Trichomoniasis in a Closed Community: Efficacy of Metronidazole

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Summary

A retrospective survey of women treated in prison for trichomonal vaginitis with metronidazole showed that 488 of 496 (98·3%) were cured after one course of drugs. Five of the eight treatment failures were successfully treated by further courses of metronidazole.

A regimen of 400 mg metronidazole twice daily for seven days is simple and effective when taken in prescribed dosage. Metronidazole is still the drug of choice for trichomonal vaginitis. No toxic reactions were observed and there was no evidence that the drug has lost efficacy in the last ten years.

Introduction

The claims that hail the advent of new drugs have sometimes testified to the enthusiasm of the promoters rather than to the actual merits of their products and may have justified the cynical advice to use a new medicine before it has lost its efficacy. Even when the initial appraisal of an active chemical compound has proved to be correct it often has had to be modified later because of the development of drug resistance or the arrival of a newer and even more effective remedy. It was natural, therefore, that many expected that sooner or later metronidazole would be eclipsed or would cease to give such high rates of cure.

The publication abroad of papers representing that metronidazole (Flagyl) had begun to fail and rumours nearer home suggested the desirability of investigations designed to compare current cure rates in trichomoniasis with those obtained in the early 1960s. Accordingly a retrospective study was made of cases treated in the closed community of Holloway Prison, and this paper embodies the findings.

In order to compare the "response rate" of patients treated with metronidazole for trichomonal vaginitis in more recent years with that of previous years (Keighley, 1962), the case papers of all women treated by me in 1967 and 1968 at H.M. Prison, Holloway, were examined and the relevant data extracted.

As in prison it is customary to issue medicines only twice a day, this practice has been followed by my clinic in the giving of metronidazole ever since 1961. The patients on treatment were brought to the clinic twice daily for seven days; 400 mg of metronidazole was given with a glass of water to the patient by the clinic sister, who watched the patient swallow her tablets. In this way it has always been checked that the patient received the correct dosage of the drug. This method of ensuring that the patient had her complete dose of metronidazole was not stressed in my paper in 1962; however, in retrospect, it appears to be *the* important factor in evaluating the efficacy of metronidazole.

When comparing results of treatment of inpatients at Holloway with those of outpatients at the Royal Northern Hospital, London, it became clear that the only difference was that the outpatients, particularly the young ones, were getting more careless every year about taking their tablets correctly. It was made as easy as possible—they were on the same twice-daily dosage as those in Holloway. But the outpatient who has forgotten to take her tablets or who has spread a week's tablets over two weeks is only too well known to us all.

The dosage in 1961-2 was 300 mg—that is 1½ tablets—twice a day for seven days. Later many of the patients refused medication because of the bitter taste of the broken tablet. For this reason only, the dosage was standardized at 400 mg (two 200-mg tablets) twice daily for seven days. No local treatment for trichomoniasis was given.

Criteria.—All patients fulfilling the following criteria were included in the present survey. (1) Those suffering from trichomonal vaginitis confirmed by wet-film microscopy and/or cytology. (2) Those treated with metronidazole 400 mg twice daily for seven days. (3) Those followed up with tests of cure for a minimum period of two weeks. Patients with less than a two-week follow-up period have been reported, but are not included in the cure rate analysis.

Retrospective Survey

During 1967 and 1968 1,110 patients with trichomonal vaginitis were treated with metronidazole—549 in 1967 and 561 in 1968. Of these, 583 fulfilled the conditions for inclusion in the survey—293 from 1967 and 290 from 1968. Their ages ranged from 15 to 50 years (Table I). The age incidence of trichomoniasis in the women in prison usually follows the pattern of the age incidence found in outpatient clinics, being highest in those aged 15-30 years, the years of highest sexual activity.

