



# Distribution of genotypes in relation to metronidazole susceptibility patterns in *Trichomonas vaginalis* isolated from South African pregnant women

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## Abstract

Reports on metronidazole resistance of *Trichomonas vaginalis* strains have been on the increase. This study investigated the in vitro metronidazole resistance patterns in *T. vaginalis* isolates obtained from South African pregnant women and the genotypes of these isolates. This study included 362 pregnant women recruited from a hospital in Durban, South Africa. The women provided self-collected vaginal swabs for the detection of *T. vaginalis* by culture in Diamonds media. Cultured isolates were then subjected to anaerobic susceptibility assays to metronidazole. For the genotyping assays, the *actin* gene was digested by *HindIII*, *MseI*, and *RsaI*. The banding patterns obtained after digestion was used to determine the genotypes. A total of 21/362 (5.8%) pregnant women tested positive for *T. vaginalis* infection. Of the 21 *T. vaginalis* isolates tested for metronidazole susceptibility, 9.5% (2/21) had a minimum inhibitory concentration (MIC) of 4 µg/ml (resistant), 38.1% (8/21) had a MIC of 2 µg/ml (intermediate), and 52.4% (11/21) had a MIC ≤ 1 µg/ml (susceptible). The dominant genotype that was identified across the isolates was genotype G. There was no correlation between genotype harboured and metronidazole susceptibility patterns. In this study, resistance to metronidazole was observed in clinical isolates of *T. vaginalis*. This study did not find a correlation between genotype harboured and metronidazole susceptibility patterns. Despite the lack of association, our study provides data on an area of research that is currently lacking in our setting.

**Keywords** *Trichomonas vaginalis* · Pregnant women · Metronidazole susceptibility · Genotyping · RFLP

## Introduction

*Trichomonas vaginalis* is an anaerobic parasitic protozoan that causes the sexually transmitted infection (STI) trichomoniasis (Akbari and Matini 2017; Ramjee et al. 2015). In sub-Saharan Africa, about 30 million infections occur annually (Naidoo and Wand 2013). Studies that have been conducted in South African pregnant women have reported prevalence rates of 15.3% (Moodley et al. 2015) and 20.2% (Morikawa et al. 2018). Trichomoniasis has been found to be associated with various health complications including pelvic inflammatory disease (PID), significant pregnancy complications, cervical cancer

prostatitis, and infertility (Johnston and Mabey 2008; Swygard et al. 2004). Significant pregnancy complications include pre-term labour, low birth weight, and premature rupture of membranes (Cornelius et al. 2012; Glehn et al. 2017).

Metronidazole and tinidazole are the only drugs approved by the United States Food and Drug Administration for trichomoniasis (Bradic et al. 2017). The World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) treatment guidelines of trichomoniasis include metronidazole or tinidazole 2 g single dose as the recommended regimens and metronidazole 500 mg, twice a day for 7-day dose as the alternative treatment regimen. Treatment with 2 g metronidazole is recommended at any time during pregnancy (Kissinger 2015a; Kissinger 2015b). Tinidazole has not been evaluated for use during pregnancy (Kissinger 2015b; Mielczarek and Blaszkowska 2015). Metronidazole resistance has been reported in clinical isolates since 1959 (Bradic et al. 2017). Most *T. vaginalis* isolates are highly susceptible to metronidazole; however, laboratory resistance and treatment failure have been reported (Bosserman et al. 2011; Kirkcaldy et al.

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2012). Two pathways of metronidazole resistance have been suggested which are aerobic and anaerobic resistance. Aerobic (clinical) resistance is observed in the presence of oxygen in which oxygen stress response genes might be involved in this type of resistance. In aerobic resistance, nitroradical anions are re-oxidised before they can impair the trichomonad; thus, metronidazole toxicity is reduced (Bouchemal et al. 2017; Sood and Kapil 2008). Anaerobic resistance has been observed in vitro. In the anaerobic resistant strains, the hydrogenosome enzymes (PFO and ferredoxin) and cytosolic thioredoxin reductase are absent. These enzymes are important for metronidazole activation (Brdic et al. 2017). Mutations in the nitroreductase genes of *T. vaginalis* have also been implicated in metronidazole resistance (Alessio and Nyirjesy 2019).

