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Growth Kinetics of Egyptian isolates of *Trichomonas vaginalis*: Possible Correlation to Clinical Presentation

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ABSTRACT

Background: *Trichomonas vaginalis* is the most common non-viral sexually transmitted infection (STI) in the world. Trichomoniasis is an important risk factor for herpes simplex type II infections, human immunodeficiency virus (HIV) acquisition and transmission, pelvic inflammatory disease and cervical neoplasia. Studying the variation in growth patterns of the parasite can be used for biological characterization of the parasites and might be related to the severity of clinical presentation.

Aim of the Work: Study the growth kinetics of Egyptian *Trichomonas vaginalis* isolates in term of log phase, growth peaks reached, division rate and generation time of each isolate and correlation of such parameters with the clinical presentation of the patients, if any.

Material and Method: The study was carried on 300 vaginal washouts collected from Egyptian women patients aged 20-45 years suspected for trichomoniasis. Positive samples for *Trichomonas vaginalis* were cultured and sub cultured on modified Diamond's medium. Growth kinetics for each isolate was done by inoculated 10^4 trophozoites/ml into 2 tubes containing 10 ml of sterile culture medium followed by counting the trophozoites every 24 hours over seven days using a hemocytometer. Growth curves were done for each isolate by plotting the parasites numbers against the time. Division rates and generation times were calculated for each isolate.

Results: Out of the 300 collected samples, twelve were found positive for *Trichomonas vaginalis* trophozoite by wet mount examination and culture technique. Comparison of the growth kinetics of the twelve *Trichomonas vaginalis* isolates revealed a salient difference among all isolates after 48 to 96 hours. Isolates 1, 2, 6, 8 and 10 had a log phase of 48 hours. Isolates 4, 5, 7, 11 and 12 had a log phase of 72 hours. Isolates 3 and 9 had a log phase of 96 hours. The fast growing isolates of *Trichomonas vaginalis* reached maximum growth after 48 hours and the highest yield was observed in isolate 10 (150.25±3.13). Slowly growing isolates reached a maximum after 96 hours with the lowest yield in isolate 3 (40.5±21). Regarding the clinical presentation, isolates 1, 2, 3, 4, 5 and 11 showed mild discharge and congested cervix. Isolates 6, 7, 8, 9, 10, and 12 showed profuse discharge with varying clinical findings on gynecological examination. Isolate 10 had the most severe pathology with cervical erosion. Isolate 9, 12 showed bleeding during examination, isolate 8 showed vaginal ulcer while isolate 6 showed just erythema of the vagina.

Conclusion: Comparison of the growth kinetics of the twelve *Trichomonas vaginalis* isolates revealed a salient difference among all isolates after 48 to 96 hours. Regarding the clinical presentation, no clear correlation was found between the growth kinetics and clinical presentation of the patients. Methods other than growth kinetics such as genotyping and determination of genetic variability are needed to verify the relation between isolates of *Trichomonas vaginalis* and clinical presentation.

Keywords: *Trichomonas vaginalis*, growth kinetics, growth curves.

INTRODUCTION

Trichomoniasis is the most prevalent non-viral sexually transmitted infection worldwide. It has been estimated that 276.4 million new infections occurred in 2008 with 11.5% increase over the 2005 incidence rate [(1)]. Worldwide, the estimated prevalence rates vary with geographic location, age, race, community and the method used for diagnosis. Accordingly, prevalence rates varying from 2% to almost 50% were recorded [(2)]. In Egypt, a prevalence rate of 8.7% among married women from Upper Egypt was recorded [(3)]. In another study a prevalence rate of 36% was encountered among symptomatic women in the child bearing age; 20-45 years [(4)]. While infections are mostly asymptomatic in men, women usually present with vaginal discharge, pruritus and dysuria. Trichomoniasis is an important risk factor for herpes simplex type II infections, human immunodeficiency virus (HIV) acquisition and transmission, pelvic inflammatory disease and cervical neoplasia. Data from studies in Africa suggest a 1.5 to 3-fold increase in HIV transmission associated with *Trichomonas vaginalis* infection. Studies done in Egypt have linked trichomoniasis to acquisition of herpes simplex type II, cancer cervix and infertility [(5,6)].

