Table 35: Continued

Strain	Clinical diagnosis	Drug tested *in vitro*	Control (No. of amoebae per 0.05 ml)	Number of parasite (% reduction in parasite count)								
				500 µg/ml	50 µg/ml	5 µg/ml	2.5 µg/ml	1.25 µg/ml	0.625 µg/ml	0.312 µg/ml	0.156 µg/ml	0.078 µg/ml
68-432	Intestinal amoebiasis	Chloroquine phosphate	115	N	N	10 (91%)	15 (87%)	26 (77%)	30 (74%)	48 (58%)	60 (48%)	68 (41%)
		Tinidazole		**N**	**N**	**N**	**N**	**N**	**N**	**1 (99%)**	**6 (95%)**	**12 (90%)**
		Metronidazole		N	N	N	N	N	N	N	N	4 (97%)
68-604	Intestinal amoebiasis	Chloroquine phosphate	240	N	N	12 (95%)	16 (94%)	22 (91%)	26 (89%)	68 (92%)	75 (70%)	81 (66%)
		Tinidazole		**N**	**N**	**N**	**N**	**1 (99%)**	**5 (98%)**	**21 (91%)**	**26 (89%)**	**31 (87%)**
		Metronidazole		N	N	N	N	N	N	N	N	6 (97.5%)
66-837	Intestinal amoebiasis	Chloroquine phosphate	182	N	N	2 (99%)	4 (98%)	10 (95%)	11 (94%)	14 (92%)	27 (85%)	77 (58%)
		Tinidazole		**N**	**N**	**N**	**N**	**N**	**N**	**1 (99%)**	**27 (85%)**	**32 (82%)**
		Metronidazole		N	N	N	N	N	N	N	N	3 (98%)
67-2468	Intestinal amoebiasis	Chloroquine phosphate	240	N	N	N	15 (97%)	30 (87%)	80 (67%)	90 (62.5%)	110 (46%)	120 (50%)
		Tinidazole		**N**	**N**	**N**	**N**	**N**	**N**	**1 (99%)**	**4 (98%)**	**7 (99%)**
		Metronidazole		N	N	N	N	N	N	N	N	18 (92 5%)
69-3787	Amoebic colitis	Chloroquine phosphate	239	N	N	86 (64%)	110 (54%)	120 (50%)	139 (42%)	178 (25.6%)	180 (25%)	186 (22%)
		Tinidazole		**N**	**N**	**N**	**N**	**1 (99%)**	**7 (97%)**	**78 (67%)**	**ND**	**ND**
		Metronidazole		N	N	N	N	N	N	1 (99%)	36 (85%)	45 (81%)

The test was read after 48 hours of incubation at 37°C, N = No growth; ND = Not done

2.2.2 Activity against bacteria:
The sponsor has included studies conducted in different laboratories in support of the *in vitro* activity of tinidazole against *Helicobacter pylori,* and anaerobic bacteria associated with bacterial vaginosis.

The sponsor has included 15 studies conducted in 11 different laboratories in support of the *in vitro* activity of tinidazole against various anaerobic bacteria associated with bacterial vaginosis. A majority of the studies examined the activity of tinidazole against *Gardnerella vaginalis* and *Bacteroides* sp. only. Of the studies included in this submission, only one performed susceptibility testing using the National Committee for Clinical Laboratory Standards (NCCLS) recommended media for anaerobic bacteria. Also, the inoculum size and incubation period varied in the different studies. The NCCLS recommends the use of Brucella agar or broth supplemented with 5% laked sheep blood, vitamin K (1 μg/ml) and hemin (5 μg/ml) and an inoculum size of 10^5 cfu per spot or 10^6 cfu/ml for susceptibility testing of anaerobic bacteria by the agar and the broth dilution methods, respectively. For the purpose of this review, the minimum inhibitory concentration (MIC) refers to the concentration of the drug required for complete inhibition of growth unless specified otherwise. The terms MIC_{50} and MIC_{90} indicate the concentration of drug required for inhibiting 50% and 90% of the isolates tested, respectively. The minimum bactericidal concentration (MBC) refers to the concentration of the drug required to kill 99% of the original inoculum upon subculture.

***Gardnerella vaginalis*:**

The *in vitro* activity of tinidazole against 510 clinical isolates of *G. vaginalis* (characterized by appearance on colistin-nalidixic acid blood agar, gram's stain, absence of catalase production, β-hemolysis on human blood agar, hippurate hydrolysis and starch fermentation) was examined using the agar dilution method[62]. Metronidazole and its metabolites were used as comparators. The drugs were dissolved in N, N-dimethylformamide and diluted in distilled water. For susceptibility testing, the Diagnostic sensitivity test (DST) agar with an inoculum of 10^6 cfu/ml was used. The cultures were incubated in the presence of drug at 37°C for 48 hours in 5% CO_2 and the MIC determined. *Bacteroides fragilis* ATCC 25285 (recommended by NCCLS) and *G. vaginalis* ATCC 14018 were included as quality control strains. The results in Table 36 show that the MIC_{90} of tinidazole (4.44 μg/ml) against the *G. vaginalis* isolates was similar to metronidazole (4.09 μg/ml) and 4-fold higher than the hydroxy metabolite of metronidazole (1.12 μg/ml). The acid metabolite of metronidazole had minimal activity against *G. vaginalis.* The MIC data of the control strains were not included, however, the sponsor has stated that the MICs against the control strains were in the expected range.

Table 36: Susceptibility of 510 *G. vaginalis* isolates to four nitroimidazole drugs.

Drugs	MIC (µg/ml)		
	MIC_{50}	MIC_{90}	Range
Metronidazole	3.08	4.09	1-16
Tinidazole	2.79	4.44	1-32
Hydroxy metabolite of metronidazole	0.71	1.12	0.125-8
Acid metabolite of metronidazole	109.87	226.55	8-256

In another study[63], the *in vitro* activity of tinidazole and 16 other antimicrobial agents was measured against 25 clinical isolates of *G. vaginalis* by the agar dilution method. For this, overnight cultures of *G. vaginalis* (10^4 cfu/spot) were used to inoculate chocolate agar containing two-fold dilution of the drug. The cultures were incubated at 37°C for 48 hours under anaerobic conditions and the MIC determined. The quality control strains were same as those used in the previous study but the MIC data for the control strains were not provided. The tinidazole MIC_{90} against the *G. vaginalis* isolates was 4-fold higher (32 µg/ml) than that observed in the previous study (4.44 µg/ml; Tables 36 and 37). However, the activity of tinidazole against the *G. vaginalis* isolates was similar to metronidazole.

Table 37: Susceptibility of 25 clinical isolates of *Gardnerella vaginalis* to 17 antimicrobial agents.

Antimicrobial Agent	MIC mg l		
	Range	MIC_{50}	MIC_{90}
Penicillin	0.015–0.25	0.03	0.12
Ampicillin	0.007–4	0.03	0.25
Cephalothin	0.25 –2	1	1
Cefoxitin	0.007–16	0.06	0.5
Ticarcillin	0.12 –16	1	2
Piperacillin	0.015–1	0.25	0.5
Cefotaxime	0.03 –8	0.12	1
Cefoperazone	0.01 –1	0.12	0.5
N-formimidoyl thienamycin	0.12 –0.5	0.25	0.25
Clindamycin	0.007–0.06	0.007	0.03
Erythromycin	0.007–0.06	0.015	0.06
Chloramphenicol	0.25 –8	1	2
Tetracycline	0.25 –32	8	16
Gentamicin	1 –32	8	32
Rosoxacin	16 –32	16	32
Metronidazole	4 –128	8	32
(hydroxy-metabolite)	0.5 –8	2	4
Tinidazole	2 –128	8	32
(hydroxy-metabolite)	0.25 –2	0.5	2

In another study by the same group of investigators[64], the susceptibility of 11 strains of *G. vaginalis* to tinadazole, metronidazole and its metabolites was measured using the agar dilution method. It is unclear if the isolates used in this study were same as in the previous study. An inoculum of 10^6 cfu/ml was used for testing. However, the media used for testing was not specified. The MICs were determined after incubating cultures in the presence of drug under anaerobic conditions for 48 hours. As in the previous studies, the activity of tinidazole against *G. vaginalis* was similar to metronidazole (Table 38).

Table 38:

MIC (mg/l) OF METRONIDAZOLE (M) AND TINIDAZOLE (T) AND METABOLITES FOR TEN STRAINS OF G VAGINALIS

Strain	M	T	M(acid)	Metabolites M(hydroxy)	T(hydroxy)
1	16	16	512	2	1
2	4	4	128	1	0·5
3	16	16	256	2	0·5
4	8	4	128	1	0·5
5	4	2	128	0·5	0·25
6	4	8	256	1	0·5
7	8	8	256	2	1
8	16	4	256	2	0·5
9	4	8	256	1	0·5
10	4	4	256	2	0·5
ATCC 14018	4	2	256	2	0·5

In another study by a different group of investigators[65], the *in vitro* activity of tinidazole against 93 isolates of *G. vaginalis,* collected between 1987-1988 from South Africa, was examined. The activity of tinidazole was compared to 24 other antimicrobial agents. The identification of *G. vaginalis* was based on the following characteristics: diffuse β-hemolysis on blood agar, variable gram stain, positive α-glucosidase and negative β-glucosidase activity. Cultures of *G. vaginalis* (10^5 cfu/spot) were used to inoculate blood (human) agar containing tinidazole, metronidazole or hydroxy metabolite of metronidazole and MIC determined after incubation at 37°C for 48 hours in an anaerobic jar. For all other antimicrobial agents, the cultures were incubated in an atmosphere of 6% CO_2. The tinidazole MIC_{90} against the 93 *G. vaginalis* isolates was 8 µg/ml and within the range observed in previous studies. The tinidazole MIC values against these isolates were similar to those of metronidazole (Table 39).

Table 39: *In vitro* susceptibilities of 93 strains of *G. vaginalis* to 25 antimicrobial agents.

Test agent	MIC (µg/ml) Range	50%	90%
Metronidazole	2.0–128.0	8.0	16.0
2-Hydroxy[a]	0.25–16.0	1.0	4.0
Tinidazole	1.0–128.0	8.0	8.0
Penicillin G	0.015–0.5	0.12	0.5
Ampicillin	0.03–1.0	0.5	0.5
Cefamandole	0.12–2.0	1.0	2.0
Cefoxitin	0.06–4.0	1.0	1.0
Cefuroxime	0.06–4.0	1.0	4.0
Cefotaxime	0.25–4.0	2.0	2.0
Ceftriaxone	0.06–4.0	0.5	2.0
Aztreonam	4.0–32.0	32.0	32.0
Imipenem	0.06–1.0	0.25	1.0
Tetracycline	2.0–128.0	64.0	64.0
Minocycline	0.12 16.0	2.0	16.0
Erythromycin	0.007–0.06	0.03	0.06
Clindamycin	0.007–0.03	0.01	0.03
Vancomycin	0.12–0.5	0.25	0.5
LY146032	0.5–8.0	4.0	8.0
Chloramphenicol	0.5–2.0	1.0	2.0
Amikacin	8.0–128.0	32.0	128.0
Rifampin	0.5–0.5	1.0	2.0
Ciprofloxacin	1.0–4.0	1.0	2.0
Sulfamethoxazole	128.0–128.0	>128.0	>128.0
Trimethoprim	0.5–4.0	2.0	4.0
Co-trimoxazole[b]	4.0–64.0	64.0	64.0

[a] 2-Hydroxymetabolite of metronidazole [1-(2-hydroxyethyl)-2-hydroxymethyl-1,5-nitroimidazole].
[b] Sulfamethoxazole-trimethoprim in a 19:1 ratio

In another study[66], the susceptibility of a *G. vaginalis* isolate to tinidazole or metronidazole was measured. In addition, the effect of the drug on bacterial morphology was examined by scanning electron microscopy (SEM). Susceptibility testing was performed by the agar dilution method using peptone-starch-dextrose (PSD) agar and an inoculum of 10^5 cfu/ml. Cultures were incubated in the presence of drug at 37°C either aerobically or anaerobically (10% CO_2 atmosphere) for 48 hours and the MIC determined. The tinidazole MIC against *G. vaginalis* under anaerobic (1.6 μg/ml) conditions was lower than under aerobic conditions (12.5 μg/ml). Similar observations were made with metronidazole. The metronidazole MIC against the *G. vaginalis* isolate was 2-fold higher than tinidazole (3.1 μg/ml) under anaerobic conditions but 2-fold lower (6.2 μg/ml) than tinidazole under aerobic conditions.