The genetic characterisation of *T. vaginalis* isolates from various regions shows that there is significant genetic diversity in this organism (Meade and Carlton 2013), thereby impeding vaccine development. *T. vaginalis* is said to contain an actin protein which is coded by a 9-membered family, having nucleotide resemblances in 5 genes which are similar to mammalian *actin* genes. When *T. vaginalis* attaches to the surface of the host cell, it causes the parasite to transform from a tetanus form to amoebic form resulting in appendage formation, i.e. pseudopodia (Shahraki et al. 2020). Both attachment and deformation regarding the host cell can be associated with the pathogenicity of *T. vaginalis*. The *actin* gene thus plays a significant role in the parasite's pathogenicity, thereby making it the gene of choice for molecular typing techniques (Rezaeian et al. 2009).

To date, there is a lack of data on the distribution of genotypes in relation to metronidazole susceptibility patterns for pregnant women in our setting, thus the rationale for this study. This study investigated the in vitro metronidazole resistance patterns in *T. vaginalis* isolates obtained from South African pregnant women, and the genotypes of these isolates were determined by restriction digestion profiles of the *actin* gene.

## Materials and methods

### Study participants recruitment

This was a cross-sectional study which included 362 pregnant women recruited from the King Edward VIII hospital antenatal clinic in Durban, South Africa, during October 2018–March 2019. The women who were enrolled in the study were 18 years and older, willing to provide written informed consent and willing to provide a self-collected vaginal swab sample to be tested for *T. vaginalis* infection. Laboratory testing was conducted at the School of Clinical Medicine Research Laboratory at the Nelson R. Mandela School of Medicine, University of KwaZulu-Natal. All women presenting with

symptoms of vaginal discharge syndrome were treated using the syndromic approach.

### Specimen collection

Women were educated on self-collection of vaginal swabs (sterile individually wrapped Dacron swab). The collected vaginal swabs were immediately inoculated into a 15-ml sterile tube containing Diamond's TYM medium (Diamond 1957). Diamond's TYM medium was supplemented with amikacin (4 µg/ml), amphotericin B (5 µg/ml), ampicillin (1 mg/ml), chloramphenicol (1 µg/ml), ciprofloxacin (2 µg/ml), and vancomycin (5 µg/ml). The tubes were inoculated with the vaginal swabs at the clinic and then transported within 1 h of inoculation to the School of Clinical Medicine Research Laboratory, University of KwaZulu-Natal, for processing.

### Diagnosis and propagation of *T. vaginalis* clinical isolates

Upon arrival at the laboratory, the culture tubes were incubated at 37°C for 7 days. Cultures were examined daily from day 2 to 7 by wet mount microscopy. A specimen was considered positive for *T. vaginalis* infection if cells with a jerk motility were observed. A sample was considered negative if no trophozoites were observed at day 7. A sub-culture of positive samples was performed by transferring 500 µl of the culture into 5 ml fresh Diamond's TYM medium supplemented with amikacin, amphotericin B, ampicillin, chloramphenicol, ciprofloxacin, and vancomycin at 48-h intervals until non-contaminated axenic cultures were obtained. Once axenic cultures were obtained, metronidazole susceptibility assays and DNA extraction were performed.

### Metronidazole susceptibility assay

Metronidazole susceptibility was performed in 96 well flat-bottomed microtiter plates under anaerobic incubation conditions. Twofold serial dilutions of metronidazole were performed in Diamond's TYM medium. The resulting concentrations ranged from 0.25 to 16 µg/ml. *T. vaginalis* cultures were then standardised to an inoculum of  $1.5 \times 10^4$  trichomonads/well. Each *T. vaginalis* isolate inoculum was then added into each well excluding the ATCC control wells. The *T. vaginalis* ATCC 50148 strain was used as a control strain and untreated cultures of the respective isolates were used as growth controls. Plates were incubated in air-tight anaerobic jars containing Oxoid™ AnaeroGen™ 2.5L gas pack (ThermoFisher Scientific, USA) and Oxoid™ Resazurin Anaerobic indicator strip (ThermoFisher Scientific, USA) at 37°C for 48 h. *T. vaginalis* motility and growth were assessed using the inverted microscope at  $\times 400$  magnification.