Variation in phenotypic behavior of *Trichomonas vaginalis* such as pathogenicity, metronidazole sensitivity, clinical manifestations

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and outcome of infection has been previously reported [7,8]. Despite the public health importance and global distribution of trichomoniasis, little knowledge is known of the various features of the infection with *Trichomonas vaginalis*. Moreover, the pathogenicity, drug resistance and other epidemiological aspects of *Trichomonas vaginalis* have still not been clarified [9].

Study of biological characteristics of clinical isolates, including growth kinetics, might help in understanding the outcome of infection, hence the aim of the present study is to correlate the growth kinetics of *Trichomonas vaginalis* isolates from Egyptian women attending the out-patient clinics of Ain Shams Maternity Hospital with clinical presentation.

MATERIAL AND METHOD

Trichomonas vaginalis isolates

The clinical isolates for the present study were collected from women aged 20-45 years, complaining of vaginal discharge and other symptoms as pruritus, burning micturition and dyspareunia suggestive of trichomoniasis. The patients were attending the Early Cancer Detection Unit, Gynecology and Obstetrics Hospital, Ain Shams University. Vaginal washouts were collected from patient's vagina (posterior fornix) under aseptic conditions. Symptoms and clinical findings as found by a gynecologist were recorded for each patient according to a pre-designed questionnaire. The vaginal washouts were examined for *Trichomonas vaginalis* trophozoites within one hour by direct wet mount technique.

Trichomonas vaginalis culture

Specimens proved positive for *Trichomonas vaginalis* trophozoites by direct wet mount were cultured in modified Diamond's medium, supplemented with 10 % bovine serum albumin, 100 U penicillin, 100 µg of streptomycin and 50 µg amphotericin B/ml of culture medium [10]. Examination started after 24 hours incubation at 37°C for the presence of viable motile *Trichomonas vaginalis* trophozoites using x10 and x40 objectives of an inverted microscope. Microscopic examination was repeated every 24 hours for 7 days. Positive cultures containing good numbers of viable motile trophozoites with no bacterial contamination were maintained by sub-culturing every 24-48 hours into a new culture tube.

Comparison of Growth Characteristics of *Trichomonas vaginalis* Clinical Isolates

For each isolate the number of parasite/ml was counted using a hemocytometer. Then 10^4 trophozoites of each isolate were inoculated into 2 tubes containing 10 ml of sterile culture medium. The growth kinetics for each isolate was followed by counting the trophozoites every 24 hours over seven days. Two counts were made from each tube, i.e. 4 counts for each isolate inoculated into 2 culture tubes. The length of the log phase was determined as the time required for the parasite, after inoculation, to reach maximum growth. The means of growth peaks of the isolates at the end of the cultures' log phase was estimated and compared with each other. Growth curves were done for each isolate by plotting the parasites numbers against the time. Generation time (GT) defined as the time interval for one division was calculated as follows: GT=T Log2/ (Log N–Log N0), where: T= time elapsed in hours between the number of the parasite at the beginning of a time interval N0 and the number, N, at the end of the time interval. The number of divisions was calculated as T/GT, and the division rate was calculated as 1/GT [11].

Statistical analysis

The data were tabulated and analyzed by using Statistical Package for Social Science (SPSS version 16, for windows). ANOVA test was used to generate a one-way analysis of variance between different isolates and differences were considered significant if the P values were less$\leq$ 0.05, followed by using the Fisher's Least Significant Difference (LSD) as a Post-hoc test to calculate the smallest significant between two means in order to make direct comparisons between two means from two individual groups. Any difference larger than the LSD is considered a significant result.

Ethical considerations

The study design was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University, according to the regulation of Egyptian Ministry of Higher Education. Informed consent was obtained from each participant in the study after clear explanation of the study objectives. Infected females were treated by the gynecologist

RESULTS

Clinical presentation enrolled patients

Out of the 300 collected samples, twelve were found positive for *Trichomonas vaginalis* trophozoite by wet mount examination. The twelve specimens were cultured on modified Diamond`s medium. The recorded clinical presentations of the twelve patients enrolled in the study are shown in table (1).