The activity of tinidazole against *G. vaginalis* was also determined by the agar diffusion method using the same media and culture conditions except that the cultures were incubated for 24 hours. At the end of the 24 hour incubation, the agar pieces were also processed for SEM. At a concentration of 100 μg/ml tinidazole and 120 μg/ml metronidazole, the diameter of the zone of growth inhibition was 25 and 20 mm, respectively. Within 10 mm from the zone of growth inhibition, no effect was observed on the morphology of *G. vaginalis* cells exposed to metronidazole by SEM. However, the figure was unclear for an independent review. The authors have stated that tinidazole gave similar results.

In another study[67], the *in vitro* activity of tinidazole and metronidazole against 51 clinical isolates of *G. vaginalis* was examined by the broth dilution method. The trypticase-soy-broth and an inoculum of 10^5 to 10^6 cfu/ml was used for susceptibility testing. The MICs were determined at the end of 24 hours of incubation while the MBCs were determined by subculturing at the end of 48 hours of incubation. The tinidazole MICs against the 51 clinical isolates (0.5 to 12 μg/ml) were similar to metronidazole and within the range observed in the studies described above (Table 40). The tinidazole MBCs against the *G. vaginalis* isolates were 2 to 4 fold higher than the MICs (Table 41).

Table 40: MICs of tinidazole against *G. vaginalis*.

Concentration	Tinidazole			Metronidazole		
μg/ml	N°	%	Cumulative %	N°	%	Cumulative %
0.5	2	3.8	3.8	—	—	—
1	—	—	—	—	—	—
2	8	15.6	19.4	11	21.5	21.5
3	14	27.5	46.9	13	25.5	47.0
6	19	37.5	84.4	12	23.5	70.5
12	8	15.6	100	14	27.5	98.0
25	—	—	—	1	2.0	100
Total	51	100	100	51	100	100

Table 41: MBCs of tinidazole against *G. vaginalis*.

Concentration µg/ml	Tinidazole N*	%	Cumulative %	Metronidazole N*	%	Cumulative %
2	—	—	—	1	1.9	1.9
3	6	11.7	11.7	1	1.9	3.8
6	15	29.4	41 1	13	25.5	29.3
12	16	31.4	72.5*	14	27.5	56.8
25	8	15.7	88.2	10	19.7	76.5
50	6	11.8	100.0	12	23.5	100.0
Total	51	100 0	100 0	51	100.0	100 0

*$p<0.01$ tinidazole vs. metronidazole

In another study[68], the *in vitro* activity of tinidazole, metronidazole and nimorazole against 20 clinical isolates of *G. vaginalis* was examined by the agar dilution method using DST agar supplemented with 10% lysed blood and an inoculum of 10^4 cfu/spot. MICs were determined after 48 hours of incubation. The results in Figure 10 show that the tinidazole MICs against the 20 isolates (0.3 to 12.5 µg/ml) were within the range observed in previous studies. The tinidazole MIC_{90} was 4 fold lower than the metronidazole MIC_{90}.

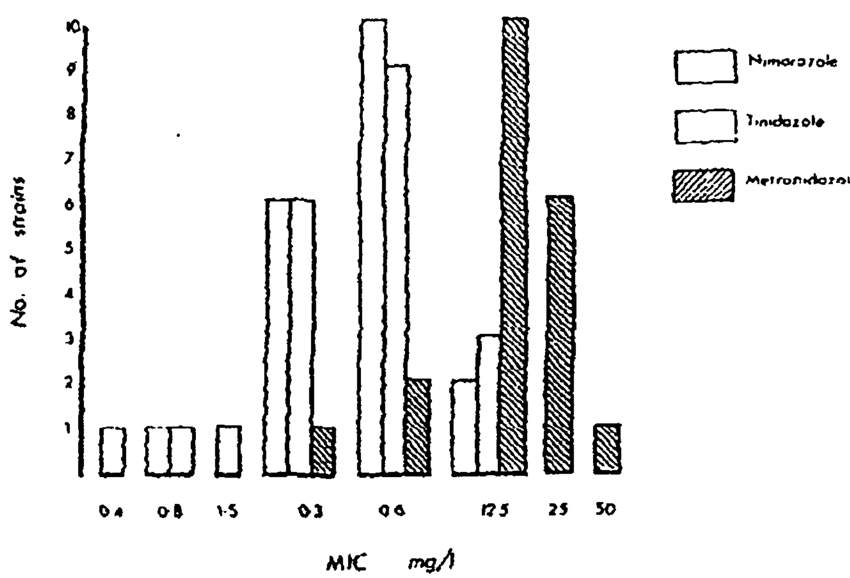


Figure 10 Susceptibility of 20 isolates *in vitro* to three imidazoles.

In another study[69], the *in vitro* activity of tinidazole and metronidazole against 102 isolates of *G. vaginalis* was examined. The standard NCCLS agar dilution method (M11-A5) using Brucella agar supplemented with hemin and Vitamin K was used to measure susceptibility to the drug. Briefly, cultures were incubated anaerobically in the presence of drug at 37°C for 2 days. The MIC defined as the concentration of the drug which showed marked reduction in growth, marked reduction in haze, or reduction in growth to few large colonies was determined. The results in Table 42 show that the activity of tinidazole is similar to metronidazole against *G. vaginalis*. However, the MIC_{90} values (>256 µg/ml) were much higher than those observed in previous studies (≤ 32 µg/ml).

Table 42: Antimicrobial susceptibilities of anaerobic bacteria to tinidazole and metronidazole.

Organism	MIC_{50}	MIC_{90}	Range (µg/ml)	Mode	% Resistant
Gardnerella vaginalis					
Tinidazole (102)	32	>256	[redacted]	4	53.9%
Metronidazole (102) (MIC ≥ 32)	64	>256	[redacted]	64	67.6%
Anaerobic Gram Positive Cocci					
Tinidazole (76)	2	4	[redacted]	2	3 9%
Metronidazole (76)	1	4	[redacted]	1	1 3%
Prevotella bivia					
Tinidazole (59)	4	8	[redacted]	4	0%
Metronidazole (59)	4	8		4	0%
Prevotella sp.*					
Tinidazole (97)	2	4	[redacted]	2	0%
Metronidazole (97)	2	8	[redacted]	2	0%
Pigmented *Prevotella**					
Tinidazole (62)	2	4	[redacted]	2	1 6%
Metronidazole (62)	2	4	[redacted]	2	0%
Porphyromonas sp.*					
Tinidazole (43)	1	1		1	0%
Metronidazole (43)	0.5	2		0 5	0%
Bacteroides fragilis					
Tinidazole (9)	2	2	[redacted]	2	0%
Metronidazole (9)	2	4	[redacted]	2	0%
Lactobacillus sp.*					
Tinidazole (24)	>256	>256	[redacted]	>256	96%
Metronidazole (24)	>256	>256		>256	100%

**Prevotella sp* includes the following *Prevotella oralis, Prevotella buccalis, Prevotella veroralis, Prevotella oulorum, Prevotella oris, Prevotella buccae, Prevotella capillosus,* and *Prevotella disiens*

*Pigmented *Prevotella* includes the following. *Prevotella intermedia, Prevotella corporis, Prevotella denticola, Prevotella loeschii, and Prevotella melaninogenica.*

**Porphyromonas sp.* includes the following: *Porphyromonas asaccharolytica, Porphyromonas endodontalis, and Porphyromonas gingivalis.*

**Lactobacillus sp.* includes the following: *Lactobacillus gasserii, Lactobacillus jensenii,* and *Lactobacillus crispatus.*

Overall, tinidazole MICs against 812 isolates of *G. vaginalis* tested in 7 laboratories were similar to metronidazole MICs and ranged between [redacted] using different susceptibility testing methods (Table 43). The tinidazole MBCs against *G. vaginalis* isolates were 2 to 4 fold higher than the MICs. In one study, the effect on cell morphology was analyzed *in situ* on agar surfaces by SEM. Over a 24 hour growth period, no apparent change in *G. vaginalis* cell morphology was observed when grown in the presence of tinidazole or metronidazole.

Table 43: Summary of the tinidazole MIC/MBCs (mg/ml) against *G. vaginalis*.

Reference	No. of strains/ isolates	Method	Media	Inoculum (cfu/ml or cfu/spot)	Incubation period	Tinidazole MIC_{90}	Metronidazole MIC_{90}
Bannatyne *et al*,. (1987)[62]	510	Agar dilution	DST agar	10^6	48	4.4	4.1
Shanker *et al.*, (1982a)[63]	25	Agar dilution	Chocolate agar	10^4	48	32.0	32.0
Shanker *et al.*, (1982b)[64]	10	Agar dilution	NA	10^6	48	16.0	16.0
Kharsany *et al.*, (1983)[65]	93	Agar dilution	Blood agar	10^5	48	8.0	16.0
Skarin and Mardh (1981)[66]	1	Agar dilution	PSD agar	10^5	48	—	3.1[#]
Carmona *et al.*, (1983)[67]	51	Broth dilution	TSB medium	10^5 - 10^6	24	12.0	12.0
Mohanty and Deighton (1987)[68]	20	Agar dilution	DST agar	10^4	48	12.5	50.0
Hillier (2002)[69]	102	Agar dilution	Brucella agar+ hemin+ vitaminK	10^5	48		
Total	**812**						

\# one isolate tested; DST = Diagnostic Sensitivity test
PSD = potato-starch-dextrose; TSB = trypticase soy broth; NA = not available

***Bacteroides* species (mostly *B. fragilis*):**

The activity of tinidazole and other antimicrobial agents against 40 clinical isolates of *Bacteroides* species was tested by the agar dilution method[70]. The authors have stated that the isolates were penicillin resistant (penicillin MICs were not specified). The testing was performed using DST agar supplemented with 10% lysed human blood and a lower concentration of the inoculum (10^2 cfu/spot i.e., 10^5 cfu/ml) than that recommended by NCCLS. The MIC defined as 90% reduction of the original inoculum (≥ 10 cfu) was determined after incubation of cultures anaerobically at 37°C for 18 hours. The results in Table 44 show that the tinidazole MICs against the 40 isolates of *Bacteroides* sp. were ≤ 0.12 µg/ml. The activity was similar to metronidazole and nimorazole but 266-fold lower than spectinomycin.

Table 44: Activity of 4 antimicrobial agents against 40 isolates of *Bacteroides* sp.[a]

Drug	MIC (µg/ml)										
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Tinidazole	6	34									
Nimorazole		13	25	2							
Metronidazole		5	35								
Spectinomycin										40	

[a] Agar dilution method, 10^2 cfu/spot (10^5 cfu/ml)

In another experiment, the effect of inoculum size on the tinidazole MIC was examined using 20 of the 40 *Bacteroides* isolates. The tinidazole MIC against the 20 *Bacteroides* isolates increased 16 - 33 fold with increase in inoculum size from 10^5 to 10^8 cfu/ml (Table 45). In addition, the tinidazole MBC against 3 of the 40 *Bacteroides* isolates in the presence of 25% serum, 100% serum or 5% lysed human blood was determined. An inoculum of 10^5 cfu/ml was used for MBC measurement. The tinidazole MBC values against the 3 isolates in the presence of 5% lysed human blood were 4 to 8-fold higher than the MIC values (Table 45). The tinidazole MBC values in the presence of 25 and 100% serum were also 2 to 16-fold higher than the MIC values.

Table 45: Effect of inoculum and serum on MIC/MBC of tinidazole against *Bacteroides* sp.

	Number tested	MIC or MBC, mg/l								
		0.06	0.12	0.25	0.5	1	2	4	8	16
MIC 10^5/ml[a]	40	6	34							
10^8/ml[b]	20						17	3		
MBC 10^5/ml[a]										
5% LHB[c]	3				2	1				
100% serum	3					2	1			
25% serum	3			3						

[a] Equivalent to spot inoculum of 10^3 cfu.
[b] Equivalent to spot inoculum of 10^5 cfu.
[c] LHB = Lysed human blood.

