

Characterisation of vaginal *Prevotella* strains from a cohort of South African women with and without bacterial vaginosis

Welp K¹, Passmore JS^{1,2,3}, Paul L^{1,3}, Jaspán HB^{4,5}, Gamielien H¹, Vasse M⁶, Gill K⁷, Bekker LG, Balle C¹, Happel A¹, Froissart R⁶ and Kullin B¹.

¹Department of Pathology, Institute of Infectious Disease and Molecular Medicine (IDM), University of Cape Town, South Africa; ²NRF-DST CAPRISA Centre of Excellence in HIV Prevention, Cape Town, South Africa; ³National Health Laboratory Service (NHLS), Cape Town, South Africa; ⁴Seattle Children's Research Institute, Seattle, WA, USA; ⁵University of Washington Department of Paediatrics and Global Health, Seattle, WA, USA; ⁶UMR MIVEGEC CNRS-IRD-UM, University Montpellier, Montpellier, France; ⁷Desmond Tutu HIV Foundation, University of Cape Town, Cape Town, South Africa

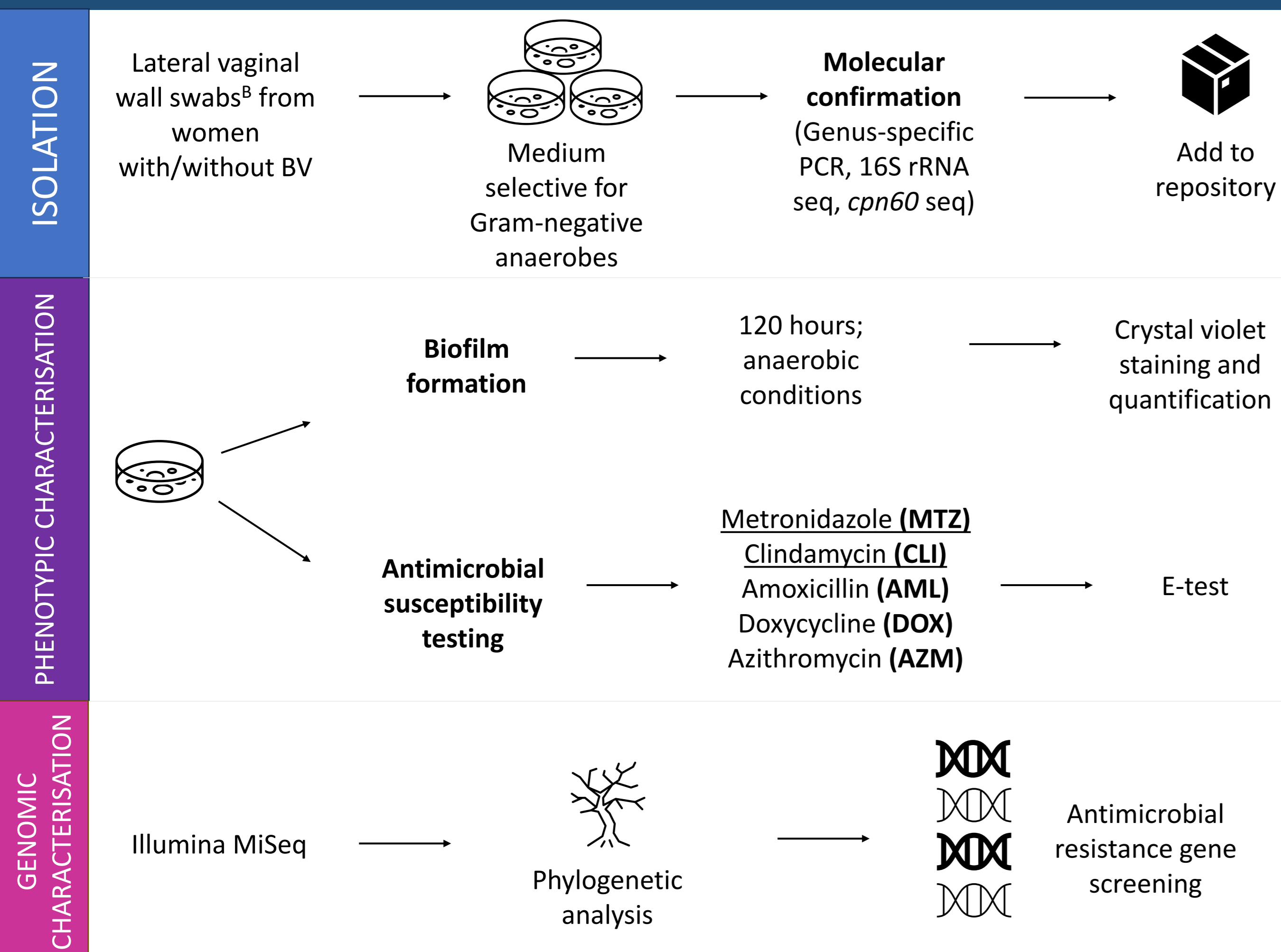
INTRODUCTION

- Bacterial vaginosis (BV)** is a commonly described condition in reproductive age cis-gender women, with a specifically high reported prevalence of **34-58% in sub-Saharan Africa (SSA)**^A.
- BV is treated with antibiotics, metronidazole and/or clindamycin, but often recurs within a few months of clearance.
- BV is underpinned by a microbiological change:
 - ↓ beneficial Lactic Acid Bacteria (LAB)
 - ↑ diverse anaerobic bacteria, including *Prevotella bivia*.
- P. bivia* is a Gram-negative anaerobe: it interacts with other BV-associated bacteria and forms part of the BV-biofilms.
- Despite the prevalence of BV in SSA and the role of *P. bivia* in BV pathogenesis, to date, very few isolated strains have been characterised from SSA.