TABLE I—*Age Incidence*

	15–20	21–30	31–40	41–50	>50
1967	72	134	41	42	4
1968	103	126	35	21	5
Total	175	260	76	63	9

The 1967 figures shown in Table I give the usual picture; the sharp rise in those aged 15-20 in 1968 was due to the opening of a new remand centre for the under-twenties in September 1967. The teenagers from various parts of England were concentrated in this one remand centre, inflating our figures and distorting the normal picture.

Of the 583 cases documented, 241 (41·3%) were prostitutes, either married or single, and 99 (17%) were promiscuous. One girl aged 18 was virgo intacta. Marital status and details of sexual activity are shown in Table II. The higher the sexual activity the higher the incidence of *Trichomonas vaginalis* infestation, and therefore it is to be expected that those women engaged in prostitution will have a high rate of infestation. In fact it was found that the incidence of trichomoniasis in prostitutes was 54% in both 1967 and 1968

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TABLE II—*Marital Status and Details of Sexual Activity*

Year	Virgo Intacta	Single			Married		Divorced, Separated with Various Partners	Widows	Lesbians	Prostitutes	Totals
		Regular Partner	Various Partners	Promiscuous	Marital Partner Only	Extramarital Consorts					
1967	1	10	33	46	47	11	22	5	2	116	293
1968	0	8	45	53	31	12	16	0	0	125	290
Total	1	18	78	99	78	23	38	5	2	241	583
%	0·2	3·1	13·4	17·0	13·4	3·9	6·5	0·9	0·3	41·3	100

TABLE III—*Analysis of Results*

Year	Total No. Recorded	Excluded from Analysis		No. Assessed	Results			Additional Diagnoses						
		Less than Two Weeks Follow-up	Positive on Cervical Cytology Only		Possible Treatment Failure	Cures		Gonorrhoea	Candidial Vaginitis	*Syphilis			Carcinoma of Cervix	Pregnant
						No.	%			P.	A.S.	L.		
1967	293	51	8	234	6	228	97·4	64	10	0	0	6	4	17
1968	290	23	5	262	2	260	99·2	49	15	1	2	3	2	23
Total	583	74	13	496	8	488	98·3	113	25	1	2	9	6	40

*Syphilis: P. = Primary. A.S. = Acute secondary. L. = Latent.

TABLE IV—*Length of Observation in 488 Cases Treated Successfully*

	Total No. Treated Successfully	Post-treatment Follow-up						
		Weeks			Months			
		2	3	4	-2	-3	-6	-12
1967 ..	228	48	40	32	59	15	20	14
1968 ..	260	56	50	58	60	7	21	8
Total	488	104	90	90	119	22	41	22
*Cumulative (%)		100·0	78·7	60·2	41·8	17·4	12·9	4·5

*These figures include patients followed-up at stated intervals and at all subsequent intervals.

(Keighley, 1969). Promiscuity, mainly in those aged 15-20, also results in a high incidence of trichomonal infestation. In 1968, of 791 girls aged 15-20 years, 23% were found to have *T. vaginalis*.

CLASSIFICATION OF CASES

Patients were classified as follows:

Excluded from Analysis.—(1) Those who had less than two weeks' post-treatment follow-up = incomplete test of cure. (2) Those who were negative for *T. vaginalis* on pretreatment direct smear but positive on cervical cytology; where no further cytology was undertaken the test of cure is taken as incomplete.

Included in Analysis.—(1) Possible treatment failures: those who failed to respond to the initial course or repeated courses of metronidazole. (2) Cures: those who responded to the *initial* course of metronidazole having had two weeks or more follow-up and no recurrence of the infestation.

Results

EXCLUDED FROM ANALYSIS

Of the 583 cases documented 87 were excluded from the "cure rate" analysis.

(1) Seventy-four patients (12·7%) had less than two weeks' follow-up (2-10 days) (Table III). All were negative for *T. vaginalis* on direct film at posttreatment examination. Of these, 62 showed immediate clinical response; inflammation and irritation had subsided and there was no discharge. In the other 12 patients some discharge persisted; most of these had concurrent infections or cervical erosion. These 74 patients were women on remand in custody, before reappearing at court, who did not return to the prison.