*T. vaginalis* growth and motility were scored according to the scoring criteria described by Upcroft (Upcroft and Upcroft 2001). Trophozoite numbers were scored 1+ (0–10 motile parasites; not more than 20% coverage of well surface and significantly less active), 2+ (20 to 50% coverage of the well surface and some trophozoite motility), 3+ (more than 50 % coverage of the well surface, almost confluent growth with much motility), and 4+ (confluent growth with full motility) (Upcroft and Upcroft 2001). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of metronidazole in which a score of 1+ was observed after 48 h of incubation. Breakpoints suggested by Upcroft were used (Upcroft and Upcroft 2001). MIC  $\leq$  1  $\mu\text{g/ml}$  was considered susceptible, MIC = 2  $\mu\text{g/ml}$  was considered intermediate (low-level resistance), and MIC  $\geq$  4  $\mu\text{g/ml}$  was considered resistant (Upcroft and Upcroft 2001). All experiments were performed in triplicate for each *T. vaginalis* isolate.

### DNA extraction from *T. vaginalis* isolates

*T. vaginalis* DNA was extracted using the phenol-chloroform method (Shaio et al. 1997). Briefly, *T. vaginalis* cells were washed twice in phosphate-buffered saline (pH 7.4) by centrifugation at 1500 $\times$ g for 10 min. DNA was extracted from the cell pellets by adding 500  $\mu\text{l}$  of lysis buffer and incubated at 65°C for 30 min. The lysis buffer was prepared from concentrated stock solutions to obtain final concentrations of 100  $\mu\text{g}$  of proteinase K, 450 mM NaCl, 15 mM sodium citrate, and 0.2% sodium dodecyl sulphate (SDS) per ml. The extracted DNA was then purified twice by adding an equal volume of phenol-chloroform (1:1; vol/vol) and centrifuged at 1500 $\times$ g for 10 min. DNA was then purified once with chloroform only. DNA extracts were then precipitated with 2 volumes of 95% ethanol (vol/vol) and 0.1 volume of 3 M sodium acetate (pH 5.2). The DNA pellets were then washed with 70% (vol/vol) ethanol, air-dried at room temperature, and dissolved in 50  $\mu\text{l}$  of TE buffer. The concentration and purity of the extracted DNA were measured using the NanoDrop Spectrophotometer (ThermoFisher Scientific, USA).

### Confirmation of *T. vaginalis* by 18S ribosomal RNA PCR

A forward primer S1 (5'-TCCCGGATAATTGAAACGGA-3') and a reverse primer S2 (5'-GAATGTGATAGCGA AATGGG-3') were used to amplify a region of approximately 413-bp within the 650-bp repeat region. PCR was performed in a total volume of 50  $\mu\text{l}$ . The reaction mixture for each *T. vaginalis* isolate contained 16  $\mu\text{l}$  of nuclease-free PCR water, 25  $\mu\text{l}$  of the DreamTaq PCR Master Mix (ThermoFisher Scientific, United States), 2  $\mu\text{l}$  of each primer, and 5  $\mu\text{l}$  template DNA. The cycling conditions were initial denaturation at 94°C for 3 min followed by 40 cycles at 94°C for 45 s, 50°C

for 40 s, 72°C for 1 min, and a final extension at 72°C for 7 min. PCR amplification was performed in a T100 thermocycler (BioRad, USA). PCR products were analysed by electrophoresis on a 1% agarose gel in 0.5X TBE buffer at 80 V and viewed under a UV illumination (Gene Genius System).

### Detection of the *actin* genes from *T. vaginalis*

A conventional nested PCR assay was used for the amplification of the *actin* genes (outer and inner regions) using oligonucleotide primers, published by Espinosa et al. (Espinosa et al. 2001) and Khalili et al. (Khalili et al. 2017). The primers used for amplification of inner and outer *actin* genes are shown in Table 1.