OBJECTIVES

- To **isolate** vaginal *Prevotella* spp. from South African women with and without BV to establish a biorepository
- To determine the **antimicrobial susceptibility** profile of *P. bivia* isolates to standard of care (SOC) antibiotics
- To determine *in vitro* **biofilm forming abilities** of different *P. bivia* isolates
- To use a **whole genome sequencing** approach to determine the molecular epidemiology of South African *P. bivia* strains and their **antimicrobial resistance (AMR)** genes

METHODS



Isolation of *Prevotella* spp.

- Lateral vaginal wall swabs with highest number of *P. bivia* reads were targeted (based on 16S rRNA metagenomic data); In total, 71 strains isolated from samples from 30 women with and without BV (as determined by Nugent scoring)
- 37 strains were taken forward for full phenotypic and genotypic characterisation** (n=36 *P. bivia*, n=1 *Prevotella* spp. related to *P. melaninogenica*)

References

- ^A Kenyon C et al. Am J Obstet Gynecol. 2013.
^B Gill K et al. JIAS. 2020, 23, 10.
^C The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1, 2023.
^D Veloo ACM et al. J Antimicrob Chemother. 2018, 73: 2687-2690.
^E Tonkin-Hill G et al. Genome Biol. 2020, 22;21
^F Pruitt K.D et al. Nucleic Acid Res. 2007, 35.
^G <https://github.com/tseemann/abricate>

RESULTS

While almost one third of *Prevotella* isolates formed more robust biofilms than the type strain, there was no difference in biofilm-forming ability stratified by BV status