(2) Thirteen patients were negative for *T. vaginalis* on pretreatment direct film examination but were found to be positive on cervical cytology. After treatment with metronidazole smears were negative, but as no further cytology was undertaken the test of cure is taken as incomplete.

INCLUDED IN ANALYSIS

Response to Treatment.—Of the 496 cases taken into the cure rate assessment, 488 (98·3%) were classified as "cures"; these were negative for *T. vaginalis* on direct film examination after one course of metronidazole and remained negative throughout the observation period. The length of observation in this group is shown in Table IV.

Treatment Failures.—Eight patients were classed as "treatment failures" and re-treated with the same dosage of metronidazole. Of these, six received a second course (four responded to treatment, one left the prison, and one refused further treatment) and two required a third course (one was negative on direct film examination throughout but positive on cervical cytology on two occasions (first and fifth weeks) and became negative on cytology after a third course of metronidazole; the other was re-treated twice but left the prison before follow-up examination was carried out). Details and summary of the pathological results in these eight cases are given in Table V.

CLINICAL RESPONSE IN ALL CASES IN SURVEY

Before treatment 95% of patients complained of a discharge and irritation; 30 (5%) had no complaints and were symptom-free. After treatment with metronidazole, inflammation and irritation subsided and discharge cleared within four weeks in 418 patients (71·7%). In 61 a scanty discharge remained which cleared at varying periods up to 12 weeks. In 74 (12·7%) some discharge persisted, mostly due to other complicating conditions (Table VI). Time of examination after the start of treatment, when the patient was free of vaginal discharge, is shown in Table VI.

ADVERSE REACTIONS

There were no complaints of intolerance. I consider this to be due to the regular life of eating and sleeping and to the fact that the patient always received her tablets after a meal.

TABLE V—*Pathological Results in Eight Patients Needing Re-treatment*

No.	Details of Patients*			Weeks after Treatment								Results of Re-treatment with Metronidazole
	Age and Marital Status	Sexual Activity	Additional Diagnoses	1	2	3	4	5	6	8	12	
1 ..	25 S.	Pro.	Preg. Gon.		+							Left the prison: no further details
2 ..	22 S.	Pro.	Gon.		—		+	—	—			Responded to second course
3 ..	29 S.	Promiscuous			+	—	—					Responded to second course
4 ..	21 M.		Can. vag.		—	+						Refused further treatment
5 ..	30 M.	E.M.C.		—		+	—	—				Responded to second course
6 ..	26 S.	E.M.C.		Smear — Cytol. +		—		—	—	—	—	Responded to third course
7 ..	21 M.				+	+		+		—	—	Left prison: no further details
8 ..	29 S.	Pro.	Chronic gon. Salpingitis at 4 weeks		—		+	—	—	—		Responded to second course

*S. = Single. M. = Married. Pro. = Prostitute. E.M.C. = Extramarital consorts. Preg. = Pregnant. Gon. = Gonorrhoea. Can. vag. = Candidial vaginitis.

TABLE VI—*Clinical Response to Treatment*

	Symptom-free Before Treatment	Time of Examination when Free from Vaginal Discharge					Persisting Discharge (74 patients)								Total
							Additional Diagnoses*							T.V. Only	
		7–10 days	2 weeks	–4 weeks	–8 weeks	–12 weeks	Gon.	Can. Vag.	Cer. eros.	C.P.S.	Ca cerv.	Salp.	Late preg.		
No.	30	84	173	161	49	12	16	10	15	1	1	1	6	24	583
%	5·2	14·4	29·7	27·6	8·4	2·0									
		71·7					12·7								

*Additional diagnoses: Gon. = Gonorrhoea. Can. vag. = Candidial vaginitis. Cer. eros. = Cervical erosion. C.P.S. = Chronic pelvic sepsis. Ca. cerv. = Carcinoma of cervix. Salp. = Salpingitis. Late preg. = Late pregnancy.

