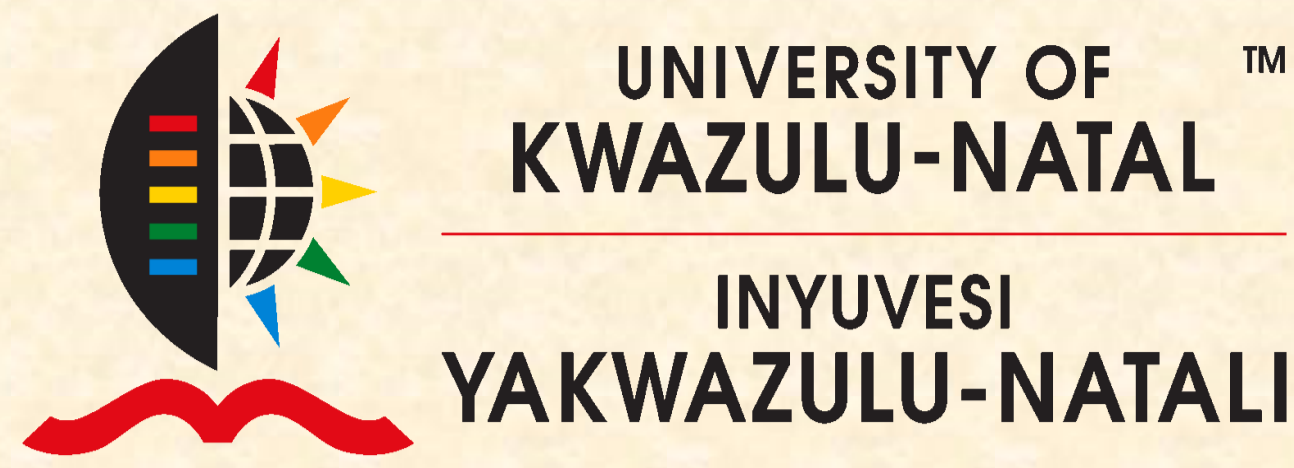


Investigating the antimicrobial resistance patterns in clinical isolates of *Gardnerella vaginalis*



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Introduction

A healthy vaginal microbiome is dominated by *Lactobacilli* species and has a low pH. When there is a fluctuation in pH, infections arise. Bacterial vaginosis (BV) has emerged as a global health threat which affects women of reproductive age. It causes abnormal discharge and is characterised by an unpleasant odour due to the decrease in the number of *Lactobacilli* accompanied by the overgrowth of anaerobic bacteria. Untreated cases of BV may lead to preterm delivery and increases the risk of contracting other sexually transmitted infections such as HIV, herpes simplex type 2 infection and gonorrhoea.

Treatment of BV includes the use of broad-spectrum antibiotics such as metronidazole and clindamycin.

Many studies have proven that one of the main microorganisms that plays a crucial role in BV is *Gardnerella vaginalis*. *G. vaginalis* is a facultative, anaerobic, non-motile and non-spore forming bacteria. *G. vaginalis* can be detected by molecular techniques such as the Polymerase Chain Reaction (PCR). The *16S ribosomal RNA* PCR and clade PCR has been used for the genotyping and identification of *G. vaginalis* via the amplification of specific genes.

In this study, pure isolates of *G. vaginalis* obtained from pregnant women were genotyped and assigned into specific clades

In this study, AMR patterns to various antibiotics were determined. and the link between AMR patterns and *G. vaginalis* clades were also described in this study.

Methods

Study Design & Sample size

Total of 150 pregnant women attending care at King Edward VIII hospital, South Africa. Two self-collected swabs, data on socio-demographics and clinical history were obtained. One swab was placed into Amie's transport media and other was smeared onto microscopic slide.

Diagnosis of BV status

Gram-staining -> Nugent scoring

Confirmatory assays

Gram staining, amplification of *16S rRNA* gene using specific primers, catalase and oxidase tests.

DNA isolation

Was performed at room temperature. Culture colonies were placed into 2ml BHI broth. Pellets were used for extraction using the Quick DNA™ Miniprep Kit.