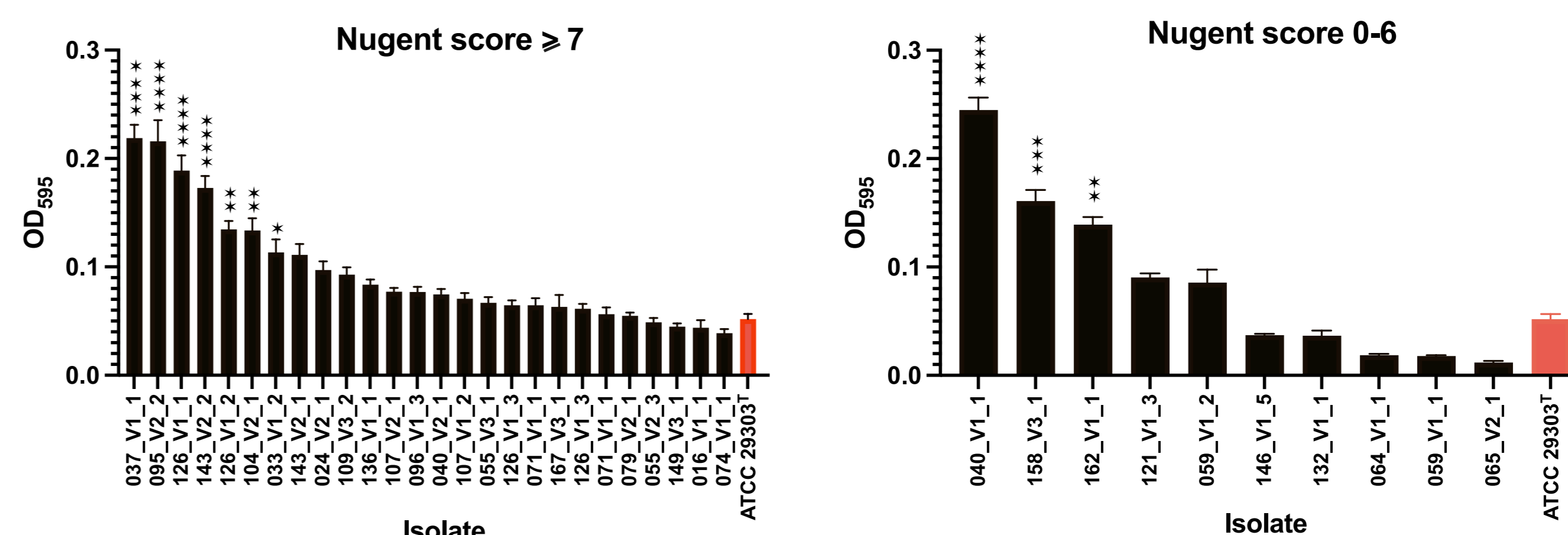


Figure 1: **Biofilm formation by clinical isolates.** Absorbance at 595nm represents biofilm biomass of isolates, stratified by BV status (Nugent score>7 BV positive; Nugent score 0-6 BV intermediate/negative). Mean absorbance is based on 3 biological replicates, with corresponding standard error. Clinical isolates differ significantly in biofilm-forming ability (p<0.0001, Kruskal-Wallis). Certain strains form significantly larger biofilms than the type-strain (ATCC 29303^T) (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Some South African *Prevotella* Isolates Are Resistant To BV Antibiotics Clindamycin And Metronidazole