Antibiotics were never given concurrently with metronidazole; if a specific infection was present it was treated before the trichomonal vaginitis.

ADDITIONAL DIAGNOSES

In 113 patients gonorrhoea as well as trichomonal vaginitis was present. Twenty-five had a concurrent candidial vaginitis for which they were given nystatin pessaries. Six patients were suffering from all three infections—that is, trichomonal gonorrhoeal, and candidial. Eight patients were treated topically with podophyllin for vaginal warts.

Conclusions

Finally, it is a good thing to pause and contemplate the change that oral medication for trichomonal vaginitis has made in women's lives. 'Flagyl' is now taken as a matter of course, and a whole generation has no knowledge of the sufferings of women with trichomoniasis before its introduction—the indignities and discomfort of the perpetual local treatment, douches, paintings, insufflations, and insertions of pessaries, etc. All these things women suffered for months and sometimes years on end, only to relapse when the treatment was discontinued.

The results of this survey leave no doubt in my mind that metronidazole has not lost its efficacy and is still the drug of choice.

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In Vitro Activity of Clindamycin, Imipenem, Metronidazole, and Piperacillin-Tazobactam against Susceptible and Resistant Isolates of *Bacteroides fragilis* by Anaerobic Kill-Kinetics

Running title: Activity of four antibiotics against *B. fragilis*

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In Vitro Activity of Clindamycin, Imipenem, Metronidazole, and Piperacillin-Tazobactam against Susceptible and Resistant Isolates of *Bacteroides fragilis* by Anaerobic Kill-Kinetics

Abstract

The aim of the present study was to investigate the activity of clindamycin, imipenem, metronidazole, and piperacillin-tazobactam against 12 *Bacteroides fragilis* isolates (resistant and susceptible strains) by kill-kinetics over 24 hours. In contrast to the other antimicrobial agents, clindamycin did not affect strains with MIC > 8.0 µg/ml. For those strains with MIC ≤ 8.0 µg/ml, all employed antibiotics except clindamycin showed nearly bactericidal activity. Metronidazole proved to be the most active antimicrobial agent.

Antimicrobial regimes for infections involving *Bacteroides fragilis* have generally been limited as they are potentially resistant to a broad range of antibiotics (28). Drugs with known activity against *B. fragilis* are some β-lactams, nitroimidazoles such as metronidazole, certain newer quinolones, chloramphenicol and clindamycin (17, 20, 23, 24, 28, 29). A diminution of susceptibility to clindamycin has been reported in various countries (1, 7, 21, 26). Resistances against metronidazole still seem to be rare (1, 3). Golan et al. found an increasing fluoroquinolone resistance among *Bacteroides* since 1994 (9). Conversely, Snydman et al. reported 2002 decreased geometric mean MICs among *B. fragilis* for piperacillin-tazobactam (26). Resistance to carbapenems can be found occasionally (28). Thus, there is a great need for knowledge of resistant patterns to accomplish an adequate prophylaxis and treatment of anaerobic or mixed aerobic/anaerobic infections. This seems even more important as there are great differences in the level of antimicrobial resistance between certain geographic areas and even from one hospital to another (7, 10, 17). Kill-kinetic-curves over time provide more information than the widely used MIC-determination and allow a comparison of different antimicrobial classes (16, 27). Thus, the aim of the present study was to investigate the *in vitro* activity of clindamycin, imipenem, metronidazole, and piperacillin-tazobactam against *B. fragilis* isolates by kill-kinetics over time. The strains were kindly provided either by Elli Goldstein, R. M. Alden Research Laboratory, Santa Monica, California, USA or were isolates of an international anaerobe-study.

Brucella broth (Becton Dickinson, Cockeysville, Maryland, USA) supplemented with vitamin K_1 (Sigma Chemical Co.), and haemin (Serva Feinbiochemica, Heidelberg, Germany) was used as growth medium, in the following mentioned as supplemented Brucella broth. Aliquots were plated on Columbia agar (Oxoid Ltd., Basingstoke, Hampshire, UK) supplemented with sheep blood (Oxoid GmbH, Wesel, Germany), vitamin K_1 and haemin, in the following mentioned as supplemented Columbia agar.