Antimicrobial susceptibility testing

Performed using the Sensititre plate method by ThermoFisher Scientific. Colonies were emulsified in 5ml Mueller Hinton Broth -> 100µL transferred into 11ml Brucella Broth-> 100µl was transferred into each well.

Results

Out of the 150 vaginal swabs, 17 isolates of *G. vaginalis* were successfully isolated from primary swabs. All isolates were catalase and oxidase negative.

Out of the 17 isolates, 5 isolates were BV positive, 2 were BV negative and 9 were BV intermediate.

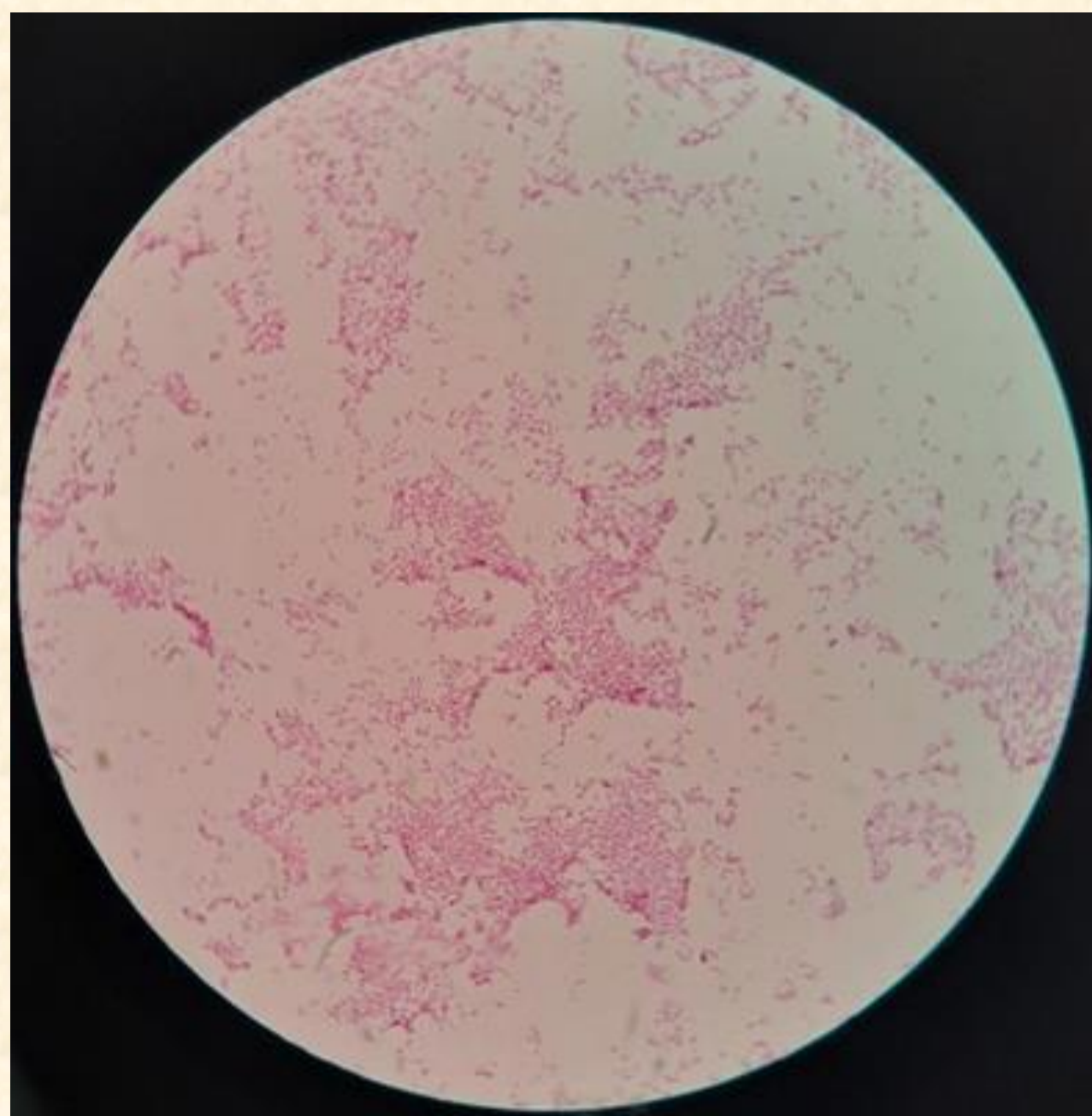


Figure 1: Gram-staining showing *G. vaginalis* under the oil immersion lens

Table 2: BV status in correlation to metronidazole susceptibility and clinical symptom