Table (1): Comparison between mean peak growth, log phase duration of the twelve *Trichomonas vaginalis* isolates and clinical presentation.

Isolate	Mean peak growth	Log Phase Duration	Vaginal discharge			Gynecological Examination
			Amount	consistency	color	
1	120.5 ±2.4	48 hr	Mild	Watery	White	Congested cervix
2	81. ±2.3	48 hr	Mild	Watery	White	Congested cervix
3	40.5±21	96 hr	Mild	Mucoid	Clear	Congested cervix
4	80±3.45	72 hr	Mild	Watery	Yellow	Congested cervix
5	110±2.90	72 hr	Mild	Watery	Yellow	Congested cervix
6	95.45±45	48 hr	Profuse	Watery	Yellow	Erythema of vagina
7	139.8±0.97	72 hr	Profuse	Watery	Yellow	Congested cervix
8	100±1.2	48 hr	Profuse	Mucoid	Clear	Ulcers at vagina
9	63.5±1.7	96 hr	Profuse	Watery	Bloody	Bleeding during examination
10	150.3±3.1	48 hr	Profuse	Watery	Yellow	Cervical erosion
11	81.3±2.4	72 hr	Mild	Mucoid	White	Abdominal pain
12	115.5±1.8	72 hr	Profuse	Mucoid	White	Bleeding during examination

As shown isolates 1, 2, 3, 4, 5 and 11 showed mild discharge and congested cervix. Isolates 6, 7, 8, 9, 10, and 12 showed profuse discharge with varying clinical findings on gynecological examination. Isolate 10 had the most severe pathology with cervical erosion. Isolate 9, 12 showed bleeding during examination, isolate 8 showed vaginal ulcer while isolate 6 showed just erythema of the vagina.

Growth kinetics of the twelve positive isolates

The twelve isolates exhibited different growth characteristics in terms of duration of log phase and growth peaks reached (table 2 and figure 1).

Table (2): Comparison between the growth kinetics of the 12 *Trichomonas vaginalis* isolates grown on modified Diamond's medium.

Isolate	Time (Hours)	Mean Trichomonas vaginalis count (x 10^4 / ml) ±SD*					
		24	48	72	96	120	144
1		6.5 ± 1.29	120.50 ± 2.38	23.00 ± 16.33	3.25 ± 14.14	0.00 ± 0.00	0.00 ± 0.00
2		3.50 ± 0.95	81.00 ± 2.31	65.00 ±12.91	43.50 ± 4.57	15.25 ±8.54	0.600 ± 4.08
3		6.25 ± 2.62	27.25 ± 0.84	33.5 ± 1.72	40.5 ± 21	19.60 ± 2.50	2.50 ± 1.25
4		1.75 ± 0.95	9.45 ± 1.25	80 ± 3.45	7.78 ± 2.23	2.5 ± 1.25	1.25 ± 0.57
5		4.5 ± 1.52	90 ± 6.65	110 ± 2.90	78.75 ± 3.87	4.2 ± 1.25	0.75 ± 1.25
6		3.5 ± 1.28	95.45 ± 45	88 ± 3.43	78.1 ± 5.78	14 ± 3.74	5.25 ± 0.95
7		4.6 ± 1.52	91.7 ± 0.56	139.75 ± 0.97	78 ± 3.70	5.5 ± 1.25	0.00 ± 0.00
8		12.25 ± 2.06	100 ± 1.25	88.6 ± 3.80	65.8 ± 1.70	12 ± 0.23	0.00 ± 0.00
9		16.5 ± 4.07	32.25 ± 4.77	40 ± 2.09	63.5 ± 1.72	10.2 ± 3.00	2.00 ± 0.09
10		6.55 ± 1.23	150.25 ± 3.13	32.5 ± 1.67	13.25 ± 0.78	2.00 ± 1.34	0.00 ± 0.00
11		8.25 ± 1.22	77.75 ± 3.80	81.25 ± 2.40	40 ± 0.55	10.22 ± 1.25	4.00 ± 3.09
12		3.5 ± 1.29	27.1 ± 1.95	115.47 ± 1.77	30.5±3.00	13.34 ± 1.22	3.25 ± 0.70
	P value	≤ 0.001	≤ 0.0001	≤ 0.0001	≤ 0.0001	≤ 0.001	≤ 0.01

* Data shown in the table are mean ± SD x10^4 / ml from 4 counts, 2 from each culture tube.

