to assess its spectrum of activity against this species. Testing three isolates revealed pMIC values comparable to ATCC 14018 (Fig. 6a) and were below

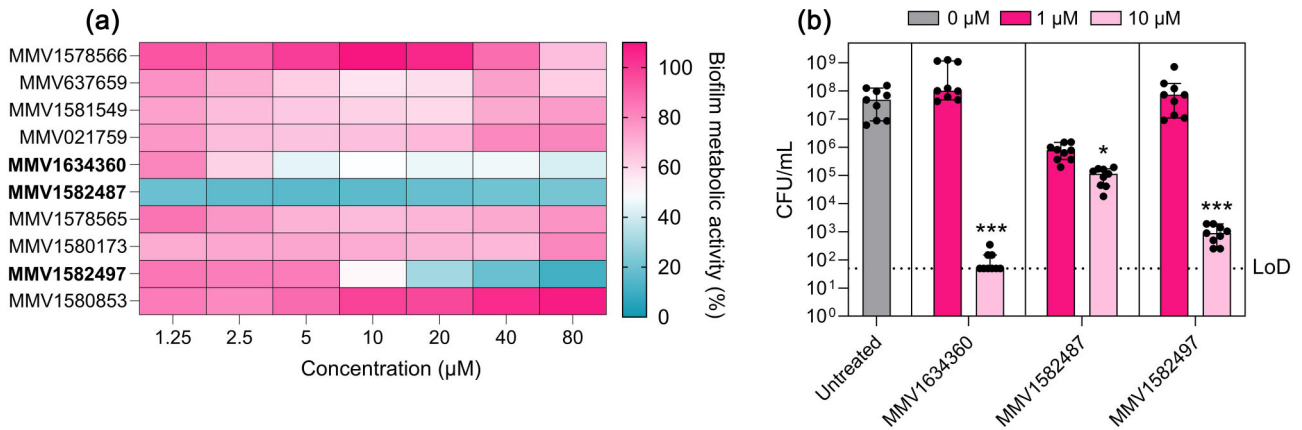


***G. vaginalis* ATCC 14018      *L. crispatus* CCUG 42898**  
***L. jensenii* CCUG 21961T      *L. gasseri* CCUG 44059**

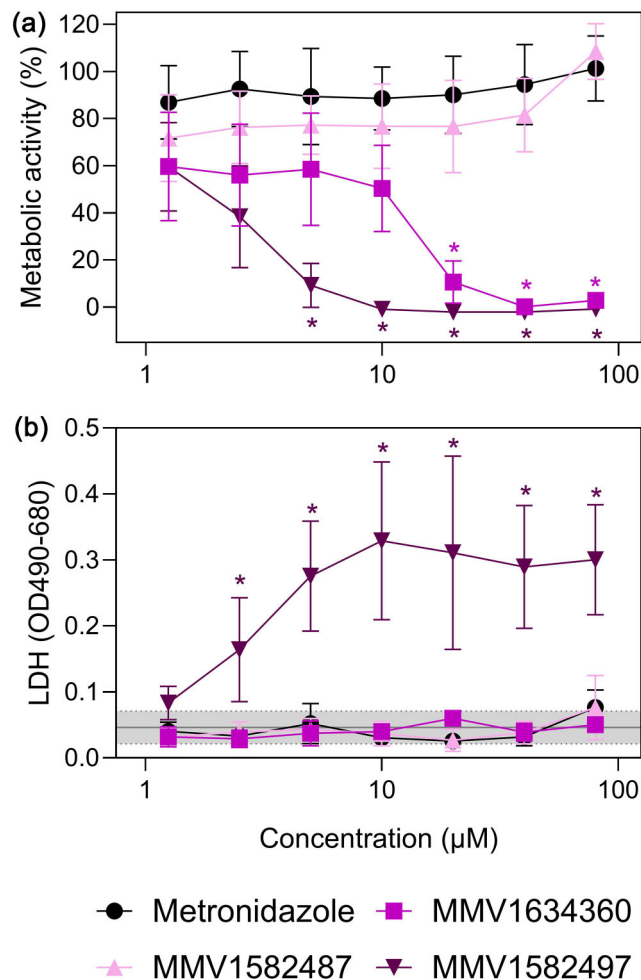
**Figure 3** Planktonic efficacy of selected MMV compounds against *G. vaginalis* ATCC 14018, *L. crispatus* CCUG 42898, *L. jensenii* CCUG 21961T, and *L. gasseri* CCUG 44059. Graphs show planktonic MIC results as percentage growth relative to untreated controls, presented as mean  $\pm$  SEM. The MMV compound identifier and trade name (where applicable) are indicated above each graph. Compounds without an assigned trade name are labelled 'none'.  $N = 3$ .