Isolate	BV status	Susceptibility profile	Clinical symptom (Abnormal discharge)
ATCC	Positive	Susceptible	-
35	Positive	Resistant	Absent
48	Negative	Susceptible	Absent
49	Negative	Resistant	Present
55	Intermediate	Resistant	Present
60	Positive	Susceptible	Present
61	Intermediate	Susceptible	Absent
62	Positive	Susceptible	Present
63	Positive	Susceptible	Absent
109	Intermediate	Resistant	Present
113	Intermediate	Susceptible	Present
125	Intermediate	Resistant	Present
129	Intermediate	Susceptible	Present
137	Intermediate	Susceptible	Absent
138	Positive	Resistant	Present

Table 3: Metronidazole susceptibility in relation to *G. vaginalis* clades

Isolate	Susceptibility profile	Clade
35	Resistant	1
48	Susceptible	1, 4
49	Resistant	1
55	Resistant	1
60	Susceptible	1, 4
61	Susceptible	1, 2, 4
62	Susceptible	1, 2, 4
63	Susceptible	1, 2
109	Resistant	1, 4
113	Susceptible	1, 2
125	Resistant	1
129	Susceptible	1, 4
137	Susceptible	1, 2, 4
138	Resistant	1

- The frequencies for each clade were as follows; 100% for clade 1, 37.5% for clade 2 and 43.75% for clade 4. Thus the most common clade being clade 1, followed by clade 4, then clade 2.
- Multiple clades were found in 62.5% of women. Out of the BV positive women, 40% of women harboured *G. vaginalis* strains that only fell into one clade (clade 1), 20% of women harboured *G. vaginalis* strains that fell into clades 1 and 4, 20% of women harboured *G. vaginalis* strains that fell into clades 1 and 2, and 20% of women harboured *G. vaginalis* strains that fell into clades 1,2 and 4.
- 60% of women were susceptible to metronidazole.
- Within clade 1, the frequency of metronidazole resistance was 42.85% . In clade 2, metronidazole resistance was 0%. Within clade 4, the frequency of metronidazole resistance was 7.14%. Isolates belonging to clade 1 showed the highest percentage of metronidazole resistance, followed by clade 4 then clade 2.

Discussion and Conclusion

In our study, the antimicrobial resistance patterns were observed. Our study's findings suggest that there is a correlation between clade 1 and metronidazole resistance. This can be due to *G. vaginalis* strains belonging to clade 1 possess a higher abundance of resistance genes. However, to confirm this, isolates need to be observed at genomic level. There is also a correlation between BV intermediate women and metronidazole resistance. Since metronidazole is the only approved antimicrobial used on pregnant women in South Africa, if infection has to progress to BV positive status due to an overgrowth of *G. vaginalis*, the individuals will not receive effective treatment due to resistant *G. vaginalis* strains.

Approaches to approve the administration of clindamycin and vancomycin should be taken into effect as there was no observed resistance to these antimicrobials.

