

Original Research Article

IN VITRO COMPARISON OF METRONIDAZOLE AND TINIDAZOLE ACTIVITY AGAINST TRICHOMONAS VAGINALIS STRAINS IN MAIDUGURI, NIGERIA

ABSTRACT

Metronidazole and tinidazole are the 5-nitroimidazole derivatives that are main antitrichomonal agents of choice for treatment of trichomoniasis. Treatment failure with metronidazole has been reported since 1960s and recently drug resistance is now on the rise in the world. This study aimed at ascertaining the in vitro susceptibility of *Trichomonas vaginalis* isolates to metronidazole and tinidazole from internally displaced women in Maiduguri, Nigeria. We recorded 53 *T. vaginalis* strains for metronidazole with minimum inhibitory concentration (MIC) range from 0.4 - 25 $\mu\text{g/mL}$ and low-level resistance of isolates to metronidazole observed 3.8% at 25.0 $\mu\text{g/mL}$ with minimum latent concentration (MLC) 50.0 $\mu\text{g/mL}$, while 49 *T.*

vaginalis strains were observed on MIC range from 0.4 $\mu\text{g/mL}$ to 12.5 $\mu\text{g/mL}$ indicates that all strain isolates were susceptible to tinidazole at above 12.5 $\mu\text{g/mL}$ MLC. This study showed in vitro low-level resistance to metronidazole in a few *T. vaginalis* strains, while all the tested isolates were sensitive to tinidazole. (Authors did not consider materials and methodology, results and conclusion).

(Authors should state statistical package used for sample analysis)

Keywords:-In vitro, *Trichomonas vaginalis*, Metronidazole and Tinidazole, Nigeria

1.0 INTRODUCTION

Trichomonas vaginalis is a flagellated unicellular, anaerobic protozoan with four free flagella and one free flagellum attached to the undulated membrane, it is the aetiologic agent of the disease trichomoniasis that is commonly non-viral sexually transmitted infection and suspected of increased risk of infection with other microbes considerably HIV, cervical neoplasia and adverse pregnancy outcomes^[1]. Trichomoniasis spread predominantly through unprotected sexual intercourse with an infected partner or via the fingers after masturbation^[2]. *Trichomonas* infection has been associated with discomfort, psychosocial distress, pelvic

inflammatory disease, post abortion infection, post-caesarean, preterm birth, low birth weight infants and preterm labour untreated infection can persist up to 5 years^[3]. Other complications caused by the organism include pelvic inflammatory disease and tubal infertility, vaginitis and cervicitis^[4]. The patient clinically presents with profuse creamy-greenish-yellowish vaginal discharge and frothy, malodour, dysuria, itching or irritation etc. In some women, the infection results in burning sensation when urinating, pain during sexual intercourse or abdominal discomfort^[5].

The effective drugs against trichomoniasis are the 5-nitroimidazoles, specifically metronidazole and tinidazole^[6]. Metronidazole is for many years the principal drug used to treat trichomoniasis^[7]. Most isolates of *Trichomonas vaginalis* are highly susceptible to metronidazole but resistance has been reported^[8,9]. In vitro susceptibility of *Trichomonas vaginalis* shows that some strains tend to have resistance to some antimicrobial agents such as metronidazole and tinidazole. This may be due to non-adherence to treatment, poor absorption of the drug and its inability to reach the site of infection^[10, 1]. Cross-resistance between different nitroimidazoles has been reported and is consistent with earlier studies^[11]. Metronidazole is a prodrug, which need to be activated by enzymes before it can act on the desired target^[12].

2.0 MATERIALS AND METHODS

2.1 *Trichomonas vaginalis* Isolates and Culture

High vaginal swab samples were collected from female internally displaced persons in Maiduguri Nigeria with/without clinical symptoms from July to November 2016. High vaginal swab samples were carefully and aseptically obtained from the posterior fornix of the vagina using a well labelled, sterile, non-abrasive high vaginal swab stick from each subject by a qualified research assistant. The tips of the other swab sticks were immediately cut and inoculated in OXOID *Trichomonas* medium supplemented with horse serum that was inactivated at 56°C for 30 minutes. The pH of 6.0 was obtained using diluted hydrochloric acid (HCL) and added to the medium after cooling to 50°C. Thereafter, 80mls of horse serum for enrichment, 10mls of Oxoid penicillin-streptomycin for suppressing bacterial growth and 10 ml of Nystatin Solution for fungi growth suppression were added to 1000 ml of the medium. The cultures were viewed under microscope to observe the motile trichomonas trophozoites. (Procedure not referenced and ethical approval was not considered).