the CLSI resistance thresholds for anaerobic bacteria (metronidazole  $\geq 32 \mu\text{g/ml} \approx 187 \mu\text{M}$ ; clindamycin  $\geq 8 \mu\text{g/ml} \approx 18.8 \mu\text{M}$ ), which have been applied in recent studies assessing *G. vaginalis* susceptibility (Landlinger et al. 2021, Johnston et al. 2023). When assessing biofilm formation, two of the clinical isolates (Gv\_1 and Gv\_2) produced significantly greater biofilm biomass com-

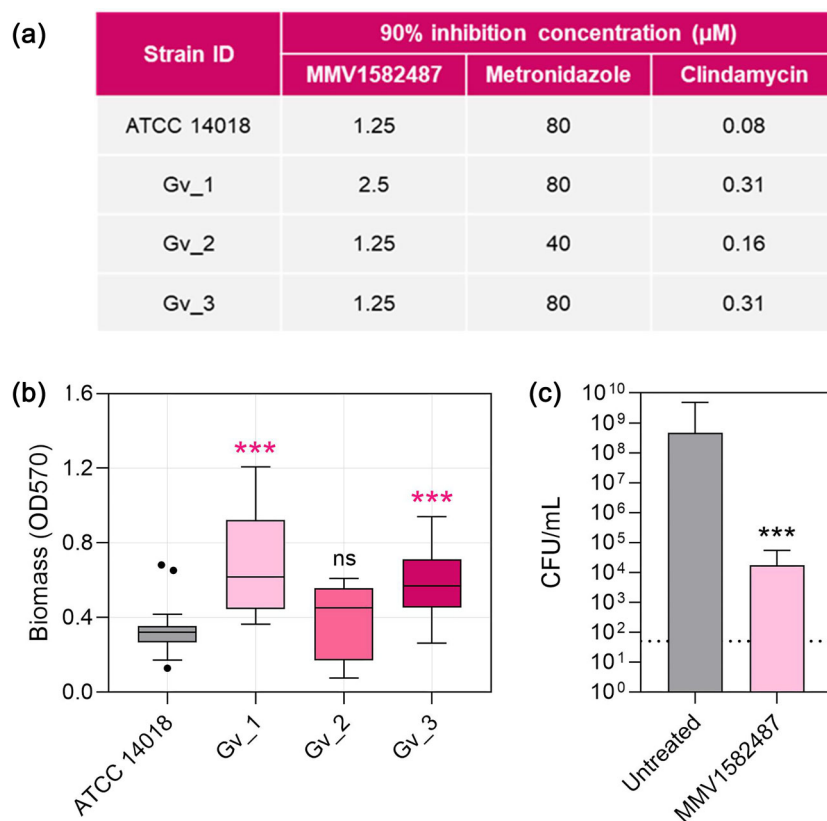
pared to ATCC 14018 (Fig. 6b). To determine whether MMV1582487 retained activity against more robust biofilms, we treated the high-biofilm-forming isolate Gv\_1 with  $10 \mu\text{M}$  MMV1582487. Encouragingly, even against this more resilient biofilm, treatment resulted in a significant  $>4\log_{10}$  CFU/ml reduction ( $P < 0.001$ ) (Fig. 6c).



**Figure 4** Biofilm efficacy of selected MMV compounds against *G. vaginalis* ATCC 14018. Initial screen of all 10 compounds against *G. vaginalis* biofilms, tested at concentrations ranging from 1.25 to 80  $\mu\text{M}$  (a). Biofilm metabolic activity was assessed using resazurin and is presented as a heatmap showing the median percentage activity relative to untreated controls. The heatmap uses a gradient from low (blue) to high (pink) metabolic activity, with white values corresponding to the midpoint of the gradient (~50% activity). Compounds in bold were selected for further testing. The bactericidal efficacy of selected compounds against biofilms was further evaluated by colony counting at 1 and 10  $\mu\text{M}$  (b). Graphs display individual data points with overlaid median and 95% confidence intervals. Statistical comparisons were performed using Kruskal–Wallis with Dunn’s post-hoc test, comparing each treatment to untreated controls, \* $P < 0.05$  & \*\*\* $P < 0.001$ .  $N = 3$ . LoD, limit of detection.