Figure 2: *16S rRNA* gene amplification plot

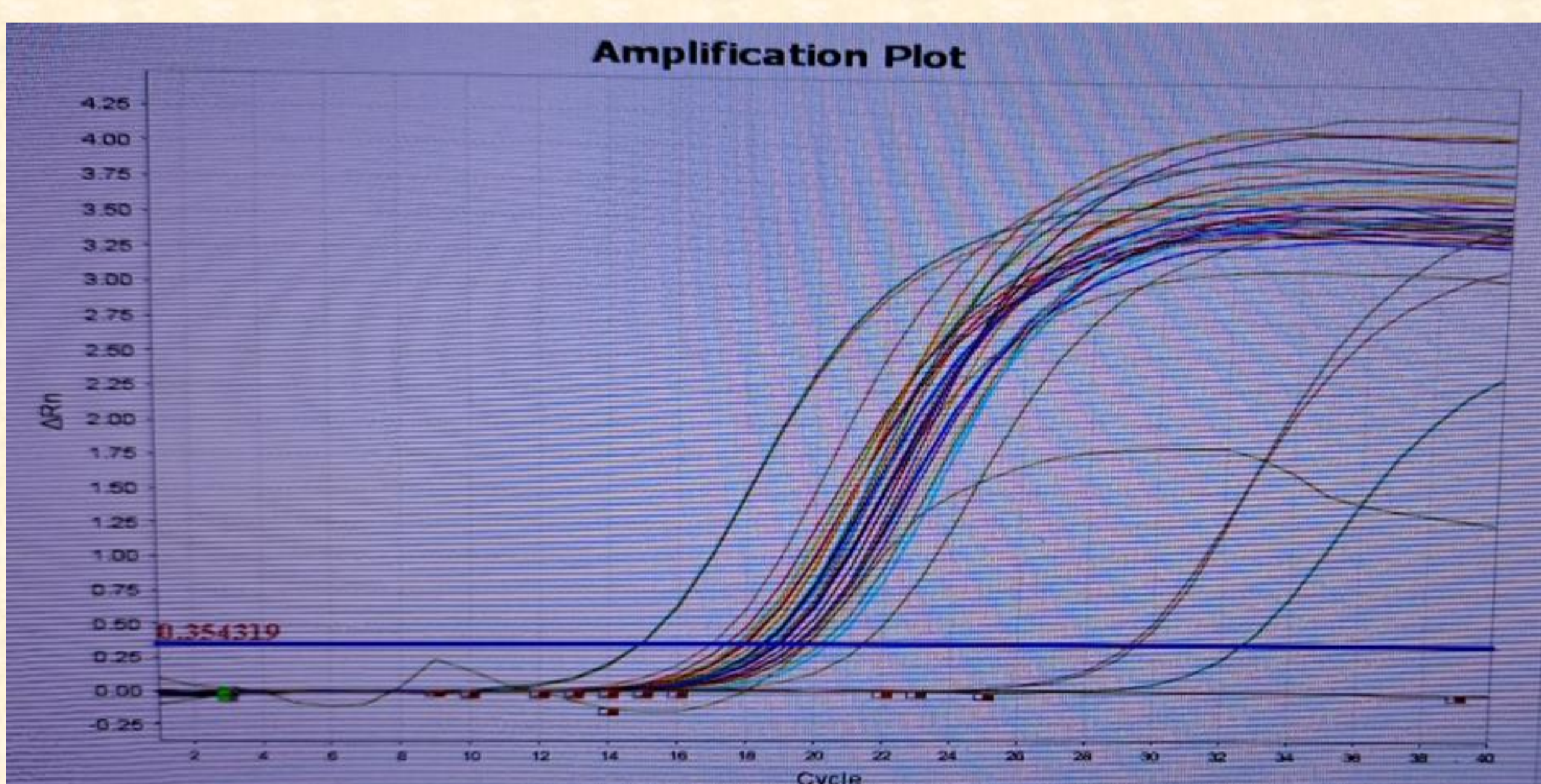


Table 1: The BV status of each isolate in correlation with the assigned clade

Isolate	BV status	Clade
35	Positive	1
48	Negative	1, 4
49	Negative	1
54	Intermediate	1
55	Intermediate	1
60	Positive	1, 4
61	Intermediate	1, 2, 4
62	Positive	1, 2, 4
63	Positive	1, 2
109	Intermediate	1, 4
113	Intermediate	1, 2
114	Intermediate	1, 2
125	Intermediate	1
129	Intermediate	1, 4
137	Intermediate	1, 2, 4
138	Positive	1