### Amplification of the outer *actin* gene

The PCR amplification reactions were performed with a total volume of 25  $\mu\text{l}$ . The reaction contained 12.5  $\mu\text{l}$  DreamTaq master mix (ThermoFisher Scientific, USA), 9.5  $\mu\text{l}$  distilled water, and 0.5  $\mu\text{l}$  of each primer (reverse and forward), and 2  $\mu\text{l}$  of template DNA was used. The negative control contained 23  $\mu\text{l}$  of PCR mixture and 2  $\mu\text{l}$  of distilled water. Thereafter, the PCR tubes were placed into the thermal cycler, and the following conditions was performed, for gene amplification initial denaturation at 94°C for 5 min, thereafter 30 cycles: denaturation at 94°C for 1 min, annealing 54°C for 1 min, elongation 72°C for 1 min, and final elongation at 72°C for 5 min.

### Amplification of the inner *actin* gene by nested PCR

The nested amplification reactions were performed with a total volume of 25  $\mu\text{l}$ . The reaction contained 12.5  $\mu\text{l}$  DreamTaq master mix, 9.5  $\mu\text{l}$  distilled water, 0.5  $\mu\text{l}$  of each primer (reverse and forward), and 2  $\mu\text{l}$  of outer PCR product. The negative control contained 23  $\mu\text{l}$  of PCR mixture and 2  $\mu\text{l}$  of distilled water. Thereafter, the PCR tubes was placed into the thermal cycler, and the following conditions was performed, for gene amplification initial denaturation at 94°C for 5 min, thereafter 30 cycles: denaturation at 94°C for 1 min, annealing 45°C for 1 min, elongation 72°C for 1 min, and final elongation at 72°C for 5 min.

### Sequence confirmation of the *actin* gene

A subset of PCR positive amplicons was sequenced to confirm the presence of the gene prior to the genotyping analysis. Sanger DNA sequencing was performed on the inner *actin* PCR amplicons. Each amplicon was sequenced in both directions to cover the full-length *actin* gene. The sequencing was conducted using the BrilliantDye™ Terminator v3.1 Cycle

**Table 1** Description of primers used for the amplification of the *actin* genes in the studied population

| Primer name                 | Primer sequence              |
|-----------------------------|------------------------------|
| Outer actin forward (Tv8S)  | 5'-TCTGGAATGGCTGAAGAAGACG-3' |
| Outer actin reverse (Tv9R)  | 5'-CAGGGTACATCGTATTGGTC-3'   |
| Inner actin forward (Tv10S) | 5'-CAGACACTCGTTATCG-3'       |
| Inner actin reverse (Tv11R) | 5'-CGGTGAACGATGGATG-3'       |

Sequencing on an ABI3500XL genetic analyser. The sequencing was performed at Inqaba Biotechnical Industries (Hatfield, Pretoria, South Africa). The ABI sequencing files were edited on CHROMAS (Technelysium, Queensland, Australia). The forward and reverse sequences were aligned using the DNAMAN software (Lynnon Biosoft, CA, USA). The identity of the edited sequences was confirmed using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).

### Restriction fragment length polymorphisms (RFLP)

The genotyping of the *actin* gene was performed using the RFLP technique. Restriction enzymes, *HindII*, *MseI*, and *RsaI*, were used to generate the banding profiles. The inner *actin* amplicons were digested with the individual enzymes. The digestion mix was made up to a final volume of 20  $\mu$ l. Each reaction consisted of 0.5  $\mu$ l enzyme, 2  $\mu$ l enzyme buffer, 0.2  $\mu$ l bovine serum albumin (BSA), 7.3  $\mu$ l distilled water, and 10  $\mu$ l of the PCR amplicon. The digestion reactions were incubated for 1 h under the following temperature conditions: 37°C for all enzymes followed by heat inactivation at 65°C for 20 min for *HindII* and *MseI*. Following incubation, the digests were run on a 2% agarose gel. The enzymes banding patterns and assignment of genotypes based on a composite of the patterns was determined according to Khalili et al. (Khalili et al. 2017).