The cells shaded in grey show the peak growth of isolates at the end of the log phase.

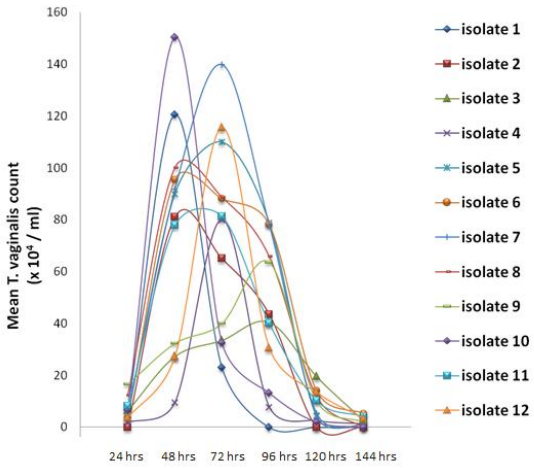

Figure (1): Comparison between the growth kinetics of the 12 *Trichomonas vaginalis* isolates grown on modified Diamond's medium

Regarding the log phase, 5 isolates; 1, 2, 6, 8, and 10 had a short log phase duration of 48 hours. Isolates 3 and 9 had a long log phase duration of 96 hours. The remaining 5 isolates; 4, 5, 7, 11, and 12 had a log phase duration of 72 hour. Comparison of the peak number of isolates at the end of log phases showed that the greatest significant yield was observed in isolate 10 (150.25±3.13) after 48 hours, and the least significant yield was in isolate 3 (40.5±21) after 96 hours. The difference between isolate 10 and 3 was

considered statistically different using the Fisher's Least Significant Difference (LSD) which was 18.99.

The division rate, generation time and number of divisions of the twelve isolates are shown in table 3.

Table (3): Comparison between the division rate, number of divisions and generation times among the 12 *Trichomonas vaginalis* isolates grown on modified Diamond's medium.

Isolates	Division rate (division/hour) mean ± SD	Number of divisions (during log phase) mean ± SD	Generation time (hour: minute) mean ± SD
1	0.16 ± 0.02	8.00 ± 0.18	6:00 ± 00:27
2	0.18 ±0. 001	8.84 ± 0.09	5:26 ± 00:03
3	0.09 ± 0.004	8.51 ± 0.14	11:16 ±00: 07
4	0.13 ± 0.02	9.27 ± 0.16	7.76 ±00:12
5	0.17 ± 0.011	12.20 ± 0. 16	5:55 ± 00:12
6	0.18 ± 0.002	8.69 ± 0. 06	5:34 ± 00:08
7	0.17 ± 0.03	12.22 ± 0.09	5:54 ± 00:19
8	0.13 ± 0.01	6.39 ± 0.13	7: 30 ± 00:15
9	0.09 ± 0.002	9.15 ± 0.02	10:31 ± 00:04
10	0.18 ± 0.01	8.73 ± 0.13	5:30 ± 00:14
11	0.13 ± 0.01	9.47 ±0. 01	7:36 ± 00:07
12	0.12 ± 0.03	8.78 ±0. 12	8:12 ± 00:08
P value	≤ 0.001	≤ 0.001	≤ 0.001

Table (3) shows variable parameters for the different isolates ranging from 0.09 to 0.18 mean division/hour and generation time ranging between means of 5:26- 11:16 hours: minutes. The number of divisions during the logarithmic phase ranged between means of 6.39-12.22.