MIC values were determined by E-test (AB Biodisk, Solna, Sweden) for all selected *B. fragilis* strains and clindamycin, imipenem, metronidazole and piperacillin-tazobactam according to the manufacterer`s instructions as described previously (25). For the *B. fragilis* strains with MIC $\leq$ 8.0 µg/ml, the killing activity for clindamycin (Sigma Chemical Co., St. Louis, USA), imipenem (Merck & Co., Inc., West Point, USA), metronidazole (Sigma Chemical Co.), and piperacillin (Sigma Chemical Co.)-tazobactam (Otsuka Chemical Co. Ltd., Osaka, Japan) were assessed using 0.5, 1, 2 or 4 $\times$ MIC. In case of strains with MIC > 8.0 µg/ml, concentrations at 0.5, 1, 2 or 4 $\times$ C_{max} values were employed. The following concentrations were used as C_{max} values: clindamycin: 16 µg/ml (8); imipenem: 32 µg/ml (22); metronidazole: 16 µg/ml (11, 13, 15); piperacillin: 60 µg/ml (2) and tazobactam: 25 µg/ml (12, 14, 19).

Antibiotic-free growth control was performed parallel to each experiment. The final inocula contained approximately 1.5×10^7 CFU/ml. At 0, 2, 4, 6, 12 and 24 hours after incubation at 37° C aliquots were plated on the supplemented Columbia agar. CFU were counted after 48 hours incubation. Detection limit was 10^2 CFU/ml. All experiments were carried out in an anaerobic chamber (Heraeus, Hanau, Germany) containing 5% H_2, 15% CO_2 and 80% N_2.

For all strains and their respective antimicrobial agents the mean value and standard deviation were calculated. Statistical analysis was done with SPSS-software. In those cases where the number of strains exceeded 3, Paired-Sample Wilcoxon Signed Rank Test was employed to identify significant differences. In each case at t = 6 hours and t = 24 hours differences were calculated. A p-value < 0.05 was considered to be significant.

Table 1 shows the MIC values for the tested *B. fragilis* strains for the respective antimicrobial agent and the breakpoints according to EUCAST (6). The investigated strains were divided by a cut off at 8.0 µg/ml into two groups: susceptible/wild type group and resistant group, respectively. For clindamycin the same cut off is used independent of the

breakpoint (4 µg/ml) according to EUCAST (6). The chosen cut off at 8.0 µg/ml sperates also two different groups, the wild type and the resistant group.

The pooled kill-kinetic-curves for *B. fragilis* strains with MIC ≤ 8.0 µg/ml are shown in Fig. 1. At concentrations above MIC clindamycin showed bactericidal activity only against 5 out of 10 strains. Imipenem was bacterical against 8 out of 9 strains, metronidazole against 10 out of 10 strains. Piperacillin-tazobactam showed bactericidal activity against 6 out of 8 strains investigated. Piperacillin-tazobactam was the only antibiotic regime where statistical significant differences were found after 6 hours of incubation. The use of 4 × MIC resulted in a higher killing rate than the use of 1 × MIC ($p < 0.05$). A significant higher killing rate using 1 × MIC or 4 × MIC instead of 0.5 × MIC and clindamycin or imipenem, respectively, occurred at t = 24 hours ($p < 0.05$). Between 1 × MIC and 4 × MIC no statistical significances were found for clindamycin or imipenem, respectively. In contrast, increasing concentrations of metronidazole or piperacillin-tazobactam resulted in significant higher killing rates after 24 hours ($p < 0.05$).