2.2 Inoculum Standardization and Preparation

All isolates of *Trichomonas vaginalis* were inoculated in duplicate in OXOID Trichomonas medium and incubated at 37°C in aerobic condition for 48 hours. For the inoculum preparation, 500 µl of *Trichomonas vaginalis* culture was added to 5 ml of antibiotic-free OXOID Trichomonas medium and incubated aerobically at 37°C. After 48 hours, the number of viable trophozoites was counted after 1:2 dilution of the cell suspension with 0.4% trypan blue solution. Trypan blue differentiates between dead and viable cells. Viable cells appear colourless and dead cells stain blue. Approximately 10 µl of this dilution were added to one of the chambers of the haemocytometer. The cells were viewed under a microscope at 100x magnification. The full grid on the haemocytometer contains a total of 9 squares, each of which is 1 mm² and were counted and calculated.

2.3 Drug Susceptibility Assay

In vitro drug susceptibility testing was conducted in accordance with Meingassner method modified by the CDC^[13,25]. Metronidazole and Tinidazole powder (Sigma Aldrich USA) was dissolved in sterile distilled water and stored at 4 °C. Serial twofold drug dilutions, ranging from 200 µg/ml to 0.4 µg/ml were prepared. The anaerobic susceptibility assays for the trichomonad trophozoites cells were 1 × 10⁵ per 10 µg/ml. Eight polypropylene conical screw-cap tubes were used to prepare the dilution series for both metronidazole and tinidazole. A volume of 9 ml of antibiotic-free medium was added to the first tube, while 5 ml of the same medium was dispensed to each of the remaining 7 tubes. One millilitre of antibiotic pre-diluted stock solution was then dispensed aseptically into the first tube and mixed thoroughly. Two-fold serial dilutions were performed by transferring 5 ml from the first to the last tubes. The OXOID trichomonas antibiotic free medium was used as growth control. An amount of 100 µl of the standardised trichomonad suspension was added to 5 ml of each of the tubes. The tubes were incubated at 37°C in 5% CO₂ for 48 to 72 hours in the incubator.

2.4 Determination of End Points

After 48 hours of incubation, the contents of each tube were mixed by inversion. An aliquot was removed aseptically and viewed microscopically under x100 objective to determine if the cells showed motility (Consider sentence; x100 can not be use for motility visualization). The MLC was done by comparing the MIC with the control to determine the highest dilution of the drug that had parasite growth-inhibitory effect, considered as MLC^[14]. All susceptibility tests of each isolate were performed two times at least compared with control.

3.0 RESULTS

Table 1 showed the in-vitro susceptibility of *Trichomonas vaginalis* strains from internally displaced women in Maiduguri, Nigeria to Metronidazole and Tinidazole. The minimum inhibitory concentration for tinidazole ranged from $< 0.4\mu\text{g/mL}$ to $12.5\mu\text{g/mL}$ using 49 *Trichomonas vaginalis* strains and MLC $\geq 25\mu\text{g/mL}$ was observed. In the same manner 53 strains of *T. vaginalis* had MIC of $0.4 - 25\mu\text{g/mL}$ to metronidazole with the highest inhibitory concentration of $\geq 50.0\mu\text{g/mL}$. Low-level resistance of strains to metronidazole recorded 3.8% at $25.0\mu\text{g/mL}$.

Table 1: In-vitro susceptibility of *Trichomonas vaginalis* strains from Maiduguri, Nigeria to Metronidazole and Tinidazole

M.I.C ($\mu\text{g/mL}$)	Tinidazole		Metronidazole	
	Number of strains	% Sensitive	Number of strains	% Sensitive
0.4	14	28.6	12	22.6
0.8	12	24.5	15	28.3
1.6	9	18.4	10	18.9
3.1	7	14.3	6	11.3
6.3	4	8.1	5	9.4
12.5	3	6.1	3	5.7
25.0	0	0.0	2	3.8
50.0	0	0.0	0	0.0
100	0	0.0	0	0.0
Total	49	100	53	100

