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In Vitro Evaluation of Activities of Nitazoxanide and Tizoxanide against Anaerobes and Aerobic Organisms

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The antibacterial activities of nitazoxanide and its main metabolite, tizoxanide, were tested against a broad range of bacteria, including anaerobes. Metronidazole, amoxicillin, amoxicillin-clavulanic acid, piperacillin, cefoxitin, imipenem, and clindamycin were used as positive controls. MICs were determined by reference agar dilution methods. The 241 anaerobes were all inhibited by nitazoxanide, with the MICs at which 90% of isolates are inhibited (MIC_{90}s) being between 0.06 and 4 mg/liter with the exception of those for *Propionibacterium* species, for which the MIC_{90} was 16 mg/liter. The MIC_{90}s of nitazoxanide were 0.5 mg/liter for the *Bacteroides fragilis* group (80 strains), 0.06 mg/liter for *Clostridium difficile* (21 strains), and 0.5 mg/liter for *Clostridium perfringens* (16 strains). Metronidazole showed a level of activity comparable to that of nitazoxanide except against *Bifidobacterium* species, against which it was poorly active, and *Propionibacterium* species, which were resistant to metronidazole. The other antibiotics showed various levels of activity against anaerobes, with imipenem along with nitazoxanide being the most active agents tested. Tizoxanide was less effective than nitazoxanide except against the *B. fragilis* group, against which its activity was similar to that of nitazoxanide. Under aerobic conditions, nitazoxanide demonstrated poor activity against members of the family *Enterobacteriacae* and *Pseudomonas*, *Staphylococcus*, and *Enterococcus* species. The same results were obtained when culture was performed under anaerobic conditions with the notable exception of the results against *Staphylococcus aureus*. The MICs of nitazoxanide were in the range of 2 to 4 mg/liter for 34 clinical isolates of *S. aureus*, 12 of which were methicillin resistant, while tizoxanide was not effective.

Nitazoxanide is a 5-nitrothiazole compound (Fig. 1), first synthesized by Rossignol (18). It is effective against a wide variety of parasites and bacteria infecting animals and humans. Human clinical studies have confirmed the results of in vitro and animal testing indicating that nitazoxanide has the broadest spectrum of antiparasitic activity ever achieved with a single drug. In humans, the spectrum includes flagellate and ciliate protozoa, coccidial protozoa such as *Cryptosporidium parvum* and *Isospora belli*, microsporidiae such as *Septata intestinalis* and *Vittaforma corneae*, and the amebas. It is also a broad-spectrum nematocidal, cestiocidal, and trematocidal anthelmintic agent that is effective against the five intestinal nematodes, the four intestinal cestodes, and the liver trematode *Fasciola hepatica* (1, 7, 9, 17, 18). Nitazoxanide and its main metabolite, tizoxanide (Fig. 2). (19), have recently been reported to be effective in vitro against metronidazole-susceptible and metronidazole-resistant strains of *Helicobacter pylori* (10). Nitazoxanide is being tested in clinical trials for the treatment of *H. pylori* infection.

Nitazoxanide and tizoxanide were tested against 241 strains of anaerobic gram-positive and gram-negative bacteria. Metronidazole, amoxicillin, amoxicillin-clavulanic acid, piperacillin, cefoxitin, imipenem, and clindamycin were used as positive controls. Nitazoxanide, tizoxanide, and metronidazole were also tested against gram-positive and gram-negative aerobic bacteria under both aerobic and anaerobic conditions.

MATERIALS AND METHODS

Bacterial strains. Anaerobic bacteria were isolated from human clinical samples during the years 1994 and 1995. They were identified by classical methods and were then subcultured in a Rosenow medium (Diagnostics Pasteur, Marnes la Coquette, France). When they were not immediately used for determination of MICs, the broth was kept by freezing it at −20°C. Before being tested, the bacteria were checked for purity by subculturing on Colombia blood agar (BioMérieux, Marcy l'Étoile, France) and on either laked blood-kanamycin-vancomycin plates (Serlabo, Bonneuil/Marne, France) for *Bacteroides* spp. or josamycin-norfloxacin plates for fusobacteria (3). Purity was also checked by Gram staining. The range of anaerobic strains collected and the numbers of each strain tested are listed in Table 1.

For good quality control and assessment of reproducibility, four reference American Type Culture Collection (ATCC) control strains were added in each batch of tests. The ATCC control strains, advocated by the M11-A3 standard of the National Committee for Clinical Laboratory Standards (12), were *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124, and *Eubacterium lentum* ATCC 43055.