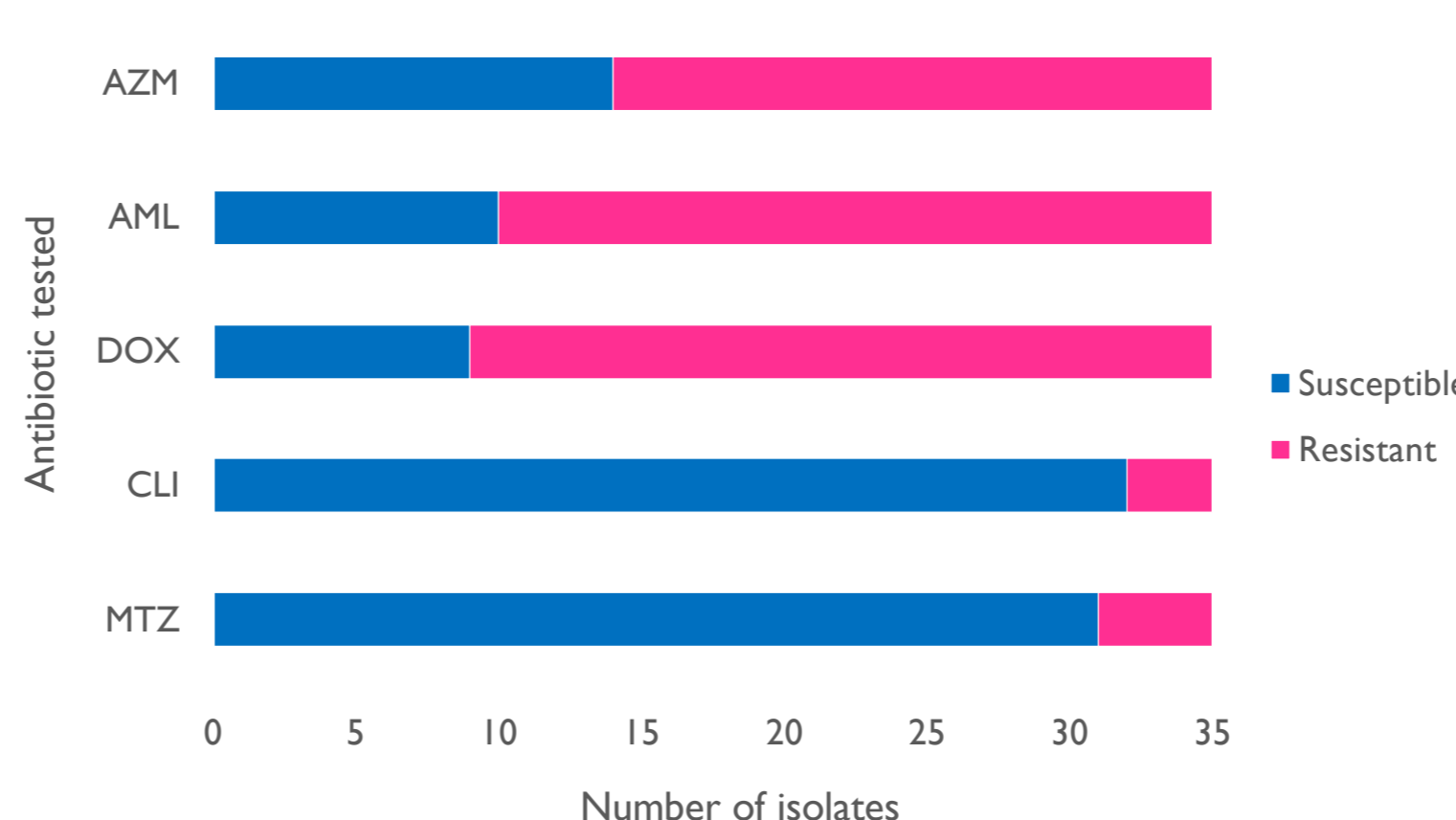


Figure 2: **Antimicrobial susceptibility of South African *Prevotella* spp. isolates to common genitourinary antibiotics, tested by E-tests (LiofilChem).** Isolates (n=37) were given AMR phenotype based on EUCAST^T classification guidelines. Tested antibiotics include SOC BV drugs, metronidazole (MTZ) and clindamycin (CLI), as well as amoxicillin (AML), azithromycin (AZM) and doxycycline (DOX).

The MTZ Resistant Strain Contains A Mobile Genetic Element With The MTZ-resistance Gene *nimK*