In another study[71], the *in vitro* activity of tinidazole, metronidazole and nimorazole against 61 isolates of *B. fragilis* was examined by agar dilution method. A 1:500 dilution of an overnight culture (grown in 1 ml thioglycollate broth anaerobically at 37°C) was used to inoculate brain heart infusion agar supplemented with 5% lysed defibrinated horse blood containing different concentration of drug (the final inoculum size i.e., cfu/spot was not specified). The cultures were incubated anaerobically at 37°C for 42 hours and the MIC determined. The results in Table 46 show that the activity of tinidazole against *B. fragilis* (MIC: 0.062 - 2 μg/ml) was similar to metronidazole (MIC: 0.125 - 4 μg/ml). The tinidazole MIC against *Bacteroides* sp. was within the range observed in the previous study.

Table 46: Activity of metronidazole, nimorazole, and tinidazole against gram negative anaerobic bacteria.

	MIC (μg/ml)							
	0·062	0·125	0·25	0·5	1·0	2·0	4·0	8·0
Metronidazole								
Fusobacterium sp	1	2	4		1			
B. fragilis		3	27	24	4		1	
Bacteroides sp			1	1				
Nimorazole								
Fusobacterium sp					2	5	1	
B. fragilis			3	7	41	6	1	1
Bacteroides sp					2			
Tinidazole								
Fusobacterium sp		2	5	1				
B. fragilis	2	1	31	15	3	1		
Bacteroides sp			1	1				
Geometric mean MIC	Metronidazole 0·34 μg/ml Nimorazole 1·05 μg/ml Tinidazole 0·28 μg/ml							

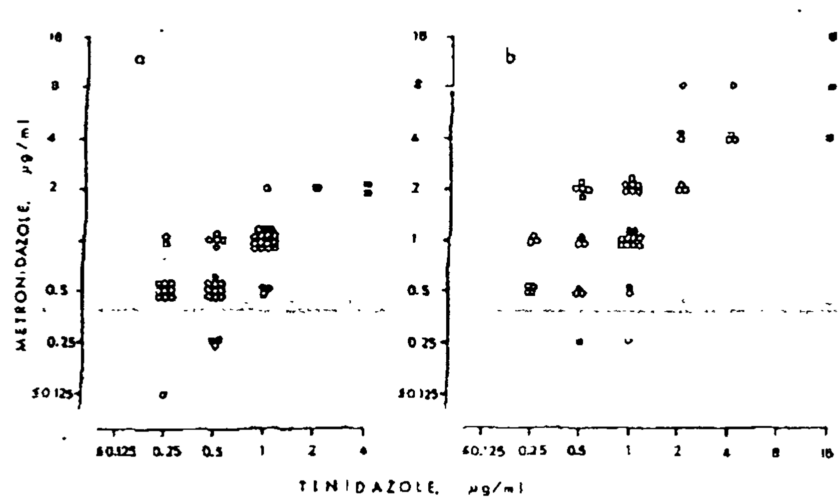
In another study[72], the activity of tinidazole against 42 clinical isolates of *B. fragilis* was tested using the agar and broth dilution methods. The Muller-Hinton agar supplemented with 10% lysed human blood and thioglycollate broth was used for testing. The inoculum size for the agar dilution method was not specified. However, an inoculum of 10^5 to 10^6 cfu/ml was used for the broth dilution method. The MIC and MBC were determined after incubating cultures in the presence of drug anaerobically at 37°C for 24 hours. The results in Table 47 show that the tinidazole MICs against *B. fragilis* isolates were ≤ 2 μg/ml and similar to metronidazole and clindamycin. The tinidazole MIC values against the *B. fragilis* isolates were similar to those observed in the previous studies.

Table 47: MIC of clindamycin, metronidazole and tinidazole for 52 anaerobic bacteria by agar dilution method.

MIC, μg/ml	Number of isolates of		
	B. fragilis	clostridia	peptostreptococci
Clindamycin			
≤0.125	12	2	4
0.25	6	0	1
0.5	16	0	1
1	7	1	0
2	1	0	0
4	0	1	0
Metronidazole			
≤0.125	0	1	1
0.25	2	0	0
0.5	15	0	4
1	20	2	1
2	5	1	0
Tinidazole			
≤0.125	0	1	0
0.25	8	0	0
0.5	20	0	3
1	10	2	3
2	4	1	0

The tinidazole and metronidazole MIC values against the *B. fragilis* isolates obtained using the broth dilution method was the same as by the agar dilution method (Table 47 and Figure 11). The tinidazole MBCs against *B. fragilis* were 4-fold higher than the MICs.

Figure 11: Comparison of the activities of metronidazole and tinidazole against 42 isolates of *B. fragilis* (o), 4 Clostridia (■), or 6 *Peptostreptococci* (●). MIC (a) and MBC (b) were determined by the tube dilution method.



In another study by the same group of investigators[73], the effect of inoculum size on the *in vitro* activity of tinidazole against 50 isolates of *B. fragilis* was examined. Metronidazole and ornidazole were used as comparators. The testing was performed using a different medium [brain heart infusion broth supplemented with yeast extract (5 μg/ml) and cysteine (1 μg/ml)]. The cultures were incubated in the presence of drug under anaerobic conditions for 24 hours. The authors have stated that activity against 50 isolates was measured. However, the results for only 20 isolates are shown. The results in Table 48 show that the MIC and MBC values for tinidazole, metronidazole and ornidazole varied 2 to 4-fold with increase in inoculum from 10^3 to 10^7 cfu/ml. The activity of tinidazole against *B. fragilis* isolates was similar to metronidazole and ornidazole. The median tinidazole MIC value against the *B. fragilis* isolates was within the MIC range observed in previous studies. The authors have stated that increasing the incubation time from 24 to 48 or 72 hours did not alter the MIC. However, the data were not included for review.

Table 48: Effect of innoculum size on the MIC and MBC of metronidazole, tinidazole or ornidazole against *B. fragilis*.

Inoculum CFU/ml in final broth	**MIC (μg/ml)***			**MBC_{99} (μg/ml)***		
	Metronidazole	**Tinidazole**	**Ornidazole**	**Metronidazole**	**Tinidazole**	**Ornidazole**
10^3–10^4	0·125	0·125	0·125	0·25	0·125	0·25
10^4–10^5	0·25	0·125	0·25	0·5	0·5	0·5
10^5–10^6	0·5	0·25	0·5	0·5	0·5	0·5
10^6–10^7	0·5	0·25	0·5	0·5	0·5	1·0

*** Median results of 20 strains. MBC_{99} is the lowest concentration that killed 99% of the original inoculum.**

In another study by the same group of investigators[74], the *in vitro* activity of tinidazole and other antimicrobial agents against 33 isolates of *B. fragilis* and 14 isolates of *Bacteroides* species (including *B. distasonis*, *B. ovatus*, *B. thetaiotamicron*, *B. uniformis* and *B. vulgaris*) was measured by the broth dilution method. The susceptibility testing was performed using a different medium (Schaedler's broth) and an inoculum of 10^3 to 10^5 cfu/ml. The cultures were incubated anaerobically in the presence of drug at 37°C for 24 hours and the MIC determined. *B. fragilis* ATCC 23745 was used as a quality control strain. The metronidazole MIC for *B. fragilis* ATCC 23745 was 0.63 μg/ml and within the range expected using NCCLS methodology (Table 49). The results in Table 50 show that the tinidazole MICs against the 16 isolates were similar to metronidazole MICs and to that observed in previous studies.

Table 49: Activity against *B. fragilis* ATCC 23745.

Compound	Geometric mean MIC*	
	μM	μg ml
Dimetridazole	10.0	1.41
Metronidazole	3.7	0.63
Secnidazole	3.7	0.68
Ornidazole	3.7	0.81
Tinidazole	0.8	0.20
Carnidazole	6.3	1.54
Panidazole	2.2	0.50

* Three experiments each with the seven compounds in parallel

Table 50: Activity against 16 clinical isolates of *B. fragilis*

Compound	MIC			
	μM		μg ml	
	Geometric mean	Range	Geometric mean	Range
Dimetridazole	6.39	2.0–20	0.90	0.28–2.82
Metronidazole	2.37	0.5–5.0	0.41	0.09–0.86
Secnidazole	2.59	0.5–5.0	0.48	0.09–0.93
Ornidazole	1.45	0.5–5.0	0.32	0.11–1.10
Tinidazole	0.49	0.1–2.0	0.12	0.02–0.49
Carnidazole	5.23	2.0–20	1.28	0.49–4.89
Panidazole	1.15	0.5–5.0	0.27	0.12–1.16

In another study[66], the susceptibility of one *B. fragilis* isolate (obtained from a patient with intestinal surgery) to tinidazole and metronidazole was performed by the agar dilution method using blood agar base No. 2 with 2% defibrinated horse blood and an inoculum of 10^7 cfu/ml. In addition, the effect of the drug on bacterial morphology was examined by SEM. Cultures were incubated in the presence of drug in 10% CO_2 at 37°C for 48 hours and the MIC determined. The tinidazole MIC against the *B. fragilis* isolate (0.8 µg/ml) was within the MIC range observed in previous studies and 3-fold lower than the metronidazole MIC (3.1 µg/ml).

The activity was also determined by the agar diffusion method using the same media and culture conditions except that the cultures were incubated for 24 hours. At the end of the 24 hour incubation, the agar pieces were also processed for SEM. For *B. fragilis*, the diameter of growth inhibition zone at a concentration of 50 µg/ml tinidazole and 16 µg/ml metronidazole were 32 and 28 millimeter, respectively. The authors have stated that the cells of *B. fragilis* within 10 mm from the zone of growth inhibition for metronidazole and tinidazole were elongated.

In another study[69], the *in vitro* activity of tinidazole and metronidazole against 9 isolates of *B. fragilis* was examined by the NCCLS method described previously on page 46. The tinidazole MICs against the 9 *B. fragilis* isolates ranged from [redacted] (Table 42, page 47). The tinidazole MIC_{90} values against the *B. fragilis* isolates were 2-fold lower than metronidazole MIC_{90} values.

Overall, the MIC_{90} values of tinidazole against 189 isolates of *Bacteroides* species (mostly *B. fragilis*) tested in 5 laboratories using different susceptibility methods ranged from [redacted]. Also, the activity of tinidazole against *B. fragilis* was similar to metronidazole in the different studies (Table 51). The tinidazole MBCs against the *Bacteroides* species were 2 to 8 fold higher than the MICs. Elongation of *B. fragilis* cells was observed after 24 hours of exposure to tinidazole by SEM.

Table 51: Summary of the tinidazole MIC/MBCs (mg/ml) against *Bacteroides species* (mostly *B. fragilis*).