Strains of *Morganella morganii* ($n = 1$), *Escherichia coli* ($n = 2$), *Pseudomonas aeruginosa* ($n = 2$), *Staphylococcus epidermidis* ($n = 2$), and *Enterococcus faecalis* ($n = 2$) were isolated from patients in the Tourcoing Hospital, Tourcoing, France. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *E. faecalis* ATCC 29242 were added as reference strains for quality control. To assess the in vitro activity of nitazoxanide against *S. aureus*, 34 additional clinical strains, including 12 methicillin-resistant strains, were collected.

Antimicrobial agents. Nitazoxanide and tizoxanide were obtained from Romark Laboratories, Tampa, Fla. Powders of known potency were supplied by the manufacturers or their French subsidiaries, as follows: metronidazole, Specia; amoxicillin and amoxicillin-clavulanic acid, Beecham; piperacillin, Lederle; cefoxitin and imipenem, Merck Sharp & Dohme; and clindamycin, Upjohn.

MIC determinations. (i) Agar dilution method for anaerobic bacteria. MICs were determined by a reference agar dilution method according to standard M-11T method (11) of National Committee for Clinical Laboratory Standards and additional recommendations provided in standard M11-A3 (12). Stock solutions of 512 mg of nitazoxanide, tizoxanide, metronidazole, amoxicillin, amoxicillin-clavulanic acid, piperacillin, cefoxitin, imipenem, and clindamycin per liter were prepared, as was a 50%–50% concentration of nitazoxanide-tizoxanide. Nitazoxanide and tizoxanide were dissolved in dimethyl sulfoxide at 55°C and were further diluted to the appropriate concentration. Metronidazole was first dissolved in 2 ml of methanol, and then distilled water was added to the solution. Twofold dilutions were made in distilled water according to the recommendations of Ericsson and Sherris (6).

Each antibiotic was incorporated into Wilkins-Chalgren (20) agar (Oxoid-Unipath, Dardilly, France) to which 5% sterile defibrinated blood was added to

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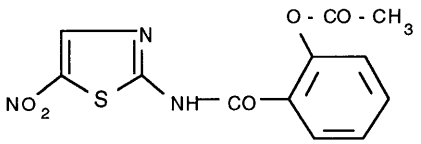

FIG. 1. Chemical structure of nitazoxanide.

provide adequate support for the growth of fusobacteria, *Peptostreptococcus* spp., and *Eubacterium* spp. Plates contained serial doubling dilutions of antimicrobial agents (from 128 to 0.003 mg/liter). All plates were used within 24 h of preparation.

An actively growing culture in Rosenow medium was diluted in a Schaedler broth (BioMérieux) to reach and match the turbidity of a 0.5 McFarland standard. The inocula were approximately 10^8 CFU/ml. For fastidious strains, the following were added to the Schaedler broth: hemin (5 μg/liter), menadione (0.1 μg/liter), sodium bicarbonate (1 g/liter), and 0.1 ml of laked blood in a 10-ml tube.

The previous inocula (2 or 3 μl) were delivered with a Steers replicator (Mast Systems, London, United Kingdom) and led to a final inoculum of 10^5 CFU per spot of inoculation on the agar plates.

At the end of each series of tests, two plates of Wilkins-Chalgren agar were inoculated but did not contain an antimicrobial agent. One plate was incubated anaerobically to determine the viability of the organisms and to serve as a control for the comparison of growth, and the other plate was incubated aerobically to indicate possible aerobic contamination. Incubation of the tested plates containing the antibiotics was done in an anaerobic chamber (Forma Scientific) at 35 to 36°C.

Reading of the MICs was done after 48 h of incubation. The MIC for an organism was the lowest concentration of an antimicrobial agent yielding no growth.

(ii) Agar dilution method for aerobic bacteria. The MICs for some aerobic bacteria were determined by a standard agar dilution method (13). The media used included Mueller-Hinton broth and Mueller-Hinton agar (BioMérieux). These organisms were incubated for 24 h at 35°C, one set under aerobic conditions and a second set inoculated at the same time and incubated under anaerobic conditions.

The activity of the combination of nitazoxanide-tizoxanide against 70 strains of the *B. fragilis* group (final inoculum, 10^5 CFU/0.1-ml well) was assessed by the classical checkerboard method by a microdilution method in Wilkins-Chalgren broth. Synergy was defined when the fractional inhibitory concentration index was ≤0.5; antagonism was defined by a fractional inhibitory concentration index of >4.0.