## Results

### Prevalence and metronidazole susceptibility patterns

A total of 21/362 (5.8%) pregnant women tested positive for *T. vaginalis* infection. The median interquartile range (Q1–Q3) age of the women who tested positive for *T. vaginalis* infection was 27.0 (24.0–36.0). The 413bp fragment corresponding to the *18S rRNA* gene from *T. vaginalis* was present in all 21 samples amplified and confirmed by DNA sequencing (100% identity to *Trichomonas vaginalis* G3, Accession number: XM\_001292180.1).

Anaerobic metronidazole MICs ranged from 0.25 to 4  $\mu$ g/ml, and the mean MICs  $\pm$  standard deviation was 1.63  $\pm$  0.95. Of the 21 *T. vaginalis* isolates tested for anaerobic

metronidazole susceptibility, 9.5% (2/21) had a MIC of 4  $\mu$ g/ml (resistant), 38.1% (8/21) had a MIC of 2  $\mu$ g/ml (intermediate), and 52.4% (11/21) had a MIC  $\leq$  1  $\mu$ g/ml (susceptible) (Table 2). The MIC of the *T. vaginalis* ATCC 50148 control strain was 1  $\mu$ g/ml which was within the expected range (Müller et al. 1988).

### Genotypes detected

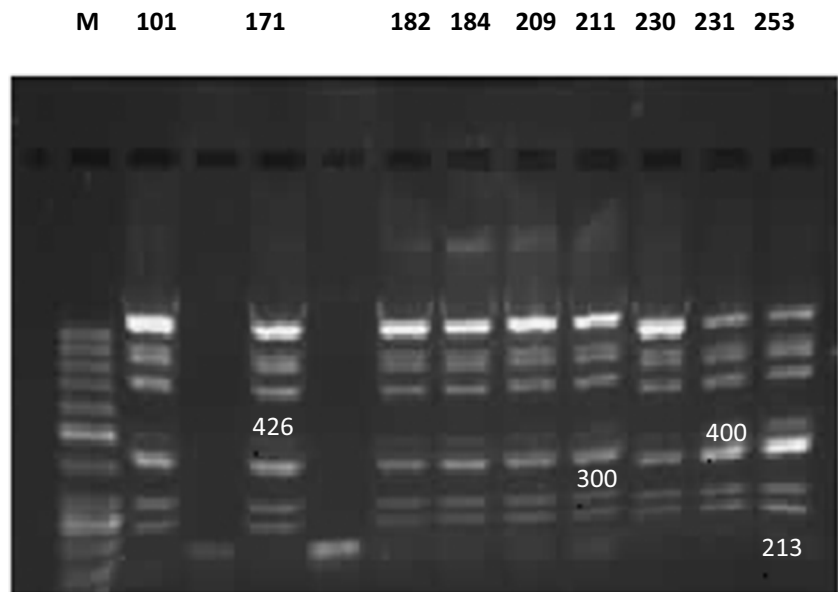
The DNA sequencing hits of the *actin* PCR amplicons showed identity (99%) to *T. vaginalis isolate 19 actin gene* (MF350343.1) and *T. vaginalis strain ATCC 30240 actin gene* (99%) (EU076579.1). Of the 21 isolates investigated in this study, 4 samples did not produce genotyping profiles and were excluded from further analysis. When digested with *HindII*, all samples produced the same banding profile and bands at positions 213bp, 300bp, 300bp, and 426bp (Fig. 1). Based on the banding profile obtained, the isolates were assigned pattern 2. Figure 2 shows the banding profile of selected isolates after digestion with *MseI*. Similar to the *HindII* digests, the same banding profile was observed for all isolates. Band sizes of 519bp and 581bp were obtained after digestion which indicated banding pattern 1 (Fig. 2). For the *RsaI* digestion, one isolate had produced a different banding profile from the other isolates. The isolate 341 had produced band sizes of 106bp, 190bp, 300bp, and 452bp. This isolate was assigned pattern 3. The remaining isolates had produced band sizes of 106bp, 250bp, and 586bp and were assigned pattern 1 (Fig. 3).