Reference	Organism	No. of isolates	Method	Media	Inoculum (cfu/ml or cfu/spot)	Incubation period	Tnz MIC_{90}	Mtz MIC_{90}
Wise *et al.*, (1977)[70]	*B. fragilis*	40[a]	Agar dilution	DST agar	10^2	18	0.12	0.25
	B. fragilis	20[a]	Agar dilution	DST agar	10^5	18	4	-
	B. fragilis	3[a]	Agar dilution	DST + 5% LHB	10^5	18	-	-
Reynolds *et al.*, (1975)[71]	*B fragilis* isolates and *Bacteroides* species	61 (45 clinical isolates of *B fragilis*)	Agar dilution	Brain heart infusion + 5% horse blood	NA	42	1.0	1.0
Jopkii and Jopkii (1977a)[72]	*B fragilis*	42[b]	Agar dilution	Muller Hinton agar + 10% human blood	NA	24	2.0	2.0
	B fragilis	42[b]	Broth dilution	Thioglycollate broth	10^5 - 10^6	24	1.0	1.0
Jopkii and Jopkii (1977b)[73]	*B fragilis*	20	Broth dilution	Brain heart infusion with yeast extract and cysteine	10^3 - 10^4	24	0.13*	0.12*
					10^4 - 10^5	24	0.13*	0.25*
					10^5 - 10^6	24	0.25*	0 5*
					10^6 - 10^7	24	0.25*	0.5*
Jopkii and Jopkii (1985)[74]	*B. fragilis*	16	Broth dilution	Schaedler broth	10^3 - 10^5	24	0.12**	0.41**
^karin and ardh (1981)[66]	*B fragilis*	1	Agar dilution	Blood agar	10^7	48	0.8[#]	3.1[#]
Hillier (2002)	*B fragilis*	9	Agar dilution	Brucella Agar + hemin + Vitamin K	10^5	48	2.0	4.0
Total		**189**					**0.12 – 4.0**	**0.25 – 4.0**

#only one strain tested; * median MIC values; ** mean MIC values;
NA = not available; Mtz = metronidazole; Tnz = tinidazole
DST = Diagnostic Sensitivity Test agar; LHB = lysed human blood
[a]same isolates tested under different conditions;
[b]same isolates tested using two methods;

Mobiluncus species:

The *in vitro* activity of tinidazole against *M. curtissi* (n = 12) and *M. mulieris* (n = 10) isolates was measured using the microbroth dilution method[75]. The testing was performed using an inoculum of 10^6 cfu/ml and Wilkins-Chalgreen broth enriched with 1% soluble starch and 2% rabbit serum. The cultures were incubated at 35°C for 48 hours in an atmosphere of 10% CO_2 and the MIC determined. *B. fragilis* ATCC 25285 strain was used as a quality control strain for testing, however, MIC data for the strain was not included. The results in Table 52 show that the tinidazole MIC_{90} value against the *M. curtissi* and *M. mulieris* isolates were >256 µg/ml and 8 µg/ml, respectively. The tinidazole MIC_{90} against *M. curtissi* was similar to that of metronidazole but 2048-fold higher than clindamycin. Against *M. mulieris*, tinidazole MIC_{90} was 32-fold lower than metronidazole but 129-fold higher than clindamycin.

Table 52: MICs (μg/ml) of 23 antimicrobial agents against 12 isolates of *M curtissi* and 10 isolates of *M. mulieris.*

Agent	MIC range tested	*M. curtisii*			*M. mulieris*		
		MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
Ampicillin	0.015–16	0.125–0.25	0.125	0.25	≤0.015–0.062	0.031	0.062
Cefazolin	0.2–200	≤0.2–1.56	0.78	1.56	≤0.2–0.78	0.2	0.4
Cefoxitin	0.062–64	1–4	2	4	0.25–0.5	0.25	0.5
Cephalothin	0.2–200	0.78–1.56	1.56	1.56	≤0.2–0.4	0.4	0.4
Chloramphenicol	0.062–64	4–8	8	8	2–4	4	4
Clindamycin	0.015–16	0.062–0.125	0.125	0.125	≤0.015–4	0.031	0.062
Colistin	4–4,096	32–64	64	64	64–256	128	256
Cycloserine	0.2–200	200–>200	200	>200	100–>200	100	>200
Erythromycin	0.2–200	≤0.2	≤0.2	≤0.2	≤0.2–>200	≤0.2	≤0.2
Hydroxymetronidazole	0.5–512	128–512	256	512	4–>512	8	128
Imipenem	0.004–4	0.031–0.125	0.062	0.125	0.031–0.062	0.062	0.062
Josamycin	0.004–4	0.008–0.015	0.015	0.015	≤0.004–>4	0.004	0.004
Metronidazole	0.5–512	32–512	128	256	2–>512	4	256
Moxalactam	0.062–64	1–4	4	4	≤0.062–0.5	0.25	0.5
Nalidixic acid	0.3–200	200–>200	>200	>200	100–200	200	200
Neomycin	0.062–64	2–8	4	8	4–8	4	8
Penicillin G	0.004–4	0.015–0.125	0.062	0.062	0.008–0.015	0.015	0.015
Rifampin	0.004–4	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004
Tetracycline	0.2–200	0.39–12.5	6.25	12.5	≤0.2–6.25	≤0.2	6.25
Tinidazole	0.5–256	>256	>256	>256	4–>256	4	8
Tobramycin	0.031–64	0.25–1	1	1	0.25–1	0.5	1
Vancomycin*	0.001–1	0.5	0.5	0.5	0.5–1	0.5	0.5
Virginiamycin	0.2–200	≤0.2	≤0.2	≤0.2	≤0.2–0.39	≤0.2	≤0.2

* Concentrations from 2 to 1,024 μg/ml were included for testing of the quality control organism only.

Fusobacterium species:

The *in vitro* activity of tinidazole, metronidazole and nimorazole against 8 isolates of *Fusobacterium* spp. was examined by the agar dilution method[71]. A 1:500 dilution of an overnight culture (grown in 1 ml thioglycollate broth anaerobically at 37°C) was used to inoculate brain heart infusion agar supplemented with 5% lysed defibrinated horse blood containing different concentration of drug (the final inoculum size i.e., cfu/spot was not specified). The cultures were incubated anaerobically in the presence of drug at 37°C for 42 hours and the MIC determined. The results in Table 46 (page 49) show that the activity of tinidazole against *Fusobacterium* species (MIC 0.125 - 0.5 μg/ml) was similar to metronidazole (MIC 0.062 - 1 μg/ml).

Peptostreptococci species:

The *in vitro* activity of tinidazole against 6 clinical isolates of *Peptostreptococci* spp. was tested using the agar and broth dilution methods[72]. The Muller-Hinton agar supplemented with 10% lysed human blood and thioglycollate broth was used for testing. The inoculum size for the agar dilution method was not specified. However, an inoculum of 10^5 to 10^6 cfu/ml was used for the broth dilution method. The MIC and MBC were determined after incubating cultures in the presence of drug anaerobically at 37°C for 24 hours. The results in Table 47 (page 50) show that the tinidazole MICs against *Peptostreptococci* isolates were ≤1 μg/ml and similar to metronidazole but 2-fold higher than clindamycin.

Pervotella and *Porphyromonas* species:

The *in vitro* activity of tinidazole and metronidazole against isolates of *Pervotella* (n = 218) and *Porphyromonas* (n = 43) species was examined using the NCCLS method described previously on page 46[69]. The results in Table 42 (page 47) show that the activity of tinidazole is similar to metronidazole against the *Pervotella* and *Porphyromonas* species.

***Lactobacillus* species:**

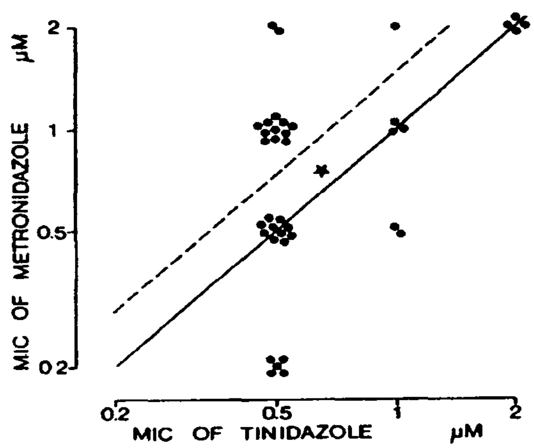
The *in vitro* activity of tinidazole and metronidazole against 24 clinical isolates of *Lactobacillus* species was examined using the NCCLS method as described previously on page 46[69]. The results in Table 42 (page 47) show that the tinidazole and metronidazole MIC_{90} values against the isolates of *Lactobacillus* species were high ([redacted] and similar to that against *G. vaginalis*. The tinidazole MIC_{90} against the *Lactobacillus* species were >32-fold higher than that for the anaerobic bacteria other than *G. vaginalis*.

***Clostridium* species:**

The *in vitro* activity of metronidazole and tinidazole against 38 strains of *Clostridium difficile* was examined[76]. In this study, cultures in Schaedler broth were incubated anaerobically using a GasPak system at 37°C for 24 hours. Following growth, cultures were serially diluted to 1:1,000 in pre-reduced Schaedler broth supplemented with metronidazole or tinidazole and incubated for 24 hours. MIC was defined as the lowest concentration without visible turbidity. The result of the study shows that the geometric mean MIC of metronidazole and tinidazole against *C. difficile* was approximately 0.74 µM (0.13 µg/ml) and 0.65 µM (0.16 µg/ml), respectively (Figure 12).

A comparative study on the activity of metronidazole and tinidazole against some clostridia species was also performed. The data compiled from 8 publications show tinidazole to be slightly less active than metronidazole against *C. perfringens* and other clostridia species (Table 53).

Figure 12:



Comparison of metronidazole and tinidazole against 38 strains of *C. difficile*. Each dot is one strain, the star is the geometric mean, and the position of equal activity of the two drugs is illustrated by the continuous line (MICs as micromolar concentrations) or the broken line (MICs as micrograms per milliliter).

Table 53: Comparisons of metronidazole and tinidazole against *C. perfringens*, and other clostridia in the literature.

Bacteria and reference no.	No. of strains	Geometric mean MIC (μg/ml)	
		Metronidazole	Tinidazole
C. perfringens			
	1	1.6	6.4
	254	0.03	0.28
	4	1.19	1.41
	9	0.50	0.54
	20	0.55	4.90
Other clostridia			
	7	1.45	3.52
	79	0.16	0.16
	14	0.33	0.42

Veillonella species:

The *in vitro* activity of tinidazole against 4 strains of *Veillonella* species was compared to ornidazole and metronidazole[77]. For this, an inoculum of 2 x 10^3 to 2 x 10^4 cfu/ml was used to spot inoculate Brain heart infusion agar supplemented with yeast extract and cultures incubated anaerobically at 37°C for 48 hours. The MIC defined as the lowest concentration of the drug showing no growth, a barely visible haze, or one discrete colony, was determined. The MBC at 48 hours was determined by replica plating onto the same media without drug. The results in Table 54 show that the activity of tinidazole (MIC = 1.6 μg/ml) was 2-fold lower than ornidazole and metronidazole (0.8 μg/ml) against the 4 *Viellonella* species.

Table 54: Inhibitory and bactericidal effect of metronidazole, ornidazole, and tinidazole on *Veillionella* species.

Drug	Cumulative number of strains inhibited (killed) at various drug concentration		
	0.4 μg/ml	**0.8 μg/ml**	**1.6 μg/ml**
Metronidazole	2 (2)	4 (4)	-
Ornidazole	2 (2)	4 (4)	-
Tinidazole	2 (2)	2 (4)	4 (4)

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A-6

Table 56:

In another study, the *in vitro* activity of eight antimicrobial agents against *H. pylori* was investigated[79]. A total of 18 clinical isolates of *H. pylori*, originally isolated from the gastric mucosa of children (ages 6-17 years), were studied. Information regarding the exact time of gastric biopsy and the procedure used for storage and transportation was not provided. However, the isolates were identified by Gram-stain, catalase, oxidase, and urease test. The cultures were passaged three times to ensure reliable growth and purity. MICs were determined by the agar dilution method with the appropriate antimicrobial agents in concentration ranging from 0.008 - 128 µg/ml. Samples were prepared by first suspending the organism in Mueller-Hinton broth and adjusting the turbidity to 1.0 McFarland standard. Approximately 10^4 CFU per spot was plated onto agar plates and incubated at 37°C for 48 hours under microaerophilic conditions. Since there are no metronidazole and tinidazole MIC interpretative criteria established for *H. pylori*, susceptible or resistant results were interpreted in accordance with the NCCLS guidelines for anaerobic organisms. The MIC values for metronidazole and tinidazole ranged between 1-16 µg/ml, and 0.25 - 2 µg/ml, respectively. The MIC_{50} and MIC_{90} for metronidazole were 2 and 4 µg/ml, respectively. The tinidazole MIC_{50} and MIC_{90} values against *H. pylori* were only two-fold lower than metronidazole (Table 57).