RESULTS AND DISCUSSION

The MICs of each antibiotic at which 50% (MIC_{50}s) and 90% (MIC_{90}s) of isolates are inhibited for each group of anaerobic bacteria are listed in Table 1. MIC_{90}s were calculated only when 10 or more isolates were tested.

Strains from the *B. fragilis* group (80 strains tested) were more susceptible to nitazoxanide than to metronidazole (MIC_{90}s, 0.5 mg/liter for nitazoxanide versus 1 mg/liter for metronidazole) and each of the other antibiotics with the exception of imipenem, the MIC_{90} of which was 0.25 mg/liter. Nitazoxanide and imipenem showed very similar results, with MIC_{50}s and MIC_{90}s of 0.25 and 0.5 mg/liter, respectively, for nitazoxanide and 0.125 and 0.25 mg/liter, respectively, for imipenem both of which were in a narrow range for susceptibility (0.015 to 2 mg/liter). Previous French reports (2, 5, 8, 14, 15) demonstrated that resistance to imipenem and metronidazole remained rare; even a decreased susceptibility (4, 16) to met-

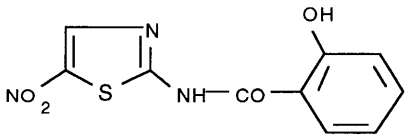

FIG. 2. Chemical structure of tizoxanide.

TABLE 1. Comparative in vitro activities of nitazoxanide, tizoxanide, and seven reference drugs against 241 clinical isolates of anaerobic bacteria

Organism (no. tested) and antimicrobial agent	MIC (mg/liter)		
	50%	90%	Range
Bacteroides fragilis (20)			
Nitazoxanide	0.5	1	0.125–1
Tizoxanide	1	2	0.5–4
Nitazoxanide-tizoxanide	0.5	1	0.06–4
Metronidazole	0.5	1	0.125–2
Amoxicillin	32	64	0.5–>128
Amoxicillin-clavulanic acid	0.125	4	0.06–64
Piperacillin	4	32	1–>128
Cefoxitin	8	16	4–32
Imipenem	0.03	0.5	0.015–2
Clindamycin	0.25	128	0.06–>128
Bacteroides thetaiotaomicron (14)			
Nitazoxanide	0.25	0.5	0.06–1
Tizoxanide	1	2	0.06–4
Nitazoxanide-tizoxanide	0.25	0.5	0.03–0.5
Metronidazole	0.5	2	0.25–8
Amoxicillin	32	64	0.5–>128
Amoxicillin-clavulanic acid	1	32	0.125–128
Piperacillin	16	>128	4–>128
Cefoxitin	16	32	8–64
Imipenem	0.125	0.25	0.03–0.5
Clindamycin	1	>128	0.06–>128
Bacteroides ovatus (8)			
Nitazoxanide	0.25	NA[a]	0.06–0.5
Tizoxanide	1	NA	0.25–2
Nitazoxanide-tizoxanide	0.125	NA	0.125–0.5
Metronidazole	0.5	NA	0.25–1
Amoxicillin	32	NA	32–>128
Amoxicillin-clavulanic acid	0.25	NA	0.125–8
Piperacillin	16	NA	8–>128
Cefoxitin	16	NA	8–128
Imipenem	0.125	NA	0.06–1
Clindamycin	1	NA	0.06–>128
Bacteroides vulgatus (21)			
Nitazoxanide	0.25	0.5	0.06–2
Tizoxanide	0.5	1	0.06–1
Nitazoxanide-tizoxanide	0.125	0.25	0.03–0.25
Metronidazole	0.25	0.5	0.06–4
Amoxicillin	64	64	0.5–>128
Amoxicillin-clavulanic acid	0.5	4	0.06–32
Piperacillin	64	>128	1–>128
Cefoxitin	8	64	2–64
Imipenem	0.125	0.5	0.03–1
Clindamycin	0.125	32	0.06–>128
Other *Bacteroides* spp. of *B. fragilis* group (17)[b]			
Nitazoxanide	0.5	0.5	0.06–1
Tizoxanide	0.5	2	0.06–4
Nitazoxanide-tizoxanide	0.25	0.5	0.06–1
Metronidazole	0.5	1	0.125–16
Amoxicillin	32	64	1–>128
Amoxicillin-clavulanic acid	0.5	16	0.06–32
Piperacillin	16	>128	4–>128
Cefoxitin	32	64	8–64
Imipenem	0.125	0.5	0.06–0.5
Clindamycin	0.25	>128	0.06–>128
Bacteroides fragilis group (80)			
Nitazoxanide	0.25	0.5	0.03–1
Tizoxanide	1	2	0.06–4
Nitazoxanide-tizoxanide	0.25	0.5	0.06–1

