GENETIC CHARACTERIZATION, METRONIDAZOLE SUSCEPTIBILITY TESTING, AND SECRETED PROTEASE ACTIVITY OF CLINICAL _Trichomonas vaginalis_ ISOLATES

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_Abbreviation:_ Trichomonas vaginalis, a protozoan parasite that infects the human urogenital tract, causes, the most common non-viral, sexually transmitted disease in the world. Over 170 million cases of trichomoniasis occur worldwide annually. Trichomoniasis is associated with vaginitis, cervicitis, chronic prostatitis, and non-gonococcal urethritis, and is a risk factor for HSV and HIV transmission. _Trichomonas_ infection increases the incidence of premature rupture of placental membranes, low birth weight infants, and can be transmitted to neonates during passage through the birth canal. Frontline treatment of _Trichomonas_ is metronidazole, a 5-nitroimidazole, and is usually very effective and well tolerated. However, an estimated 2.5-5% of cases of trichomoniasis display some degree of resistance to initial treatment. _Trichomonas vaginalis_ possesses several virulence factors which contribute to its pathology and survival in the host. Cysteine proteases are virulence factors of _Trichomonas vaginalis_ that have implicated roles in immune evasion, nutrient acquisition, adhesion, cytotoxicity, and hemolysis. CP30 is a heterogeneous mixture of 4 secreted cysteine proteases that have been shown to induce apoptosis in primary human vaginal epithelial cells and degrade human IgG and IgA. We have characterized historical reference and clinical isolates of _T. vaginalis_ based on genetic profiles, susceptibility to metronidazole, and virulence. There is currently no consensual discriminatory typing method applicable to _T. vaginalis_. Therefore, to genetically characterize the isolates, we have developed a multi-locus sequencing typing (MLST) scheme. We expect that this method will provide a more rapid, easily reproducible technique for genetic typing of _T. vaginalis_. Our scheme utilizes 7 house-keeping genes to determine the relatedness of individual _T. vaginalis_ isolates. This should provide a sufficient level of discrimination power for typing this organism. We have successfully generated 400-500 bp PCR products for 40 _T. vaginalis_ isolates. The number of single nucleotide polymorphisms (SNPs) observed at a single locus ranges from 1-10 SNPs. The Alamar Blue™ colorimetric assay was used to test isolate susceptibility to metronidazole. Susceptibility testing shows the prevalence of high drug resistance found in this study to be greater than the national average of 2.5-5%. Secreted Cysteine protease activity was assessed via a fluorometric analysis. The variation in activity among _T. vaginalis_ isolates is greater than six-fold.