Table 57:

Antimicrobial susceptibilities of 18 clinical isolates of *H. pylori* to omeprazole and other antimicrobial agents

Antimicrobial agent	MIC (µg/ml)*		
	Range	50%	90%
Ampicillin	≤0.03–0.25	≤0.03	≤0.03
Azithromycin	≤0.25	≤0.25	≤0.25
Cefixime	≤0.008–≥4	0.06	0.5
Ciprofloxacin	0.06–0.5	0.25	0.5
Doxycycline	0.25–8	0.5	2
Erythromycin	0.008–0.125	0.03	0.06
Metronidazole	1–16	2	4
Tinidazole	0.25–2	1	2
Omeprazole	16–256	64	128

* 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

In another study, the *in vitro* activity of tinidazole and 5 other antimicrobial agents, including metronidazole was tested by the agar dilution method against 15 clinical isolates of *H. pylori*[80]. Clinical isolates were obtained from gastric biopsy specimen from patients and identified by standard methods. Isolates were maintained in Tryptic soy agar supplemented with 15% glycerol at -70°C until needed.

Bacterial concentrations were established by transferring known aliquots to parallel plates without the presence of antimicrobial agents. For susceptibility testing, cultures were diluted to conform to MacFarland standard in 0.9% NaCl with the appropriate antimicrobial agents and incubated in a microaerophilic atmosphere at 36.5°C for a minimum of 3 days. However, the exact MacFarland number was not given. Antimicrobial resistance was characterized by the appearance of discrete colonies following 6 days of incubation. The metronidazole and tinidazole MICs were ≤ 4.0 µg/ml. By increasing the inoculum size from 10^3 to 10^7 cfu/spot, the average MIC was increased by a factor of 21.2 for metronidazole and 17 for tinidazole (Table 58).

Table 58: Effect of inoculum size on the mean MIC of six antimicrobial agents for *H. pylori*.

Antimicrobial agent	Mean MIC in mg/l (SE) at the following inoculum sizes (cfu/spot)				
	10^3	10^4	10^5	10^6	10^7
Ampicillin	0.03 (0 039)	0 04 (0 05)	0.04 (0.043)	0.06 (0.05)	0.07 (0.047)
Erythromycin	0 02 (0 002)	0 03 (0 005)	0 05 (0 015)	0.07 (0 02)	0.06 (0.013)
Tetracycline	0 06 (0 026)	0.17 (0.043)	0 26 (0 067)	0 32 (0.08)	0.38 (0.12)
Chloramphenicol	6.22 (2.06)	14.73 (3 20)	19 8 (3 36)	24 0 (2 70)	26.67 (3.37)
Metronidazole	0 11 (0 014)	0 21 (0 067)	0.39 (0 131)	1 71 (0 38)	2.33 (0.61)
Tinidazole	0 13 (0 010)	0 18 (0.029)	0.56 (0 126)	1 20 (0.28)	2.2 (0.488)

The pharmacodynamic effect of metronidazole and tinidazole alone and in combination with clarithromycin on *H. pylori* (NCTC 11637) was investigated[81]. Briefly, the study evaluated the initial killing, post-antibiotic effect (PAE), effective re-growth time (ERT), and control-related effective re-growth time (CERT). However, instead of monitoring endpoints such as MICs and MBCs, the study monitors the time course of bacterial responses to ATP and relies on information obtained by monitoring the bioluminescence of intracellular ATP.

Mueller-Hinton broth, supplemented with calcium, magnesium and 1% fetal calf serum was used as growth medium. MICs were determined by the E-test method following 3 days of incubation at 37°C under microaerophilic conditions. Intracellular ATP was monitored by Luciferase reagents and ATP monitoring reagents. Sample ATP levels were calculated by using standard amounts of ATP as reference[82]. The result of the study shows that the clarithromycin, metronidazole and tinidazole MICs were 0.032, 0.094 and 0.094 µg/ml, respectively. Clarithromycin, metronidazole and tinidazole induced CERT that is both concentration and exposure time dependent (Figure 13, Table 59).

Figure 13: CERTs for clarithromycin, metronidazole, and tinidazole, clarithromycin in combination with metronidazole, and clarithromycin in combination with tinidazole after 5 h of exposure. The concentrations of the antibiotics singly and in the combinations in cultures 1-8 are given in Table 59 below.

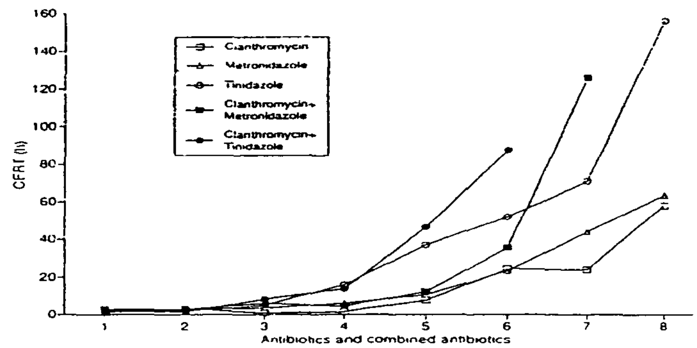


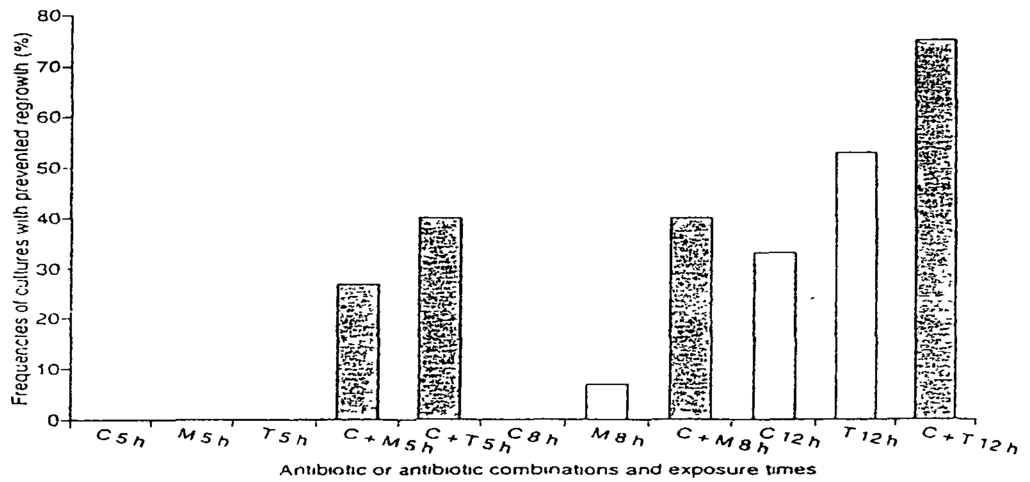
Table 59: CERTs induced by clarithromycin, metronidazole, timidazole, clarithromycin combined with metronidazole and clarithromycin combined with tinidazole after different incubation times.

Antibiotic and culture no	Concn (μg/ml)	Incubation times (h)	CERT[a] (h) Ranges	Medians
Clarithromycin				
1	0 0075	5, 8, 12	4–6 0–2, 0–5	4, 2, 2
2	0 015	5 8 12	0–3, 0–5, 0–12	2, 5, 4
3	0 03	5 8 12	0-6, 0–4, 0–7	3, 4, 2
4	0 06	5 8, 12	7–7, 1–3, 0-25	7, 2, 5
5	0.125	5, 8 12	9-16, 5–7, 7-88	12, 6, 43
6	0 25	5, 8, 12	23–26, 16–23, 30–77[c]	24, 23, 53[e]
7	0 5	5 8, 12	16–32, 24–55, 87–>240[c]	24, 44, >240[c]
8	1	5, 8, 12	54–62, 70–94, 142–>240[c]	58, 86, >240[c]
Metronidazole				
1	0 06	5, 8	2–3, 1–9	2, 2
2	0 125	5, 8	2–3, 2–3	2, 2
3	0 25	5, 8	3–4, 1–3	3, 1
4	0.5	5, 8	6–6, 2–13	6, 7
5	1	5, 8	2–19, 7–52	11, 10
6	2	5, 8	19–28, 25–82	24, 40
7	4	5, 8	30–58, 58–109	44, 86
8	8	5, 8	53–74, 75–>240[b]	64, 79[b]
Tinidazole				
1	0 06	5, 12	1–3, 0–3	2, 2
2	0.125	5, 12	2–3, 0–3	2, 1
3	0.25	5 12	3–6, 2–11	5, 6
4	0 5	5, 12	8–17, 10–38	16, 38
5	1	5 12	25–48, 22–>240[b]	37, 49[b]
6	2	5, 12	32–60, 109–>240[c]	52, 240[c]
7	4	5, 12	65–84, >240[d]	71, >240[d]
8	8	5, 12	156, 125–>240[c]	156, >240[c]
Clarithromycin-metronidazole				
1	0 075 + 0 06	5 8	0–9 2–7	0, 2
2	0 015 + 0 125	5 8	0–9, 2–7	0, 4
3	0 03 + 0.25	5, 8	0-10, 3–6	8 3
4	0 06 + 0 5	5, 8	0–10, 6–12	3, 12
5	0 125 + 1	5, 8	6–20, 16–42	11, 28
6	0 25 + 2	5, 8	19–49, 60–>240[b]	39, 94[b] (S[e])
7	0.5 + 4	5, 8	85–167[h] (S), 135–>240[c] (S)	167[b] (S), >240[c] (S)
8	1 + 8	5, 8	>240[d] (S), >240[d] (S)[f]	>240[d] (S), >240[d] (S)[f]
Clarithromycin-tinidazole				
1	0 075 + 0 06	5, 12	1–4, 0-4	2, 2
2	0 015 + 0 125	5, 12	0–4 2–19	1 9
3	0 03 + 0 25	5 12	0 18, 5–36	8, 21 (S)
4	0 06 + 0.5	5 12	2–24, 18–>240[b]	13, 23[b] (S)
5	0 125 + 1	5, 12	13–100, 65–>240[b]	46 72[b] (S)
6	0 25 + 2	5 12	84–>240 (S), >240[c]	87[b] (S), >240[d] (S)
7	0 5 – 4	5 12	>240[d] (S),[h] >240[d]	>240 (S),[h] >240[d]
8	1 + 8	5 12	200–>240[c] (S),[g] >240[d]	>240[d] (S),[g] >210[d]

[a] The values are the means of three experiments
[b] No regrowth in one of three experiments
[c] No regrowth in two of three experiments
[d] No regrowth in three of three experiments
[e] S, synergism
[f] $P < 0.005$ (two-tailed paired t test)
[g] $P < 0.01$ (two-tailed paired t test)
[h] $P < 0.001$ (two-tailed paired t test)

When single antimicrobial agents were tested, clarithromycin induced the longest CERT followed by metronidazole and tinidazole. Clarithromycin, metronidazole and tinidazole did not prevent re-growth following 5 hours of exposure time (Figure 14). However, metronidazole prevented re-growth of < 7% of the culture after 8 hours of exposure. Tinidazole was not tested at 8 hours.

Figure 14: Frequencies of prevention of regrowth in cultures after different times of exposure to the five highest concentration of clarithromycin (C), metronidazole (M), tinidaxole (T), clarithromycin in combination with metronidazole (C + M), and clarithromycin in combination with tinidazole (C + T).



In drug combination studies, CERTs induced by clarithromycin in combination with metronidazole or tinidazole, were found to be concentration and exposure time dependent (Table 59 and Figure 14). CERTs induced by clarithromycin and tinidazole were longer than the combinations of clarithromycin and metronidazole, indicating that this is a more effective combination. In summary, the effect produced by the drug combination is greater than the effect produced by the drug alone. Please note that the number of cultures tested against each drug or the combination of drugs was not specified.

2.2.3. Effect of protein binding:

The effect of serum proteins on the activity of tinidazole was investigated against 3 *Bacteroides* isolates by the agar dilution method[70]. The testing was performed in the presence of 25% serum, 100% serum or 5% lysed human blood and an inoculum of 10^5 cfu/ml was used for MBC measurement. In the presence of 5% lysed human blood, MBC was 4 to 8-fold higher than the MIC values (Table 45, page 49). Please note that the MIC values were measured in media in the absence of lysed human blood or serum.

The tinidazole MBC values in the presence of 25 and 100% serum were 2 to 16-fold higher than the MIC values under similar experimental conditions. In the presence of 100% serum, MBC was 4 to 8-fold higher than in the presence of 25% serum. It appears that the activity of tinidazole may be decreased in the presence of serum proteins.