Based on the patterns generated, genotypes were assigned. Table 3 describes the assignment of the genotypes based on banding patterns. Of the 17 isolates, 16 isolates (94.1%) harboured genotype G, and the one isolate harboured genotype I. The distribution of the genotypes across the MIC profiles is shown in Table 4. According to Table 4, the I genotype

**Table 2** Distribution of anaerobic MICs obtained with metronidazole ( $n = 21$ )

| Antibiotic    | No of isolates with MIC ( $\mu$ g/ml) |     |   |   |   |   |    |
|---------------|---------------------------------------|-----|---|---|---|---|----|
|               | $\leq 0.25$                           | 0.5 | 1 | 2 | 4 | 8 | 16 |
| Metronidazole | 1                                     | -   | 9 | 9 | 2 | - | -  |

**Fig. 1** *Hind*II RFLP pattern of the digested *actin* gene amplicon resolved on a 2% agarose gel. M: O'GeneRuler 50bp DNA Ladder (ThermoFisher Scientific) and banding profiles of the *T. vaginalis* isolates. Size fragments of 213bp, 300bp, 400bp, and 426bp were observed for the respective isolates



was carried by an intermediate isolate, and the G genotype was distributed across susceptible, intermediate, and resistant isolates. There was no correlation between genotype harboured and metronidazole susceptibility patterns.

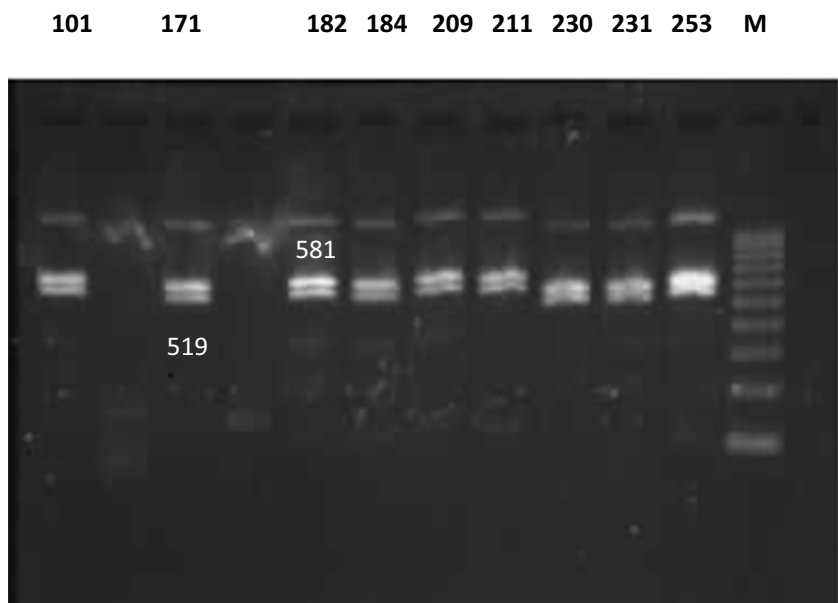
## Discussion

Trichomoniasis is the most common STI with an estimated annual incidence of 276.4 million cases globally (Akbari and Matini 2017; Bouchemal et al. 2017; Gatti et al. 2017; Ramjee et al. 2015). To the best of our knowledge, this study was the first to investigate the in vitro metronidazole resistance patterns in *T. vaginalis* isolates obtained from South African