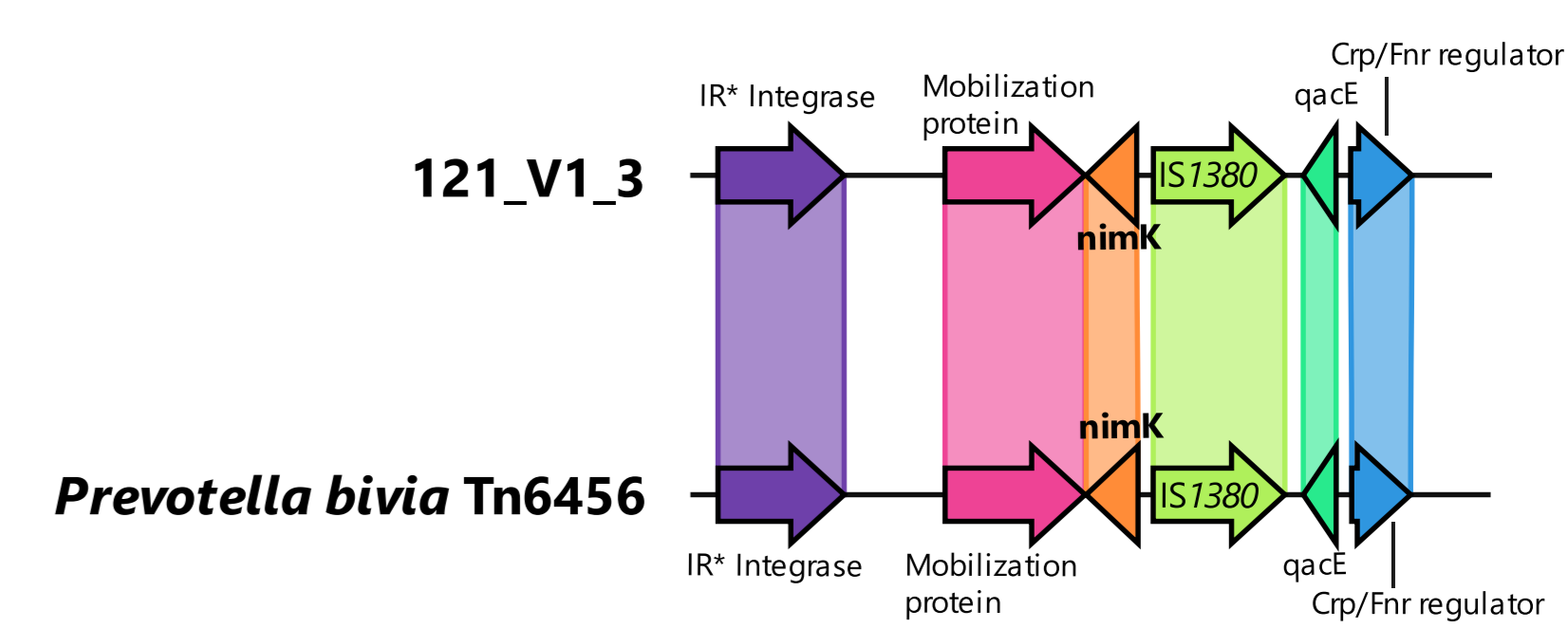


Figure 3: **Identification of a previously described *nimK* containing mobile genetic element.** This element was previously identified by Veloo (2018)^D in 3 *P. bivia* strains with MTZ resistance. The alignment to the previously published transposon was performed and visualized using Clinker. Strain 121_V1_3 (MIC for MTZ = 8ug/ml) contained this element, and no major amino acids differences were noted. The schematic shows the identified genes including repeat regions, an integrase, mobilization protein, *nimK*, an insertion sequence (IS1380), a SMR transporter (*qacE*) and a Crp/Fnr regulator.

Core Genome Alignment And Identification Of AMR Genes In Clinical And Previously Published *Prevotella* Isolates