Chi-Square (χ^2) = 25.895, $p = .001$

4.0 DISCUSSION

Isolates were assayed for metronidazole and tinidazole susceptibility under anaerobic conditions, according to the method developed by Meingassner and Thurne using serial dilutions of drug concentrations from 0.4 to $100\mu\text{g/mL}$. The minimum lethal concentration (MLC) was the lowest dilution at which no motile trichomonads could be observed from an isolate assay. The strain isolates showed a relative resistance to metronidazole, all isolates were susceptible to tinidazole at less than $25\mu\text{g/mL}$ MLCs. This study showed more

susceptibility of tinidazole than metronidazole, this supports the idea that tinidazole should be prescribed for *Trichomonas vaginalis* infected persons. The results showed a statistical significance in the minimum inhibitory concentration of tinidazole and metronidazole at (p value < 0.05) critical level. This finding is consistent with results of previous studies which showed that tinidazole had better in vitro activity than metronidazole at similar molar concentrations^[15]. Clinical resistance and treatment failure have occurred with *Trichomonas vaginalis* infections for which MLCs of nitroimidazoles to the parasite were as low as 12.5 µg/mL, and treatment success has occurred in infections with *Trichomonas vaginalis* infections for which MLCs of nitroimidazoles to the parasite were 100–200 µg/mL^[16]. According to Robert *et al.*, the susceptibility to metronidazole and tinidazole are defined as MLC >25 µg/mL, low-level resistance as MLC 50–100 µg/mL, moderate-level resistance as MLC 200 µg/mL, and high level resistance as MLC >400 µg/mL^[24]. Although metronidazole has been used to treat *Trichomonas vaginalis* infections for ≈40 years, we found a low prevalence of in vitro metronidazole resistance. MLCs of tinidazole were lower than MLCs of metronidazole, and we did not detect tinidazole resistance. Krashin *et al.* did not detect tinidazole resistance^[17] detected one strain (0.6%) that exhibited low-level tinidazole resistance (MLC 50 µg/mL) among the 178 strains tested^[13]. Among 91 strains collected in Spain during 1995 and 1999, 2.2% exhibited low-level resistance to metronidazole^[18]. Mohammad *et al.*^[19] reported an anaerobic MIC of resistant isolate at 3.2 µg/ml in an in vitro susceptibility of Iranian isolates of *T. vaginalis* to metronidazole. The prevalence of in vitro metronidazole and tinidazole resistance is consistent with previously published US estimates. Three studies conducted in the southeastern United States among women attending STD or Gynecology clinics from 1997 through 2005 found a metronidazole- resistance prevalence of 2.4%–9.5%^[13,17,23]. Ikeh and Bello reported > 2.0 µg/mL MIC emergence of metronidazole resistant strains in Jos, Nigeria^[10]. A small study conducted among women from Papua New Guinea found 21 (91%) of 23 studied isolates had MLCs of metronidazole of >50 µg/mL, including 4 (17%) with MLCs of 200 µg/mL^[20]. A recent evaluation of the utility of susceptibility testing in women for whom clinical treatment has failed found that treatment recommendations based on susceptibility results may have a beneficial role in informing the clinical management of some women with persistent infection^[21]. Although tinidazole and metronidazole are the only nitroimidazoles available in the United States, ornidazole, tenonitrazole, and nimorazole are available in Europe and could be alternatives to metronidazole.

These agents are of the same drug class as metronidazole, however, and the emergence of clinically notable nitroimidazole resistance would be expected to adversely influence the treatment effectiveness of each of these agents^[22]. The problem of assessing *Trichomonas vaginalis* resistance to metronidazole is compounded by the lack of universally recognized breakpoints for clinical resistance. Many different methods, reporting on either MICs or minimum lethal concentrations (MLCs), are used to test for drug susceptibility. Furthermore, it is not possible to define accurate threshold values for resistant and sensitive strains without correlating outcome with pharmacokinetics, reinfection and MIC and/or MLC.

5.0 CONCLUSION

It was concluded based on the findings that tinidazole, been the most neglected drug for treatment of trichomoniasis, is required when other agents of treatment failure, especially poor drug compliance and reinfection are excluded. This study detected low metronidazole resistance IDPs *T. vaginalis* isolates.

(Authors should considered correcting grammatical errors)

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