The pooled kill-kinetic-curves for *B. fragilis* strains with MIC values > 8.0 µg/ml are shown in Figure 2. The two metronidazole resistant strains were effectively killed by metronidazole when concentrations of C_{max} (16 µg/ml) or more were used. Also, two of three imipenem resistant strains were killed by imipenem with concentrations of C_{max} (32 µg/ml) or more. Piperacillin-tazobactam showed activity against 3 of the 4 piperacillin-tazobactam resistant strains when concentrations of C_{max} (piperacillin: 60 µg/ml and tazobactam: 25 µg/ml) or higher were used. In contrast, clindamycin did not inhibit the bacterial growth of the clindamycin resistant strains even at concentrations of $4 \times C_{max}$ (64 µg/ml). Due to the limited number of strains with MIC > 8 µg/ml, only for piperacillin-tazobactam statistical analysis could be performed. However, no statistical differences in killing rates could be found even between $0.5 \times C_{max}$ and $4 \times C_{max}$.

Comparing the prior established MICs by E-test with the assessed kill-kinetics, a good correlation could be found when the organisms were susceptible for the respective antibiotic agent. Furthermore, in the present study imipenem showed a slightly better effect than piperacillin-tazobactam. In contrast, clinical trials comparing piperacillin-tazobactam with imipenem/cilastatin in patients with intra-abdominal infections revealed equal efficacy (5, 18) or slight advantages for piperacillin-tazobactam (4). Employing resistant strains, clindamycin showed no effect on those strains while metronidazole could prove a rather good effect. Thus, metronidazole appeared to be the most effective investigated substance but still needs a combination with another antibiotic to cover infections with aerobic bacteria in mixed infections.

In summary, the kill kinetics over time could provide additional information of local resistant patterns which is of utmost importance for an adequate prophylaxis and treatment in anaerobic or mixed infections. They should be performed in studies after prior establishing MIC values also choosing resistant strains.

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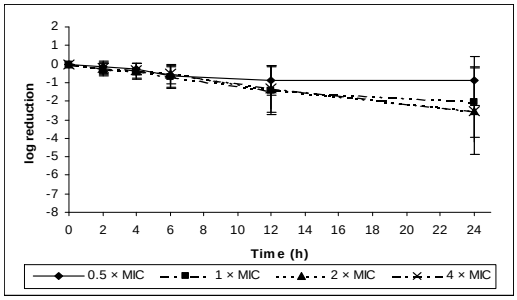
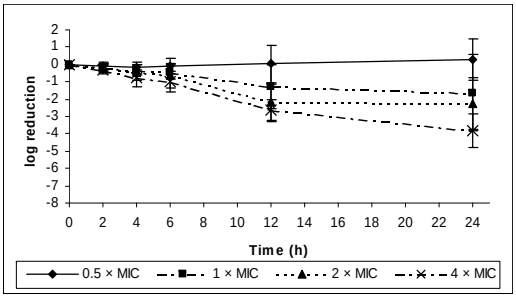
TABLE 1 MIC values (µg/ml) of the *B. fragilis* strains tested and breakpoints according to EUCAST (7)

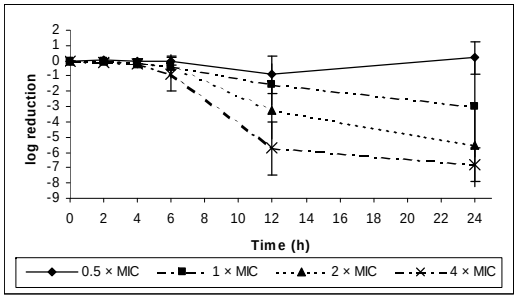
***B. fragilis* strain**	**Clindamycin** ≤ 4(s) / > 4 (r)	**Imipenem** ≤ 2(s) / > 8 (r)	**Metronidazole** ≤ 4(s) / > 4 (r)	**Piperacillin/ Tazobactam** ≤ 8(s) / > 16 (r)
WAL 13174	0.03	0.5	>256	2
RMA 5935	0.03	>32	0.25	>256
RMA 5120	1	0.25	0.5	0.125
RMA 5081	2	0,25	1	4
WAL 13054	2	0.25	>256	1
RMA 6600	2	>32	0.5	32
WAL 13267	4	0.125	0.5	0.5
RMA 0309	4	>32	0.5	>256
RMA 5798	8	0.125	0.5	4
RMA 5691	8	0.25	1	16
RMA 5138	>256	0.5	0.5	2
RMA 6791	>256	0.5	1	1