Effect of protein binding on the activity of tinidazole against protozoa was not measured.

2.2.4. Activity of the metabolites:

The activity of the metabolite against *G. vaginalis* was investigated *in vitro*[63]. The experimental procedures are reviewed in section 2.2.2. The hydroxy metabolites of tinidazole and metronidazole were more active than the parent compounds (Table 37, page 43).

In another study, the *in vitro* activity of the tinidazole, metronidazole and their metabolites was tested against ten clinical isolates and a reference strain of *G vaginalis*[64]. The details of the experimental procedures are covered in section 2.2.2. The result of the study indicates that the hydroxy metabolites of both tinidazole and metronidazole are more active than the parent compound (Table 38, page 44). The study also showed the hydroxy metabolite of tinidazole to be more active than the hydroxy metabolite of metronidazole. The MICs of tinidazole and hydroxy metabolite of tinidazole were similar to that reported in the previous study. The acid metabolite of metronidazole was inactive and no studies were conducted on the acid metabolite of tinidazole.

Activity of the metabolite against protozoa was not determined.

2.3. Activity *in vivo*:

2.3.1. *T. vaginalis* and *T. foetus*:

The *in vivo* activity of tinidazole against *T. vaginalis* and *T. foetus* (bovine strain) was examined in mice. The trichomonads were inoculated either intravaginally, intraperitoenally, or subcutaneously and the effective dose determined. The intraperitoneal inoculations produce visceral lesions and can lead to death in the mice while subcutaneous inoculations produce localized abscess. Although the intraperitoneal and subcutaneous mice models can provide some information on the activity of the drug against trichomonads, the intravaginal mice model more closely mimics the disease in human.

In another study[33], the activity of tinidazole against the *T. vaginalis,* strain TR, was examined in NMRI mice. The mice were inoculated intraperitoneally with 1-2 x 10^6 trophozoites/ml. Tinidazole or other nitroimidazole drugs were administered orally 2 hours before and after infection and on days 1 and 2 of infection. The mice were sacrificed on day 5 of infection and the peritoneal exudates or washings examined for the presence of trophozoites by wet mount and culture. The thioglycollate medium was used to culture *T. vaginalis*. The animals were considered cured if the culture of the peritoneal exudates/washings were negative. The effective dose to cure 50% (ED_{50}) and 95% (ED_{95}) animals was determined. The raw data for the parasite counts were not included. Also, metronidazole was not used as a comparator.

The tinidazole ED_{95} value against *T. vaginalis* in the intraperitoneal model was 8.8 mg/kg (Table 61). Tinidazole was less active than the experimental drug, HOE239, against *T. vaginalis* in the intraperitoneal model.

Table 61: Activity of various 5-nitro imidazole against *T. foetus* and *T. vaginalis* in mice.

			Effective doses with confidence limits (P = 0·05) (mg kg⁻¹)	
Compound parasite	*Application*	*No. animals*	*ED50%*	*ED95%*
Tritrichomonas foetus-peritonitis (NMRI-mouse)				
HOE 239		589	2·0 (1·8–2·2)	3·6 (3·0–4·3)
HOE 239 (sulphoxide)		78	1·4 (0·9–2·1)	5·6 (3·3–9·4)
HOE 239 (sulphone)	−2 h/+2 h	100	2·3 (1·8–2·9)	6·6 (4·3–10·0)
Metronidazole	Oral	880	21·0 (19·8–22·6)	44·0 (38·5–50·8)
Tinidazole		234	8·3 (7·4–9·3)	15·0 (12·9–17·7)
Ornidazole		60	11·9 (10·3–13·5)	15·3 (13·6–20·1)
Nitrimidazine		30	42·0 (33·8–53·0)	77·8 (52·0–117·0)
Trichomonas vaginalis (TR14)-peritonitis (NMRI-mouse)				
HOE 239	−2 h/+2 h	35	3·3 (2·7–3·9)	4·5 (3·4–5·9)
Tinidazole	Oral	20	7·0 (6·2–7·9)	8·8 (7·1–10·7)
Tritrichomonas foetus-sc abscess (hairless mouse)				
HOE 239	−2 h/+2 h/D+1/D+2	55	11·6 (10·2–13·3)	17·3 (13·2–22·8)
Tinidazole	Oral	44	26·6 (20·7–34·2)	53·5 (32·1–89·0)
Trichomonas vaginalis (TR14)-sc abscess (hairless mouse)				
HOE 239	−2 h/+2 h/D+1/D+2	72	7·6 (6·7–8·6)	11·1 (8·7–14·2)
Tinidazole	Oral	44	18·8 (15·4–23·0)	31·2 (22·0–44·1)

In another experiment, hairless mice were inoculated subcutaneously with the same number of trophozoites. The drugs were administered orally 2 hours before and after infection and on days 1 and 2 of infection. The animals were sacrificed 9 days post-infection. Animals that developed no abscess or were negative by culture of abscess material were considered cured. The ED_{50} and ED_{95} were determined as described above. The raw data on the parasite counts were not included. The ED_{95} value of tinidazole against *T. vaginalis* in mice infected subcutaneously was 3-fold higher than in mice infected intraperitoneally (Table 61).

In another study[83], the activity of tinidazole and other nitroimidazoles was tested against *T. vaginalis* in mice inoculated by the subcutaneous or intravaginal route. For subcutaneous infection, 4 x 10^5 trophozoites were inoculated into each shaved flank of NMRI mice. The drugs were administered orally at 2, 18 and 24 hours post-infection. After 6 days of infection, the presence of lesions or trophozoites in wet mounts of abscess material was determined. Animals with no lesions or trophozoites in abscess material were considered cured and ED_{50} values determined.

For intravaginal infection, the mice were pretreated with 40 mg/kg estradiolundecylate by the subcutaneous and intraperitoneal routes, 3 days prior to infection. The mice were infected intravaginally with 1 x 10^5 trophozoites of *T. vaginalis* mixed with *C. albicans* (80:1 v/v ratio). The treatment method was same as that described above for the subcutaneous model. The vagina of the infected animals was rinsed with CACH medium. The number of motile trophozoites was determined by incubating the vaginal wash in CACH medium at 37°C for 48 hours and the ED_{50} values calculated. The results in Table 62 show that tinidazole was more active than metronidazole, nimorazole and ornidazole. The tinidazole ED_{50} values in mice infected intravaginally (ED_{50} = 1.41 mg/kg) were 5-fold lower than mice infected subcutaneously (ED_{50} = 7.50 mg/kg). This may be due to the route of infection, altered physiology from estrogen treatment or effect of mixed *T. vaginalis/Candida* infection.

Table 62:

Efficacy of several 5-nitroimidazole-derivatives against topical and ectopical infections with T. vaginalis in mice

Substance	Dosage (mg/kg × 3[a])	Model: T. vaginalis inoculation site intravaginally[b]	s.c.[c]
Metronidazole	ED_{50}[d]	3.71 (2.76–4.99)	10.95 (9.10–13.18)
Tinidazole	ED_{50}	1.41 (1.02–1.95)	7.50 (6.19–9.10)
Nimorazole	ED_{50}	5.62 (4.15–7.61)	33.95 (30.62–37.73)
Ornidazole	ED_{50}	4.57 (3.60–5.82)	10.59 (9.07–12.37)

[a] 2, 18 and 24 h p.i. orally. [b] 3 × 9 animals at each dosage level, mean infection rate of untreated control groups 94%. [c] 3 × 6 animals at each dosage level, infection rate of untreated control groups 100%. [d] ED_{50} according to Spearman-Karber. figures in brackets are 95% confidence limits.

The activity of tinidazole against the *T. foetus* strain H was examined in NMRI mice[33]. The mice were inoculated intraperitoneally with 1-2 x 10^6 trophozoites/ml. Tinidazole or other nitroimidazole drugs were administered orally 2 hours before and after infection (2 doses) or as a single dose at 4, 24, or 48 hours after infection. The mice were sacrificed on day 5 of infection and the peritoneal exudates or washings examined for presence of trophozoites by wet mount and culture. The Standard I bouillon medium was used to culture *T. foetus*. The animals were considered cured if culture of the peritoneal exudates/washings was negative. The effective dose to cure 50% (ED_{50}) and 95% (ED_{95}) animals was determined. The results in Table 61 (page 63) show that tinidazole was more active than metronidazole and the experimental drugs, orindazole and nitrimidazine, but less active than another experimental drug, HOE239, against *T. feotus* in the intraperitoneal mouse model.. The tinidazole ED_{95} value against *T. foetus* in the intraperitoneal model was 15 mg/kg. The ED_{50} values increased in mice treated on days 1 and 2 compared to 4 hours post-infection (Table 63).

Table 63: Activity of tinidazole and HOE 239 against intraperitoneal *T. foetus* infections in NMRI-mice after single oral dose at different times post infection.

Compound	*Time of treatment*	*No animals*	*ED_{50} with confidence limits (P = 0·05) (mg kg^{-1} × 1)*
HOE 239	+4 h	41	5 9 (4·2 -8 3)
	D+1	30	11 8 (9 1- 15 4)
	D+2	24	22 3 (15·8–31·4)
Tinidazole	+4 h	30	9 9 (8 8 -11·2)
	D+1	30	14·5 (11·5–18·3)
	D+2	30	26 (18 3–36·5)

In another study[31], the *in vivo* activity of tinidazole against *T foetus* was compared to metronidazole. For this, CR female mice (4 to 6 per group) were inoculated intraperitoneally with 5 x 10^5 trophozoites. After 24 hours of infection, drugs were administered orally or subcutaneously for 3 days. Peritoneal wash was obtained at 24 hours after discontinuation of therapy using standard trypticase serum base plus 6% serum (STS) medium. If no viable organism was observed in the peritoneal wash, the mice were considered to have cleared infection. The results in Table 64 shows that the minimal effective dose for tinidazole against *T. foetus* was 12.5 mg/kg either by oral or subcutaneous routes while that for metronidazole was 100 mg/kg.

Table 64: Therapeutic activity against *Trichomonas foetus* in mice

Route	Dose	Tinidazole			Metronidazole		
		No of trials	Total no. infected	Per cent cleared	No. of trials	Total no infected	Per cent cleared
Oral	mg/kg 200	1	5	100	7	33	100
	100	2	10	100	7	33	100
	50	4	20	100	7	33	56
	25	9	44	100	5	25	4
	12 5	9	44	100	2	12	0
	6 25	9	44	12			
Subcutaneous	100				1	5	100
	50				1	5	20
	25				1	5	0
	12 5	1	5	100			
	6 25	1	5	0			

In another study[84], the activity of tinidazole against the same species was examined using the same method. Other 2 methyl-5 nitroimidazole compounds were used as comparators. The results in Table 65 show that the minimum effective dose for tinidazole against *T. foetus* was same as in the previous study (12.5 mg/kg). The minimum effective dose for metronidazole against *T. foetus* was stated to be 100 mg/kg.

Table 65: Activity of some 1-substituted 2 methyl-5-nitroimidazoles in mice infected with *T. foetus*.

ACTIVITY OF SOME 1-SUBSTITUTED 2-METHYL-5-NITROIMIDAZOLES IN MICE INFECTED WITH *Trichomonas foetus*

No	MED (mg/kg)	No.	MED (mg/kg)
1	12.5	16	100
9	12.5	17	200
10	25	18	100
11	25	19	200*
12	100	21	50
13	50	22	200
14	100	2	100
15	25		

*Complete clearance at this level, but MED not established.

Compound #1 = tinidazole
MED = minimum effective dose

In summary, a 1.41 mg/kg dose of oral tinidazole was required for 50% reduction in trophozoite count in mice infected intravaginally with a mixture of *T vaginalis* and *C. albicans*. A higher tinidazole dose (7.5 mg/kg) was required to have a similar effect in mice infected intraperitoneally. A 1.4 to 2.6 fold higher dose of metronidazole compared to tinidazole was required for treatment in these 2 mouse models. The suppression of infection in 95% of mice infected intraperitoneally with the *T vaginalis* TR strain required a dose of 8.8 mg/kg tinidazole. A 3 fold higher dose of tinidazole was required for suppression of infection in mice infected subcutaneously with the same strain. .