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TABLE 1—*Continued*

Organism (no. tested) and antimicrobial agent	MIC (mg/liter)		
	50%	90%	Range
Metronidazole	0.5	1	0.06–16
Amoxicillin	32	64	0.5–>128
Amoxicillin-clavulanic acid	0.5	8	0.06–128
Piperacillin	16	>128	1–>128
Cefoxitin	16	32	2–128
Imipenem	0.125	0.25	0.015–2
Clindamycin	0.50	1	0.06–>128
Prevotella spp. (15)[c]			
Nitazoxanide	1	4	0.125–4
Tizoxanide	0.5	8	0.06–8
Nitazoxanide-tizoxanide	0.25	4	0.03–2
Metronidazole	0.25	1	0.06–1
Amoxicillin	2	32	0.5–64
Amoxicillin-clavulanic acid	0.5	8	0.06–8
Piperacillin	2	8	0.125–8
Cefoxitin	1	4	0.5–4
Imipenem	0.03	0.06	0.03–0.125
Clindamycin	0.06	0.06	0.06–0.25
Fusobacterium spp. (23)[d]			
Nitazoxanide	0.5	2	0.06–3
Tizoxanide	0.5	1	0.06–8
Nitazoxanide-tizoxanide	0.25	1	0.03–2
Metronidazole	0.125	0.25	0.06–0.5
Amoxicillin	0.125	8	0.06–8
Amoxicillin-clavulanic acid	0.06	0.125	0.06–1
Piperacillin	0.125	2	0.125–4
Cefoxitin	0.125	1	0.125–2
Imipenem	0.06	0.25	0.03–0.5
Clindamycin	0.06	0.5	0.06–8
Veillonella spp. (7)[e]			
Nitazoxanide	1	NA	0.5–4
Tizoxanide	2	NA	0.25–4
Nitazoxanide-tizoxanide	1	NA	0.25–2
Metronidazole	0.5	NA	0.125–1
Amoxicillin	0.5	NA	0.06–2
Amoxicillin-clavulanic acid	0.5	NA	0.06–4
Piperacillin	16	NA	1–64
Cefoxitin	1	NA	0.125–4
Imipenem	0.125	NA	0.03–1
Clindamycin	0.06	NA	0.06–0.125
Other gram-negative rods (4)[f]			
Nitazoxanide	0.25	NA	0.06–4
Tizoxanide	0.125	NA	0.06–8
Nitazoxanide-tizoxanide	0.06	NA	0.03–4
Metronidazole	0.125	NA	0.06–0.25
Amoxicillin	0.25	NA	0.06–64
Amoxicillin-clavulanic acid	0.25	NA	0.06–32
Piperacillin	0.125	NA	0.125–32
Cefoxitin	0.5	NA	0.25–32
Imipenem	0.06	NA	0.03–0.125
Clindamycin	0.06	NA	0.06–64
All gram-negative anaerobes (129)			
Nitazoxanide	0.25	1	0.06–4
Tizoxanide	0.5	2	0.06–8
Nitazoxanide-tizoxanide	0.25	1	0.03–4
Metronidazole	0.25	1	0.06–1
Amoxicillin	16	64	0.06–>128
Amoxicillin-clavulanic acid	0.25	8	0.06–128
Piperacillin	8	128	0.125–>128
Cefoxitin	8	32	0.125–128
Imipenem	0.06	0.5	0.015–2
Clindamycin	0.125	64	0.06–>128
Clostridium perfringens (16)			
Nitazoxanide	0.5	1	0.25–2
Tizoxanide	0.5	1	0.25–2
Nitazoxanide-tizoxanide	0.25	0.5	0.125–2
Metronidazole	0.25	0.5	0.25–0.5
Amoxicillin	0.06	0.125	0.06–0.125
Amoxicillin-clavulanic acid	0.06	0.06	0.03–0.06
Piperacillin	0.125	0.125	0.125
Cefoxitin	0.25	0.5	0.03–0.5
Imipenem	0.03	0.06	0.03–0.06
Clindamycin	0.06	16	0.06–16
Clostridium difficile (21)			
Nitazoxanide	0.06	0.06	0.06–0.125
Tizoxanide	0.06	0.06	0.06
Nitazoxanide-tizoxanide	0.03	0.03	0.03
Metronidazole	0.25	0.25	0.06–0.5
Amoxicillin	0.5	2	0.125–2
Amoxicillin-clavulanic acid	0.5	2	0.06–2
Piperacillin	4	8	0.125–8
Cefoxitin	64	64	0.25–64
Imipenem	2	2	0.03–2
Clindamycin	1	16	0.125–128
Other *Clostridium* spp. (13)[g]			
Nitazoxanide	0.125	0.5	0.06–1
Tizoxanide	0.25	1	0.06–2
Nitazoxanide-tizoxanide	0.06	1	0.03–2
Metronidazole	0.25	0.5	0.06–0.5
Amoxicillin	0.25	0.5	0.06–1
Amoxicillin-clavulanic acid	0.25	0.5	0.06–2
Piperacillin	1	8	0.125–16
Cefoxitin	4	64	0.125–64
Imipenem	0.125	1	0.03–1
Clindamycin	0.125	1	0.06–2
Bifidobacterium spp. (8)			
Nitazoxanide	0.5	NA	0.125–8
Tizoxanide	1	NA	0.25–32
Nitazoxanide-tizoxanide	0.5	NA	0.125–16
Metronidazole	8	NA	0.5–64
Amoxicillin	0.25	NA	0.125–0.5
Amoxicillin-clavulanic acid	0.125	NA	0.06–0.5
Piperacillin	0.5	NA	0.06–1
Cefoxitin	2	NA	0.5–16
Imipenem	0.125	NA	0.03–0.5
Clindamycin	0.06	NA	0.06
Eubacterium spp. (20)[h]			
Nitazoxanide	0.5	1	0.25–4
Tizoxanide	1	1	0.25–8
Nitazoxanide-tizoxanide	0.25	0.5	0.125–4
Metronidazole	0.5	1	0.125–16
Amoxicillin	0.5	1	0.06–2
Amoxicillin-clavulanic acid	1	1	0.06–2
Piperacillin	16	16	0.125–32
Cefoxitin	8	16	0.06–128
Imipenem	0.25	0.5	0.03–1
Clindamycin	0.125	0.5	0.06–4
Propionibacterium spp. (11)[i]			
Nitazoxanide	8	16	8–16
Tizoxanide	16	32	8–32