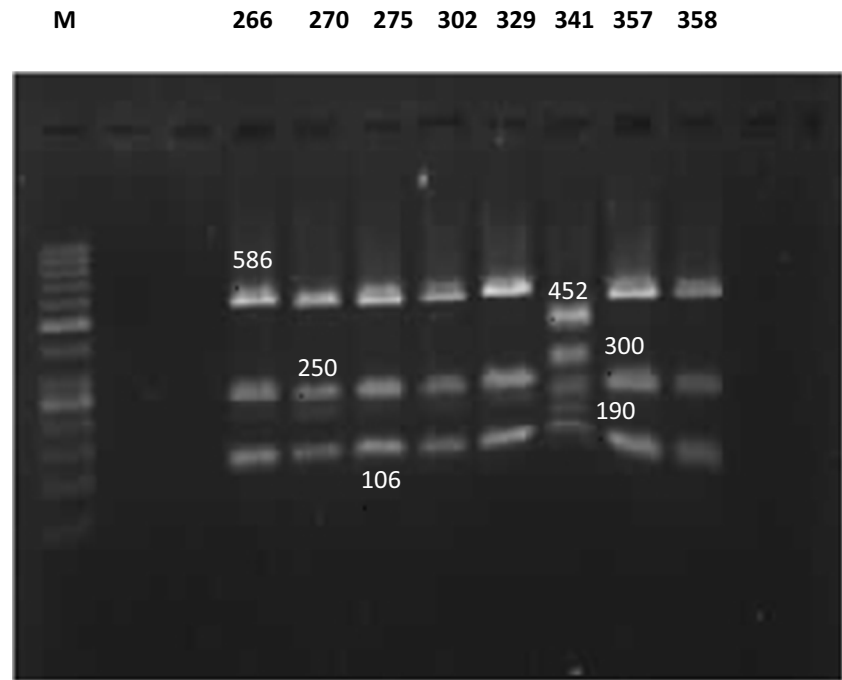
pregnant women and the correlation between the genotypes and drug susceptibility patterns.

Reports on metronidazole resistance of *T. vaginalis* strains have been increasing (Ramjee et al. 2015). Even though the metronidazole cure rates are high, clinical treatment failure is challenging (Petrin et al. 1998). Most *T. vaginalis* isolates are highly susceptible to metronidazole; however, laboratory resistance and treatment failure have been reported (Bosserman et al. 2011; Kirkcaldy et al. 2012). Approximately 2.5 to 10% of *T. vaginalis* isolates are resistant to metronidazole treatment (Kusdian and Gould 2014). In this study, 9.5% of the isolates produced resistant profiles towards metronidazole with the majority of the isolates being susceptible. In South Africa, metronidazole resistance was reported in 6% of *T. vaginalis*

**Fig. 2** *Mse*I RFLP pattern of the digested *actin* gene amplicon resolved on a 2% agarose gel. M: 100bp DNA ladder (ThermoFisher Scientific) and banding profiles of the *T. vaginalis* isolates. Fragment sizes of 519bp and 581bp were observed for the isolates



**Fig. 3** *RsaI* RFLP pattern of the digested *actin* gene amplicon resolved on a 2% agarose gel. M: 100bp DNA ladder (ThermoFisher Scientific) and banding profiles of the *T. vaginalis* isolates. Fragment sizes of 106bp, 190bp, 250bp, 300bp, 452bp, and 586bp were observed for the isolates



**Table 3** Genotypes assigned to *T. vaginalis* isolates based on *HindII*, *MseI*, and *RsaI* banding patterns ( $n=17$ )

| Isolate | <i>HindII</i><br>Band sizes (bp) | Pattern | <i>MseI</i><br>Band sizes<br>(bp) | Pattern | <i>RsaI</i><br>Band sizes (bp) | Pattern | Genotype |
|---------|----------------------------------|---------|-----------------------------------|---------|--------------------------------|---------|----------|
| TV101   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV171   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV182   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV184   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV209   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV211   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV230   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV231   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV253   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV266   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV270   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV275   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV302   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV329   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV341   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 190, 300,<br>452          | 3       | I        |
| TV357   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |
| TV358   | 213, 300, 400,<br>426            | 2       | 519, 581                          | 1       | 106, 250, 586                  | 1       | G        |