The tinidazole ED_{50} values against *T. foetus* in the intraperitoneal mouse model ranged between 10 and 26 mg/kg. The tinidazole ED_{50} values in the intraperitoneal mouse model were 2 to 3.5 fold lower than the ED_{50} values in the subcutaneous mouse model.

2.3.2. *G. lamblia*:

The activity of tinidazole and other antiparasitic drugs against the strain BRIS/83/HEPU/106 of *G. lamblia* was examined in suckling mice[85]. The mice were inoculated intragastrically with 10^5 trophozoites. After 6 days of infection, different concentrations of the drugs [dissolved in phosphate buffered saline (PBS) containing 0.2% carboxymethylcellulose] were administered intragastrically as a single dose. Vehicle treated animals were used as controls. The animals were sacrificed 2 days post-treatment and the entire small intestine placed in 5 ml of PBS and trophozoites allowed to detach for 10 minutes. The number of trophozoites was counted using a hemocytometer. The effective dose required to kill 50% trophozoites (ED_{50}) was calculated. The results in Table 66 show that the ED_{50} value for tinidazole (2.8 mg/kg) was lower than metronidazole (40.8 mg/kg). In summary, at 2 days post-treatment, tinidazole was more active than metronidazole in reducing trophozoite counts in suckling mice infected intragastrically with *G. lamblia*.

Table 66: *In vivo* activity of drugs against *G. lamblia* BRIS/83/HEPU/106 in suckling mice.

	Drug	ED_{50} (P95 limits)* (mg/kg)
5-nitroimidazoles	ronidazole	1 0 (0·3–2·3)
	satranidazole	1·4 (1 1–1·9)
	fexinidazole	1 4 (1 1–1·7)
	flunidazole	2·2 (1·7–2·7)
	tinidazole	2 8 (2·0–3·7)
	ornidazole	4·5 (3 9–5·1)
	S75 0400 A†	6 4 (4 5–10·3)
	secnidazole	9·1 (4·9–28 1)
	nimorazole	16·5 (12·5–22·0)
	metronidazole	40 8 (30·7–58 0)
	panidazole	> 100
	ketoconazole	> 200
Other compounds		
	nitridazole	3·1
	paromomycin sulphate	7 6
	furazolidone	13 5
	quinacrine dihydrochloride	32 6
	nitrofurantoin	33·3
	emetine dihydrochloride	54 5
	amodiaquine base	79 1
	berberine sulphate	> 200
	chloroquine diphosphate	> 200
	sulphasalazine	> 200

*Dose required to kill 50% of the organisms with 95% confidence limits
†1-methyl-2-(4-dimethylamino-methyleniminο-phenoxymethyl)-5-nitroimidazole hydrochloride

The activity of tinidazole against the cyst stage of *G. lamblia* was not examined *in vivo*.

2.3.3. *E. histolytica*:

The activity of tinidazole against *E. histolytica* was examined in rats[31]. The rats (5 or 6 per group) were inoculated intracecally with approximately 2 x 10^5 trophozoites/ml. Different doses of tinidazole or metronidazole were administered orally once daily for 4 days. At 24 hours post-treatment, the number of rats that cleared the infection, and the average degree of infection (ADI) defined as the sum of pathology scores of individual rats divided by the number of rats, were determined. The pathology score assigned were as follows: 0, no amoeba or gross pathology; 1, few amoeba; 2, many amoeba, no cecal inflammation; 3, many amoeba, cecal lesions or inflammation; 4, many amoeba, cecal lesions, inflammation, and mucus. Both tinidazole and metronidazole at doses $\geq$ 50 mg/kg were effective (ADI $\leq$ 0.30) in clearing the infection due to *E. histolytica* in rats (Table 67).

Table 67: Therapeutic activity against *E. histolytica* in rats (5 or 6 rats per group).

Treatment	Dose	No of rats cleared/no infected	Avg degree of infection
	mg/kg		
Tinidazole	200	9/10	0 10
	50	7/10	0 30
	25	2/6	2 00
	6 25	13/24	1 02
	3 125	3/9	2 00
Metronidazole	200	5/6	0 17
	50	7/9	0 22
	25	3/6	1 33
	6 25	11/23	1 28
	3 125	4/9	1 56
Infected control	–	8/24	1 94

In another study[86], the activity of tinidazole was compared to other anti-amoebic drugs in hamsters with amoebic liver abscess. For this, small pieces of liver infected with the KAH2 strain of *E. histolytica* containing 20,000 trophozoites were used to inoculate anaesthesized healthy hamsters through an incision below the xiphisterum. The drugs were administered twice daily for 5 days immediately after infection. Emetine and dehydroemetine were administered intramuscularly while the other drugs including tinidazole were administered orally. Untreated infected animals were used as controls. The animals were sacrificed 24 hours after end of therapy and the amoebic liver abscesses produced in the livers were scored. The results in Table 68 show that emetine and dehydroemetine at 4 mg/kg. and metronidazole and tinidazole at 100 mg/kg, were effective in preventing the development of amoebic abscesses in hamsters. However, the parasite load in the liver was not measured.

Table 68: Screening of systemically active amoebicides against experimental hepatic amoebiasis produced in hamsters by a liver passaged strain of *E histolytica* (KAH2)

Drugs	Dose mg/kg/day*	No of animals infected/inoculated	Lesion grade	Average
Control	Nil	6/6	4 4 4, 4 3	3.83
Emetine HCl	4 mg/kg	0/6	0	0
Dehydroemetine HCl	4 mg/kg	0/6	0	0
Metronidazole	100 mg/kg	0/6	0	0
Tinidazole	100 mg/kg	0/5	0	0
Chloroquine	200 mg/kg	0/6	0	0
Mepacrine	200 mg/kg	0/5	0	0
Chloroquine	100 mg/kg	2/5	2 2 0 0 0	0.8
Mepacrine	100 mg/kg	0/6	0	0
Amodiaquine	200 mg/kg	0/5	0	0
Ambilhar	200 mg/kg	Toxic		—
Amodiaquine	100 mg/kg	1/5	2 0 0, 0 0	0.4
Ambilhar	100 mg/kg	1/5	1, 0 0, 0, 0	0.2
Ambilhar	50 mg/kg	2/5	2 2 0 0 0	0.8

* Drug given from day 0 to day 4

In summary, tinidazole (≥ 50 mg/kg) was effective in decreasing the degree of *E. histolytica* infection in rats at 24 hours post-treatment. At 100 mg/kg, the drug was effective in preventing development of amoebic liver abscess in hamsters. However, the activity of tinidazole against the cyst stage of *E. histolytica* was not examined *in vivo*.

2.3.4. Bacteria:

No studies were done to measure the activity of tinidazole against bacteria *in vivo*.

2.4. DRUG RESISTANCE:

The development of resistance to tinidazole by *T. vaginalis*, *G. lamblia*, and *E. histolytica* was not examined *in vitro* or *in vivo*.

2.5. CROSS-RESISTANCE:

The development of cross-resistance between tinidazole and metronidazole in *T. vaginalis* isolates and strains was examined *in vitro* and *in vivo*.

In vitro:

The cross-resistance between tinidazole and metronidazole was examined using laboratory strains with high metronidazole MIC (strain A [redacted]) and clinical isolates obtained from patients' [redacted]

The tinidazole MIC and MLC against *T. vaginalis* strain A (metronidazole MIC of 100 μg/ml under aerobic conditions) and 4 strains with low metronidazole MIC (0.6 to 3.1 μg/ml under aerobic conditions) was examined as described on page 17. The tinidazole MIC and MLC values against strain A were higher (60 fold higher under aerobic conditions and 7 fold higher under anaerobic conditions) than against the strain with low metronidazole MIC, suggesting cross-resistance between metronidazole and tinidazole (Table 9, page 18).

[redacted]

The cross-resistance between tinidazole and metronidazole was not examined against *G. lamblia* and *E. histolytica in vitro*.

In vivo:

[redacted]

The cross-resistance between tinidazole and metronidazole was not examined against *G. lamblia* and *E. histolytica in vivo*.

3. CLINICAL MICROBIOLOGY:

3.1. Trichomoniasis:

The sponsor submitted 34 publications that describe clinical studies with 1 or 2 gm of tinidazole administered as a single dose for the treatment of trichomoniasis (summarized in Table 70). Of these 34 studies, the sponsor identified 5 studies as pivotal studies that had enrolled the largest number of patients (shown in bold, Table 70). These were blinded and controlled studies to determine efficacy of tinidazole in the treatment of trichomoniasis. Metronidazole, ornidazole or placebo was used as a comparator in the 34 studies. Please note that the inclusion criteria, efficacy endpoints, and follow-up period varied in the different studies. The primary endpoint was based on absence of trichomonad by wet mount or culture at follow-up. The follow-up period varied from 3 days to 6 weeks. A total of 3943 patients (3486 females and 457 males) from Europe, Asia, South America and Africa were enrolled in the 34 studies.

In patients with vaginitis, a successful parasitological outcome was observed in 2131/2271 (94%) patients (29 studies, reference# 34, 87-92, 94-96, 98-107, 109-116, and 118 in Table 70) treated with tinidazole using the wet mount or culture method.. Information on symptom resolution or relief was available for 357 of the 2271 patients (7 studies, reference# 87, 88, 98, 105, 106, 110, and 113 in Table 70). Of these 357 patients, 324 (91%) showed resolution of symptoms after treatment with tinidazole. A correlation was observed between clinical and parasitological outcome in these 357 patients. One study (Lyng, 1981)[88], examined the relapse rate when both female patients and their male partners were treated with tinidazole. A relapse rate of 5% was observed in female patients, at 1 month post therapy with tinidazole. The relapse rates were not examined in other studies. Tinidazole was as effective as metronidazole and other experimental drugs such as ornidazole and carnidazole.

In patients with urethritis, absence of trichomonads by wet mount or culture of urine sediments or urethral scrapings was observed in 96% (240/250) men treated with 2 gm tinidazole dose (7 studies, reference# 34, 93, 97, 108, 109, 113 and 116 in Table 70). Information on resolution of symptoms was available for 105 of the 250 patients (2 studies, reference# 93 and 113 in Table 70). Resolution of symptoms was observed in 98/105 (93%) patients treated with tinidazole. The parasitological outcome correlated with clinical outcome in these 105 patients. The relapse rates were not measured in male patients with urethritis. The efficacy of tinidazole was similar to metronidazole in patients with urethritis.

Overall, the results in Table 70 show that tinidazole was as effective as metronidazole for the treatment of trichomoniasis in both female and male patients with vaginitis or urethritis. No difference was observed in the treatment success rate when studies examining clinical and parasitological outcomes were compared with those that evaluated only parasitological outcome.

The diagnosis of trichomonas by culture is considered the gold standard[1,2]. The culture results were used to determine efficacy in only 13 studies (see Table 70, shown as underlined). Of the 13 studies, 3 used Diamond's medium for culture, 2 used [redacted] trichomonad medium, 1 used trypticase-yeast extract-iron-serum medium, and 1 used Cysteine-peptone-liver infusion-maltose medium. For the remaining 6 studies, the culture media was not specified. The details of the incubation conditions for culture methods, number of vaginal specimens examined by the culture or wet mount method, and other experimental details were not included in the publications. Based on the limited information, it is difficult to compare results across studies and interpret the sensitivity of the wet mount to culture method. Overall, the efficacy of tinidazole in 565 patients with trichomoniasis from the 13 studies using the culture method varied from 74 - 100% compared to 80 - 100% in 1963 patients evaluated by the wet mount method in the remaining 21 studies. A direct comparison between wet mount and culture methods was made in 1 study (Psaroudakis *et al.*, 1977)[89] where 40% of patients with a negative wet mount were positive by culture, suggesting that the culture method is more sensitive than wet mount. However, the raw data on the wet mount results, details of the culture methods, and number of specimens examined by wet mount were not included in the publication. Overall, the greater sensitivity of culture using Diamond's medium or In Pouch test compared to wet mount has been described in the literature[1,2].

The *in vitro* susceptibility of baseline isolates to tinidazole was examined in 2 studies. The tinidzole MICs against the 8 isolates from the study by Wallin, 1974[34] and 55 isolates from the study by Sucharit, 1979[37] ranged from 0.12 to 6 μg/ml.