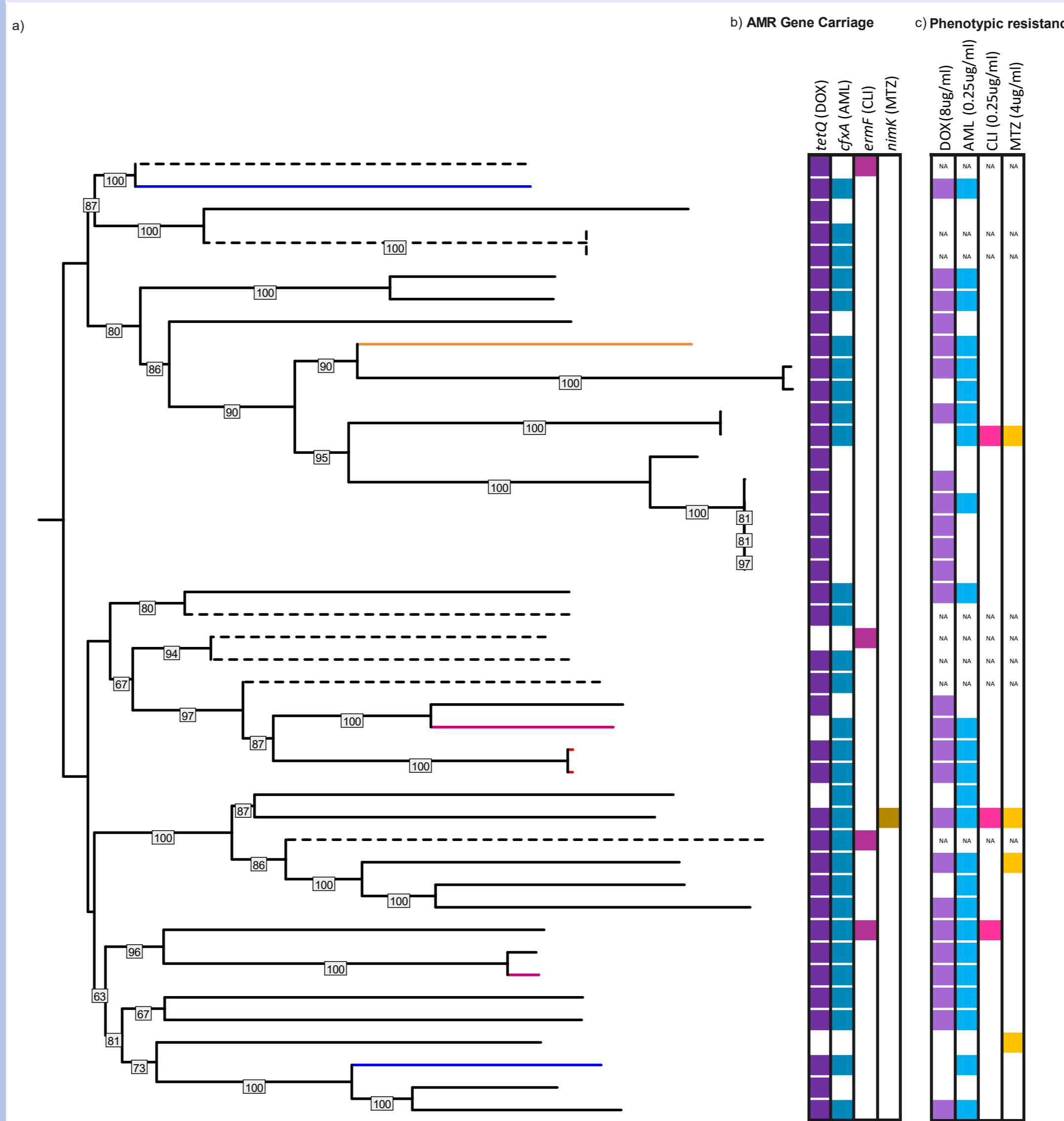


Figure 4: **Core genome alignment of *P. bivia* isolates.** a) Core genome alignment was performed with Panaroo^E and the tree was generated, with bootstrap values greater than 60 displayed on the branches. Reference *P. bivia* strains were obtained from NCBI's RefSeq^F (dotted lines). b) AMR gene presence in the genome was identified by the CARD, Argannot and Resfinder databases using ABRicate^G. The antibiotic that the gene may confer resistance to is indicated in brackets c) Strains resistant to the tested antibiotics are shown. The phenotype was assigned based on EUCAST^T breakpoint guidelines, with MIC breakpoints indicated in brackets.

DISCUSSION

- 37 *Prevotella* strains** (n=36 *P. bivia*, n=1 *Prevotella* spp.) were isolated and **fully characterised**
- Certain strains formed **significantly larger biofilms than the *P. bivia* type-strain (ATCC 29303^T)**
 - This suggests that some strains may be implicated more heavily in BV biofilms than others
- While rare, there were some clinical isolates that **were resistant to BV antibiotics**, CLI (n=3) and MTZ (n=4)
 - Additionally, the majority of isolates were resistant to **AML (71.4%), DOX (74.2%) and AZM (60%)**, antibiotics used for other genitourinary infections
- A previously described transposon, **Tn6456**, containing an **MTZ-resistance gene *nimK***, was identified in one strain
 - The *nimK* may be conferring **MTZ resistance** to this strain
 - The presence of this gene on a mobile genetic element may pose the **risk of transfer of *nimK*** to other bacteria in the female genital tract
- Addition of these isolates to databases will improve available data on *Prevotella* spp. And would **better represent South Africans in BV research**

