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In vitro Comparison of Metronidazole and Tinidazole Activity against *Trichomonas vaginalis* Strains in Maiduguri, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author HSH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IEI managed the analyses of the study. Author OVO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the *in vitro* comparison of metronidazole and tinidazole activity against *Trichomonas vaginalis* strains from internally displaced women in Maiduguri, Nigeria. Materials and Methodology: high vaginal swab samples were collected from (41) *T. vaginalis* positive women who consented for the study was cultured and isolated. The results were analyzed using SPSS statistics version 20.0. Results recorded (53) *T. vaginalis* strains for metronidazole with minimum inhibitory concentration (MIC) range from 0.4 - 25 µg/ml and low-level resistance strains to metronidazole observed 3.8% at 25.0 µg/ml with minimum latent concentration (MLC) ≥ 50.0 µg/ml. Forty-nine (49) *T. vaginalis* strains were observed on the MIC range from 0.4 µg/ml to 12.5 µg/ml, indicating that all strain isolates were susceptible to tinidazole at above 12.5 µg/ml MLC. This study showed *in vitro* low resistance to metronidazole in a few *T. vaginalis* strains, while all the tested isolates were sensitive to tinidazole.

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1. INTRODUCTION

Trichomons vaginalis is a flagellated unicellular, anaerobic protozoan with four free flagella and one flagellum attached to the undulated membrane, it is the aetiologic agent of the disease trichomoniasis that spread predominantly through unprotected sexual intercourse with an infected partner or via the fingers after masturbation and suspected to increased risk of infection with other microbes considerably HIV, [1,2]. *Trichomonas* infection has been associated with discomfort, psychosocial distress, pelvic inflammatory disease (PID), post abortion infection, post-caesarean, preterm birth, low birth weight infants and preterm labour untreated infection can persist up to 5 years [3]. In males, it is usually found in the urethra, prostate or epididymis causing urethritis and prostatitis [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].

World Health Organization (WHO), reports170 million new infections occur annually throughout the world [6] and it also estimated 276.4 million new cases of trichomoniasis occurred globally in 2008 [7]. Prevalence of 15% was reported from four villages of Ekwulumili Community Anambra State, Southeastern Nigeria [8], also 10.99% prevalence of *Trichomonas vaginalis* infection among women attending antenatal in three hospitals in Maiduguri was recorded [9].

The effective drugs against trichomoniasis are the 5-nitroimidazoles, specifically metronidazole and tinidazole [10]. Most isolates of *T. vaginalis* are highly susceptible to metronidazole but resistance has been reported [11,12]. *In vitro* susceptibility of *T. vaginalis* shows that some strains tend to have resistance to some antimicrobial agents such as metronidazole and tinidazole. Cross-resistance between different nitroimidazoles has been reported and is consistent with earlier studies [13].

2. MATERIALS AND METHODS

2.1 Ethical Consideration

Ethical approval was obtained from state Ministry of Health Maiduguri, Borno State and permits were issued from Headquarters 7 Division Nigeria Army Maimalari Cantonment, Maiduguri, National State Emergencies (NEMA) and State Emergency Management Agencies (SEMA). Informed consents were sought for from all the subjects that were willing to participate for the study.

2.2 *Trichomonas vaginalis* Isolates and Culture

High vaginal swab samples were collected in Maiduguri Nigeria from female internally displaced persons from July to November 2016. High vaginal swab samples were carefully and aseptically obtained from the posterior fornix of the vagina using a well labeled, 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℃ for 30 minutes. The pH of 6.0 was obtained using diluted hydrochloric acid (HCl) and added to the medium after cooling to 50℃. Thereafter, 80 ml of horse serum for enrichment, 10 ml 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 (manufacturer's instructions).

2.3 Inoculum Standardization and Preparation

All isolates of *T. vaginalis* were inoculated in duplicate in OXOID *Trichomonas* medium and incubated at 37℃ in aerobic condition for 48 hours. For the inoculum preparation, 500 µl of *T. vaginalis* culture was added to 5 ml of antibiotic-free OXOID *Trichomonas* medium and incubated aerobically at 37℃. After 48 hours, the number of viable trophozoites was counted after 1:2 dilution of the cell suspension with 0.4% trypan blue solution.

2.4 Drug Susceptibility Assay

In vitro drug susceptibility testing was conducted in accordance with Meingassner method modified by the CDC [14,15]. Metronidazole and Tinidazole powder (Sigma Aldrisch USA) were dissolved in sterile distilled water and stored at

4℃. 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 1×10^5 per 10 µg/ml. Eight polypropylene conical screw-cap tubes were used to prepare the dilution series for both metronidazole and tinidazole. Two-fold serial dilutions were performed by transferring 5 ml from the first to the last tubes. 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℃ in 5% CO_2 for 48 to 72 hours in the incubator.

2.5 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 the viable cells which appeared colorless while dead cells stained blue. The minimal latent concentration (MLC) was determined by sub-culturing the tubes that showed no growth to a fresh antibiotic free medium; minimum inhibitory concentration (MIC) determine the lowest dilution of the drug that had parasite growth-inhibitory effect [16].

3. RESULTS

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

4. 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 µg/ml. The *T. vaginalis* strains showed a relative resistance to metronidazole, all strains were susceptible to tinidazole at less than 25 µg/ml MLCs. This study showed more susceptibility to tinidazole than metronidazole, this supports the idea that tinidazole been the second drug of choice should be prescribed for *T. vaginalis* infected persons when metronidazole fails. 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 [17]. Clinical resistance and treatment failure have occurred with *T. vaginalis* infections for which MLCs of nitroimidazoles to the parasite were as low as 12.5µg/ml, and treatment success has occurred in infections with *T. vaginalis* infections for which MLCs of nitroimidazoles to the parasite were 100–200 µg/ml [18]. 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

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

M.I.C (µg/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 ($\chi 2$) = 25.895, p = .001

high level resistance as MLC >400 µg/ml [19]. Although metronidazole has been used to treat *T. 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 [20] detected one strain (0.6%) that exhibited low-level tinidazole resistance (MLC 50 µg/ml) among the 178 strains tested [14]. Among (91) strains collected in Spain during 1995 and 1999, 2.2% exhibited low-level resistance to metronidazole [21]. Mohammad et al. [22] 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 United States 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% [14,20,23]. Ikeh and Bello reported > 2.0 µg/ml MIC emergence of metronidazole resistant strains in Jos, Nigeria [24]. 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 [25]. 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 [26]. 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, the emergence of clinically notable nitroimidazole resistance would be expected to adversely influence the treatment effectiveness of each of these agents [27]. The problem of assessing *T. 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 susceptibility strains without correlating outcome with pharmacokinetics, re-infection and MIC and/or MLC.

5. CONCLUSION

The study detected low resistance level of metronidazole to *T. vaginalis* strains among internally displaced women in Maiduguri, Nigeria; while tinidazole been the second drug of choice for treatment of trichomoniasis appeared to be more active against *T. vaginalis* strains.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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