**Figure 5** Investigating the cytotoxicity of selected MMV compounds in vaginal epithelial VK2/E6E7 cells by measuring metabolic activity (a) and LDH release (b). Graphs show mean  $\pm$  standard deviation. In panel b, the shaded area represents baseline LDH release from untreated cells, with the mean shown as a solid line and  $\pm$ SD shown as dotted lines. Statistical significance was determined by Kruskal–Wallis test with Dunn’s multiple comparisons post-hoc, \* $P < 0.05$ .  $N = 3$ . LDH, lactate dehydrogenase.



**Figure 6** Evaluating MMV1582487 against a broader panel of *G. vaginalis* isolates. Planktonic MICs were determined for *G. vaginalis* ATCC 14 018 and three clinical isolates using MMV1582487, metronidazole, and clindamycin (a). Biofilm formation of each strain was assessed by crystal violet staining (b). Graphs show Tukey boxplots; statistical comparisons were performed using Kruskal–Wallis test with Dunn’s post hoc. Asterisks above each clinical isolate indicate comparison with the type strain: \*\*\* $P < 0.001$ ; ns, not significant. Biofilms of the high-biofilm-forming clinical isolate Gv\_1 were treated with 10  $\mu\text{M}$  MMV1582487 and evaluated by colony counting (c). Graph shows median with 95% confidence intervals; statistical comparison was performed using Mann–Whitney test, \*\*\* $P < 0.001$  vs. untreated control.  $N = 3$ .

## Discussion

Despite growing recognition of the key role that microbial biofilms play in BV, current therapies remain largely limited to conventional antibiotics (i.e. metronidazole and clindamycin). While both drugs are active against a range of vaginal pathobionts, they have notable limitations that contribute to unacceptably high recurrence rates of  $\geq 50\%$  (Vodstrcil et al. 2021). For example, many BV-associated bacteria, including certain *G. vaginalis* clades (Schuyler et al. 2016), are intrinsically resistant to metronidazole. Alternatively, clindamycin lacks selectivity in this microenvironment, displaying activity against both BV-associated bacteria and beneficial *Lactobacilli* species, potentially hindering vaginal microbiome restoration following treatment. Consistent with these limitations, a number of studies have also demonstrated that both antibiotics display limited activity against pre-formed *G. vaginalis* biofilms (Rosca et al. 2022b, Johnston et al. 2023, Johnston and Kean 2025), a recognized hallmark of BV development. As such, there is a clear need for the discovery of novel therapeutic approaches.

One promising route is drug repurposing, a strategy that reduces the time, effort and cost of the drug development pipeline (Pushpakom et al. 2019, Wall and Lopez-Ribot 2020). In BV, this approach was recently demonstrated using pyrimethamine, an anti-parasitic agent that inhibited *G. vaginalis* while sparing beneficial lactobacilli (Rosca et al. 2025). Building on this, the present study

systematically screened two MMV drug libraries; the GHPB (Adam et al. 2024) and PRB (Samby et al. 2022), which collectively contain 640 structurally diverse compounds, to identify novel candidates with selective activity against *G. vaginalis*.

Our initial experiments focused on compound selectivity, aiming to minimize collateral damage to beneficial members of the microbiome—a key characteristic of effective novel therapies for dysbiosis-associated diseases such as BV (Miethke et al. 2021). To account for this, we first screened against *L. crispatus* as a representative of CST I. Recognizing the diversity of CST’s (Ravel et al. 2011, Chee et al. 2020), we subsequently extended this to additional *Lactobacilli* spp., (*L. jensenii*, and *L. gasseri*). Following this wider investigation, some initially promising candidates, such as MMV637659 and MMV1580853, were excluded due to undesirable off-target effects on some *Lactobacilli*, reinforcing the need to test across representative species from each ‘protective’ CST.

Having identified selective compounds, we next evaluated the anti-biofilm efficacy of the top 10 candidates against *G. vaginalis*. As expected, the majority of compounds were ineffective against pre-formed biofilms, aligning with the well-documented antimicrobial tolerance conferred by microbial biofilms. Only three candidates displayed notable anti-biofilm activity which were MMV1634360 [an antiproliferative compound with previously documented anticancer and antifungal activity (Krishnan et al. 2007)], MMV1582487 [developed as an *E. coli* aminopeptidase N

inhibitor (Yang et al. 2005)], and MMV1582497 [a thymidylate kinase inhibitor developed for *M. tuberculosis* (Naik et al. 2015)]. At 10  $\mu\text{M}$ , all three compounds reduced both biofilm metabolic activity and viable counts. Interestingly, MMV1582487 also suppressed biofilm metabolism at 1.25  $\mu\text{M}$ , potentially indicating a bacteriostatic mechanism or direct impact on biofilm metabolism at lower concentrations. Notably, one of these compounds had also emerged from PRB screens against priority pathogen *Acinetobacter baumannii*, with MMV1582497 demonstrating inhibition of growth (Sivasankar et al. 2024).