**Table 4** Distribution of genotypes based on metronidazole susceptibility patterns

| Isolate | MIC ( $\mu\text{g/ml}$ ) | Susceptibility profile | Genotype |
|---------|--------------------------|------------------------|----------|
| TV101   | 2                        | Intermediate           | G        |
| TV171   | 1                        | Susceptible            | G        |
| TV182   | 2                        | Intermediate           | G        |
| TV184   | 1                        | Susceptible            | G        |
| TV209   | 2                        | Intermediate           | G        |
| TV211   | 2                        | Intermediate           | G        |
| TV230   | 2                        | Intermediate           | G        |
| TV231   | 1                        | Susceptible            | G        |
| TV233   | 2                        | Intermediate           | G        |
| TV253   | 4                        | Resistant              | G        |
| TV266   | 1                        | Susceptible            | G        |
| TV270   | 4                        | Resistant              | G        |
| TV275   | 1                        | Susceptible            | G        |
| TV302   | 1                        | Susceptible            | G        |
| TV329   | 1                        | Susceptible            | G        |
| TV341   | 2                        | Intermediate           | I        |
| TV357   | 1                        | Susceptible            | G        |
| TV358   | <0.25                    | Susceptible            | G        |

strains isolated from women attending an antiretroviral clinic (Rukasha et al. 2013). A study conducted by Abdel-Magied et al. (Abdel-Magied et al. 2017) reported a prevalence of 8.2% for metronidazole resistance in non-pregnant women from Egypt. This study builds on the data by Mabaso et al. (2020) who have reported on metronidazole resistance in pregnant women, and now the current study shows the association between resistance profiles and prevalent genotypes.

The genetic characterisation of *T. vaginalis* isolates from various regions show that there is significant genetic diversity in this organism (Meade and Carlton 2013). In this study, genotype G was shown to be the most prevalent in the isolates investigated. Only a single isolate carried genotype I. Our findings of the dominant genotype G have also been reported for other populations. A study conducted in Zambia on female sex workers revealed the presence of eight different genotypes based on the *actin* gene, the most common genotype was G (Crucitti et al. 2008). In another study conducted in Ndola, Zambia, involving adolescent girls, pregnant women as well as sex workers, Crucitti et al. (2010) identified nine different genotypes, with genotype G being the most frequent across all three study groups. A study conducted by Momeni et al. (Momeni et al. 2015) identified five different genotypes with genotype G being the most prevalent in a population of Iranian men and women.

In this study, based on the high dominance of genotype G (94.1%) in the isolates tested, there was no observed correlation between genotypes and metronidazole resistance patterns. In addition, there is a lack of data on the correlation of drug susceptibility patterns in relation to genotyping based on the *actin* gene.

Our study now provides data on this correlation. Past studies have found significant associations between drug susceptibility patterns and genotypes based on other gene targets. A study conducted by Abdel-Magied et al. (Abdel-Magied et al. 2017) reported on a correlation between metronidazole susceptibility patterns and genotypes of *T. vaginalis* based on restriction analysis of the *ITS1* gene. In another study by Conrad et al. (2012) where the authors used multilocus sequence typing and microsatellite genotyping of *T. vaginalis*, a correlation between genotypes and metronidazole susceptibility patterns was observed using microsatellite genotyping. A future research direction for our research group would be to determine genotypes based on microsatellite genotyping and then attempt to link the data to patterns of susceptibility/resistance in order to determine correlations.

This study was limited in terms of due to the cross-sectional nature of this study, the women were not followed up in order to determine test of cure, and therefore, the clinical response could not be matched with in vitro susceptibility. Another limitation is that women were recruited from one hospital; however, the King Edward VIII Hospital is a tertiary hospital, and it hosts a wider population of Durban. Future collaboration studies are planned which will include *T. vaginalis* clinical isolates from other Districts in South Africa.

## Conclusion

In this study, resistance to metronidazole was observed in clinical isolates of *T. vaginalis*. This was the first study to provide data on metronidazole susceptibility/resistance patterns in pregnant women from South Africa. Genotype G was shown to be the most dominant genotype in the study population. However, our study did not find a correlation between genotype harboured and metronidazole susceptibility/resistance patterns. Despite the lack of association, our study provides data on an area of research that is currently lacking in our setting.

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**Availability of data and material** Not applicable

**Code availability** Not applicable

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## Declarations

**Ethics approval** Ethics approval for this study was obtained from the Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal (BE296/18).

**Consent to participate** Written informed consent was obtained from the study participants.

**Consent to publish** De-identified data has been obtained from our local Institutional Review Board (IRB).

**Conflict of interest** The authors declare no competing interests.

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