Table 70: Summary of all clinical studies using single dose tinidazole (1 to 2 gm) for the treatment of trichomonaisis.

Study (country)	Design	N/Sex	Tinidazole dose	Comparator (dose)	Diagnosis	Endpoint	Parasitological and clinical outcome	
							Tinidazole (%)	Comparator (%)
Lyng, 1981 (Denmark)[88]	OL for females, DB, R, C for males	137/F 68/M	2 gm	Placebo in males	Culture of vaginal specimen using Diamond's medium	-ve culture at 1-2 weeks, 1 month	132/137 (96%)-1 week; 101/118 (86%)-1 month; relapse = 5% when partner is treated; clinical outcome not specified	-
O'Prasertsawat, 1992 (Thailand)[90]	DB, R, C	132/F	2 gm	MTZ (1.6 gm)	Culture of vaginal specimen using trypticase-yeast extract-iron-serum (TYI) medium	-ve culture at 6-16 days	65/65 (100%); 61/65 (94%) symptoms improved	MTZ: 66/67 (98.5%); 63/67 (94%) symptoms improved
Chaisilwattan, 1980 (Thailand)[91a]	DB, R, C	107/F*	2 gm	OR (1.5 gm)	Wet mount	-ve wet mount on day 4, 7, 14	51/52 (98%);	OR: 54/55 (98%)
Gabriel, 1982 (UK)[92]	SB, R, C	95/F	2 gm	MTZ (2 gm)	Wet mount (40x objective) and culture using trichomonas culture medium	-ve culture at 14 days	40/42 (95.3%); Clinical outcome not specified	MTZ: 39/40 (97.5%); Clinical outcome not specified
Hillstrom, 1977 (Sweden)[93 a]	DB, R, C	90/F	2 gm	OR (1.5 gm)	Culture of vaginal specimen using Diamond's medium	-ve culture at 1 week, 1 month	41/43 (95%)-1 week; 37/40 (92.5)-1 month	OR: 45/45 (100%)-1 week; 41/42 (97.6%)- 1 month
Weidenbach, 1974 (Germany)[94]	OL, R, C	64/F	2 gm	MTZ (2 gm)	Wet mount and culture (details not given)	-ve culture at 1 and 6 weeks	40/43 (93%)-1 week; 12/12 (100%)-6 weeks; Clinical outcome not specified	20/21 (95%)-1 week; 4/4 9100%)-6 weeks; Clinical outcome not specified
Wallin, 1974 (Sweden)[34]	OL, R	115/F 11/M	1.6 gm or 2 gm	None	Culture of vaginal specimen or urethral scraping using Diamond's medium	-ve culture at 1 and 4 weeks	45/47 (96%)-2 gm dose; 52/56 (93%)-1.6 gm dose; 10/10 males (100%); Clinical outcome not specified	-
Fantini, 1974 (Argentina)[95]	OL	25/M	2 gm	-	Wet mount and culture of urine sediment	-ve culture at 30 days	22/25 (88%) 18/25 (72%) symptoms resolved	-

N = number of patients; R = randomized; DB = double blind, SB = single blind, OL = open label; MC = multicenter,
DR= Dose ranging, C = comparative, TZ = tinidazole; MTZ = metronidazole; OR = ornidazole;
P = placebo; M = males; F = females
# 40% patients with negative wet mount positive by culture; [a] patients with other concurrent vaginal infections;
Studies that are underlined used culture for evaluating parasitological outcome and studies shown in bold were considered pivotal by the sponsor.

Table 70: (Continued)

Study (country)	Design	N/Sex	Tinidazole dose	Comparator (dose)	Diagnosis	Endpoint	Parasitological and clinical outcome	
							Tinidazole (%)	Comparator (%)
Aimakhu, 1975 (Nigeria)[96]	DB, R, C	50/F*	2 gm	MTZ (200 mg t.i.d 7 days)	Wet mount and culture (details not given)	-ve culture at 3, 5, 15 days	24/25 (96%); Clinical outcome not specified	MTZ: 25/25 (100%); Clinical outcome not specified
Ward, 1976 (Australia)[97]	OL	25/F	2 gm	None	Wet mount and culture (details not given)	-ve culture at 1 week	25/25 (100%); Clinical outcome not specified	-
Jones, 1977 (Australia)[98]	OL	50/F	2 gm	None	Wet mount and culture using Oxoid trichomonad medium	-ve culture at 1 week	39/41 (95%); Clinical outcome not specified	-
Psaroudakis, 1977 (Greece)[89]	OL	66/F	2 gm (2nd dose to failures at 3-5 days)	None	Pap smear, wet mount, and culture[#]	-ve culture at 3-5 days	43/58 (74%); 45/56 (80%)-vaginal discharge normal 48/49 (98%)- after re-treatment	-
Sucharit, 1979 (Thailand)[37]	OL	55/F	1.8 gm	None	Wet mount and culture in cysteine-peptone-liver infusion-maltose (CPLM) medium	-ve culture at 7 days	NA (100%); Clinical outcome not specified	-
Kawamura, 1978 (Japan)[99]	OL	73/M	1 gm	MTZ (1 gm)	Wet mount and culture of urine sediment	-ve culture at 7-14 days	39/39 (100%); Clinical outcome not specified	34/34 (100%); Clinical outcome not specified
Roseman, 1973 (South Africa)[100]	OL	31/F	2 gm	None	Wet mount	-ve wet mount at 1 and 4 weeks	28/31 (90.3%)-1 week; 24/24 (100%)- 4 week; symptoms resolved by 1 week in all patients	-
Milek, 1974[101a] (Switzerland)	OL, DR, MC	386/F	1.5 gm, 1.6 gm, 1.8 gm, or 2 gm	None	Wet mount	-ve wet mount at 7-10 days	82/98 (84.5%)- 1.5 gm; 66/75 (88%)- 1.6 gm; 73/79 (92.4%)-1.8 gm; 126/134 (94%)- 2 gm	-
Swarz, 1974 (Europe)[102]	OL, MC	251/F	2 gm	None	Wet mount	-ve wet mount at 1-2 weeks and 4-6 weeks	245/251 (97.6%)-1week; 216/221 (95.3%)-4 weeks; Clinical outcome not specified	-

N = number of patients, R = randomized; DB = double blind, SB = single blind, OL = open label; MC = multicenter;
DR= Dose ranging; C = comparative, TZ = tinidazole, MTZ = metronidazole; OR = ornidazole; P = placebo;
M = males; F = females; # 40% patients with negative wet mount positive by culture;
[a] patients with other concurrent vaginal infections; Studies that are underlined used culture for evaluating parasitological outcome.

Table 70: (Continued)

Study (country)	Design	N/Sex	Tinidazole dose	Comparator (dose)	Diagnosis	Endpoint	Parasitological and clinical outcome	
							Tinidazole (%)	Comparator (%)
Dellenbach, 1974 (France)[103]	OL	32/F	2 gm	None	Wet mount	-ve wet mount at 1 and 6 weeks	31/32 (97%)-1 week; 29/32 (90.6%)-6 week; Clinical outcome not specified	-
Schellen, 1974[104] (Netherlands)	OL	53/F	2 gm	None	Wet mount	-ve wet mount at 2 months	46/49 (94%); Clinical outcome not specified	-
Bedoya, 1974 (Spain)[105]	OL	15/F	2 gm	None	Wet mount	-ve wet mount at 4 days	14/15 (93%); Clinical outcome not specified	-
Rees, 1974 (Kenya)[106]	DB, C	29/F	2 gm	Placebo-ascorbic acid	Wet mount	-ve wet mount at 7 days	8/10 (80%); Clinical outcome not specified	Placebo: 0/10 (0%); Clinical outcome not specified
Mati, 1974 (Kenya)[107]	DB, C	31/F	2 gm	Placebo-yeast tablets	Wet mount	-ve wet mount at 7 days	16/16 (100%); 12/15 (80%)-symptom relief	Placebo: 4/15 (26.7%); 5/13 (38%)-symptom relief
Ali, 1975 (Bangladesh)[108]	OL	39/F	2 gm	None	Wet mount	-ve wet mount at 7 and 30 days	29/36 (80%)-7 days; 32/36 (89%)-30 days; 30/36 (83%) symptoms cured	-
Akinla, 1975 (Africa)[109]	OL	24/F	2 gm	None	Wet mount	-ve wet mount at 7 days	23/24 (94%); Clinical outcome not specified	-
Massa, 1976 (Chile)[110]	OL	30/M	2 gm	None	Wet mount of urine sediment	-ve wet mount at 7 - 14 days	25/30 (83.3%); Clinical outcome not specified	-
Pavlovic, 1976 (Croatia)[111]-abstract	OL	35/F 30/M	2 gm	None	NA	Evaluation at 8 days	32/35 (91.4%)-females; 28/30 (93%)- males; Clinical outcome not specified	-
Anjaeyulu, 1977 (India)[112a]	R, C	100/F	2 gm	MTZ (2 gm)	Wet mount	-ve wet mount at 12 days	47/50 (94%); 42/50 (84%)- symptom relief	32/50 (64%); 25/50 (50%)- symptom relief

N = number of patients, R = randomized; DB = double blind; SB = single blind, OL = open label; MC = multicenter; DR= Dose ranging, C = comparative; TZ = tinidazole; MTZ = metronidazole; OR = ornidazole; P = placebo; M = males; F = females; # 40% patients with negative wet mount positive by culture;
[a] patients with other concurrent vaginal infections;

Table 70: (Continued)

Study (country)	Design	N/Sex	Tinidazole dose	Comparator (dose)	Diagnosis	Endpoint	Parasitological and clinical outcome	
							Tinidazole (%)	Comparator (%)
Schmor, 1974 (Austria)[113a]	OL	50/F	2 gm	None	Wet mount	-ve wet mount at 6-10 days and 4-6 weeks	49/50 (98%) at both visits; Clinical outcome not specified	-
Apte and Packard, 1978 (Asia)[114]	OL, MC	859/F	2 gm	None	Wet mount	-ve wet mount at 8-21 days	818/859 (95.2%); Clinical outcome not specified	-
Beric, 1978 (Germany)[115]	OL	200/F 175/M	2 gm	MTZ (5 gm over 10 days)	Wet mount	-ve wet mount at 8 days	103/104 (99.5%)-females; 80/80 (100%)- males (parasitological and clinical)	97/100 (97%)-females; 89/91 (98%)-males (parasitological and clinical)
Rao, 1978 (India)[116]	OL, R, C	60/F	2 gm	MTZ (2 gm)	Wet mount	-ve wet mount at 4, 8 and 12 days	29/29 (100%); Clinical outcome not specified	30/30 (100%); Clinical outcome not specified
Chaudhuri, 1980 (Netherlands)[117]	DB, R	77/F	2 gm	CAR (2 gm)	Wet mount	-ve wet mount at 1 and 2 weeks	36/38 (96.5%); Clinical outcome not specified	CAR: 38/38 (100%); Clinical outcome not specified
Patil, 1983 (India)[118]	OL	45/F 45/M	2 gm	None	Wet mount of vaginal specimen in females and urine in males	-ve wet mount at 5-10 days	42/45 (93%)-females; 36/36 (100%)-males; Clinical outcome not specified	-
Bloch, 1985 (South Africa)[119a]	OL, C	161/F	2 gm	MTZ (2 gm) Benzoyl MTZ (2 gm)	Wet mount	-ve wet mount at 14 days	(NA) 95%; Clinical outcome not specified	MTZ: NA (100%) Benzoyl MTZ: NA (100%); Clinical outcome not specified
Quartararo, 1974 (Italy)[120]	OL	22/F	2gm	None	Wet mount and Pap smear	-ve wet mount at 7 days	21/22 (95%); Clinical outcome not specified	-

N = number of patients, R = randomized, DB = double blind, SB = single blind, OL = open label; MC = multicenter;
DR= Dose ranging, C = comparative, TZ = tinidazole, MTZ = metronidazole; OR = ornidazole; P = placebo;
M = males; F = females; # 40% patients with negative wet mount positive by culture;
[a] patients with other concurrent vaginal infections;