We next extended our screen to the host by evaluating compound cytotoxicity against VK2/E6E7 vaginal epithelial cells. In these experiments, two candidate compounds (MMV1634360 and MMV1582497) showed significant off-target cytotoxicity, whereas MMV1582487 exhibited no adverse effects and advanced as the lead compound. Screening against clinical isolates of *G. vaginalis* confirmed that MMV1582487 retained comparable planktonic efficacy to the type strain and remained active against a high-biofilm-forming isolate. Importantly, it also lacked activity against *Lactobacillus* species, supporting its potential for downstream use alongside probiotics aimed at restoring the vaginal microbiome (Cohen et al. 2020). Moreover, we observed slight synergism with metronidazole, suggesting possible use as an adjunct therapy.

MMV1582487 is a decyl phosphinate previously shown to competitively inhibit aminopeptidase N within *E. coli* (Yang et al. 2005). In *E. coli*, this enzyme is cytosolic and is essential in ATP-independent protein degradation. The *G. vaginalis* genome incorporates several aminopeptidases, with this enzymatic activity having previously been explored as a biochemical target for BV diagnostics (Schoonmaker et al. 1991), suggesting a potential reciprocal cellular target. However, *Lactobacilli* also possess these enzymes, which would not explain the selectivity of the compound. The lack of inhibitory activity against *Lactobacilli* remains unclear but could be speculated to be lack of competitive inhibition of the target, inhibition of a non-essential function in *Lactobacilli*, or alternative target pathway. In addition, we cannot rule out that selectivity is linked to the low pH environment generated by *Lactobacilli*, which may impact compound stability. Given that the healthy vaginal pH is  $<4.5$ , selective efficacy that is only observed under the elevated pH conditions characteristic of BV could be advantageous. Future studies should therefore seek to characterize the efficacy of this compound in acidic conditions to determine whether selectivity is retained or attenuated across a range of physiologically relevant environments.

Moving forward, although MMV1582487 emerged as a promising candidate from this study, several limitations of this study must be acknowledged. Given the number of compounds screened, our work was performed in simplified mono-species planktonic and biofilm systems. These systems were appropriate for a high-throughput screen, but do not reflect the polymicrobial nature of BV. Future studies should therefore employ multi-species biofilm models which more accurately reflect the inter-species interactions and structural complexity of biofilms found *in vivo* (Rosca et al. 2022b, Johnston et al. 2023). In a similar sense, it would also be useful to examine how treated biofilms influence host tissues, as seen in a number of recent studies (Rosca et al. 2022a, Johnston and Kean 2025). Additionally, while preliminary insights into the potential target of MMV1582487 were discussed and derived from earlier literature on *E. coli*, a more in-depth analysis of the precise mechanism of action in *G.*

*vaginalis* is required. Future studies could address this through approaches such as *in silico* docking or comparative structural analyses. Finally, although slight synergism with metronidazole and selectivity over *Lactobacillus* species were observed, additional work is needed to evaluate how MMV1582487 performs in combination with existing antibiotics and whether it is compatible with probiotic-based microbiome restoration strategies.

Collectively, these findings highlight MMV1582487 as a selective, anti-biofilm candidate against *G. vaginalis*. While its precise mechanism of selectivity remains unclear, its retention of efficacy against high-biofilm-forming isolates and lack of cytotoxicity in vaginal epithelial cells underscore a clear therapeutic potential.

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## Author contributions

William Johnston (Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing), Jamie Smith (Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing), Elisa Giammarini (Data curation, Formal Analysis, Investigation, Writing – review & editing), Amy Campbell (Formal Analysis, Investigation, Methodology, Writing – review & editing), Anthony J Slate (Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing), Ermando Canga (Formal Analysis, Investigation, Methodology, Writing – review & editing), Marino Swanzy-Krah (Formal Analysis, Investigation, Methodology, Writing – review & editing), Pranitha Murali (Formal Analysis, Investigation, Methodology, Writing – review & editing), Mark Mason (Conceptualization, Methodology, Resources, Writing – review & editing), Rebecca Metcalfe (Funding acquisition, Methodology, Resources, Writing – review & editing), Charlotte-Eve Short (Conceptualization, Funding acquisition, Resources, Writing – review & editing), Ryan Kean (Conceptualization, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing)

## Supplementary material

Supplementary material is available at *Journal of Applied Microbiology* online.

## Conflicts of interest

None declared.

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## Data availability

Data from the library screens are available in supplementary file 1. All other data is available on request to the corresponding author.

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