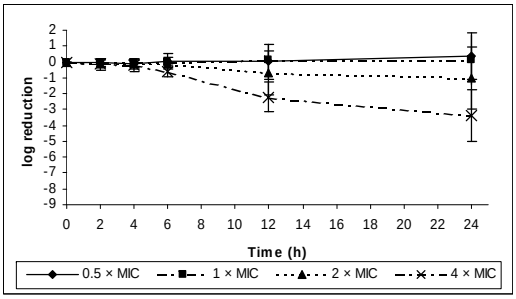
a. (clindamycin)



b. (imipenem)

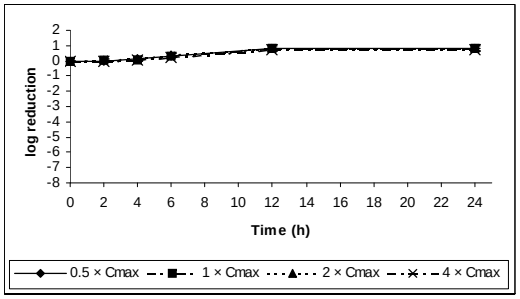


c. (metronidazole)

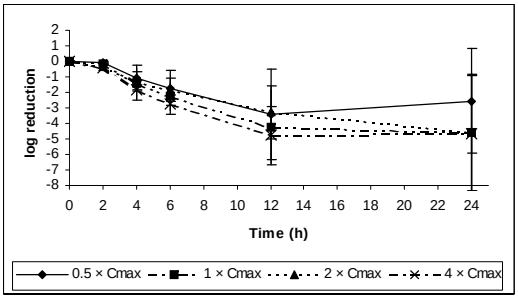


d. (piperacillin-tazobactam)

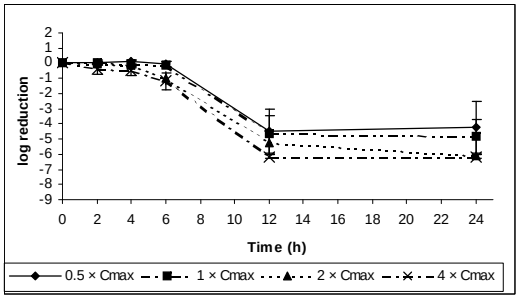
FIG. 1. Pooled kill-kinetic-curves of 10 *B. fragilis* strains and clindamycin (a), 9 *B. fragilis* strains and imipenem (b), 10 *B. fragilis* strains and metronidazole (c), and 8 *B. fragilis* strains and piperacillin-tazobactam (d) for strains with MIC ≤ 8 µg/ml.



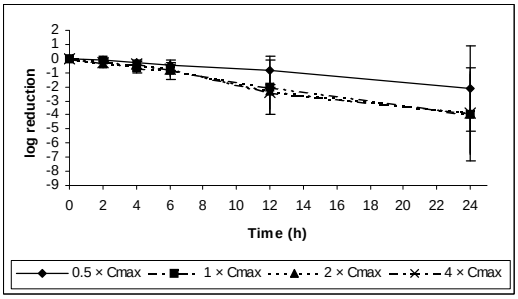
a. (clindamycin)



b. (imipenem)



c. (metronidazole)



d. (piperacillin-tazobactam)

FIG. 2. Pooled kill-kinetic-curves of 2 *B. fragilis* strains and clindamycin (a), 3 *B. fragilis* strains and imipenem (b), 2 *B. fragilis* strains and metronidazole (c), and 4 *B. fragilis* strains and piperacillin-tazobactam (d) for strains with MIC > 8 µg/ml.