DISCUSSION

The present study has shown that there was a considerable variability in the growth kinetics of *Trichomonas vaginalis* isolates. Similar findings were previously recorded by other investigators who worked on clinical and laboratory-maintained isolates [11-16]. In the present study, comparison of the growth kinetics of the twelve *Trichomonas vaginalis* isolates revealed a salient difference among all isolates after 48 to 96 hours. Isolates 1, 2, 6, 8 and 10 had a log phase of 48 hours. Isolates 4, 5, 7, 11 and 12 had a log phase of 72 hours. Isolates 3 and 9 had a log phase of 96 hours using an initial inoculum of 10^4 /ml. The fast growing isolates of *Trichomonas vaginalis* reached maximum growth after 48 hours and the greatest yield was observed in isolate 10 (150.25±3.13). Slowly growing isolates reached a maximum after 96 hours with least yield in isolate 3 (40.5±21). This is in agreement with ***Boulos et al.***[15] who compared the growth kinetics of 20 *Trichomonas vaginalis* isolates, using an initial inoculum of 10^4 /ml, the log phase varied between 48 and 96 hours. In contrast, ***Kulda et al.***[17] compared the growth of nine isolates using a higher initial inoculum and reported that the fast-growing strains of *Trichomonas vaginalis* reached maximum growth after 32 hours and 48-52 hours for the slowly growing ones. The longer duration at which the maximum growth was reached in our study may be due to the smaller initial inoculum used. In confirmation, ***Abd El Ghaffar et al.***[18] reported a correlation between the starting inoculum and the time of growth peak as when the starting inoculum was smaller, the peak growth was delayed. In contrast ***Smith*** [13], inoculated 10 ml of Hollander medium with 400000 parasites (40000/ml), which gave a higher peak count after 72 hours incubation at (37°C). The different growth characteristics of the isolates may probably be due to the effect of temperature of incubation (35°C), where lower temperature allows degradation of the nutrients in the medium hence more organisms develop and multiply. Variations could also be attributed to the different types of media used (Hollander medium).

Regarding the division rates and generation time, the present study has shown that, the twelve isolates can be categorized into three groups. The first group included isolates 1, 2, 5, 6, 7, and 10 which exhibited significantly rapid division rates that ranged from 0.16-0.18 division/hour and short generation time that ranged from a mean of 5:26-6:00. The second group included isolates 8, 11 and 12 with intermediate division rates that ranged from around 0.13 division/hour and a generation time of nearly 8 hours. The third group, included isolates 3, and 9 with slow division rates of 0.09-0.1 division/hour and generation time above 10 hours. Similar results were also found by ***Soliman and Aufy*** [19], who categorized their isolates into slow, medium and fast growing with a generation time of more than 11 hours, between 6 and 11 hours, and less than 6 hours, respectively.

No clear correlation was found between the growth kinetics of the different isolates and the clinical presentation of the patients, who exhibited in most cases variable amounts of watery to mucoid discharge with variable degrees of vaginitis and congested cervix. Isolate 10 which showed the highest growth peak of 150.25±3.13 after a log

phase of 48 hours was isolated from a female who presented with severe vaginitis, profuse discharge and cervical erosion. Isolate 3 which exhibited the lowest growth peak of 40.5±21 after 96 hours belonged to a female who presented, as others, with mild vaginitis, moderate amount of vaginal discharge and slightly congested cervix. ***Boulos et al.***[15], working on 20 Egyptian isolates found clear relationship, between severity of infection with three isolates in term of number of parasites in wet mount in vaginal discharge, short generation time of 6:34- 7:31 hours and pathogenicity in mice. Also, ***Honigberg*** [12] reported an inverse correlation between the growth rates of three isolates and pathogenicity as determined by the subcutaneous mouse assay.

In our study, it appears that the number of parasites at the end of log phase rather than the pattern of growth kinetics might be related to the severity of infection in one case however, we could not find clear correlation between the growth kinetics and clinical presentation. Apparently, methods other than growth kinetics and mice pathogenicity, such as genotyping and determination genetic variability, are needed to verify the relation between isolates of *Trichomonas vaginalis* and clinical presentation.

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