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TABLE 1—*Continued*

Organism (no. tested) and antimicrobial agent	MIC (mg/liter)		
	50%	90%	Range
Nitazoxanide-tizoxanide	8	16	4–16
Metronidazole	64	64	32–64
Amoxicillin	0.06	0.125	0.06–0.125
Amoxicillin-clavulanic acid	0.06	0.06	0.03–0.25
Piperacillin	0.25	0.5	0.125–1
Cefoxitin	0.25	0.25	0.125–1
Imipenem	0.03	0.03	0.03
Clindamycin	0.03	0.03	0.03
Peptostreptococcuss and *Ruminococcus* spp. (23)[j]			
Nitazoxanide	0.25	1	0.06–4
Tizoxanide	0.25	2	0.06–4
Nitazoxanide-tizoxanide	0.125	1	0.03–2
Metronidazole	0.5	1	0.06–2
Amoxicillin	0.125	0.25	0.06–8
Amoxicillin-clavulanic acid	0.06	0.25	0.03–0.5
Piperacillin	0.125	0.5	0.06–1
Cefoxitin	0.5	2	0.125–4
Imipenem	0.06	0.25	0.03–1
Clindamycin	0.06	1	0.06–64
All gram-positive anaerobes (112)			
Nitazoxanide	0.25	1	0.06–16
Tizoxanide	0.5	2	0.06–8
Nitazoxanide-tizoxanide	0.5	8	0.06–32
Metronidazole	0.25	1	0.06–64
Amoxicillin	0.25	1	0.06–32
Amoxicillin-clavulanic acid	0.125	1	0.03–2
Piperacillin	0.25	16	0.06–32
Cefoxitin	2	64	0.03–128
Imipenem	0.06	2	0.03–2
Clindamycin	0.06	2	0.06–128

[a] NA, not applicable.
[b] Three *B. uniformis*, six *B. distasonis*, two *B. caccae*, two *B. merdae*, one *B. eggerthii*, two *B. stercoris*, and 1 *B. fragilis* group strains were tested.
[c] Seven *P. bivia*, one *P. oris*, one *P. buccalis*, one *P. buccae*, and five *P. intermedia* strains were tested.
[d] Twenty *F. nucleatum*, and three F. necrophorum strains were tested.
[e] Five *V. parvula* and two *Veillonella* sp. strains were tested.
[f] Two *Porphyromonas asacharalytica*, one *Bacteroides splanchicus*, and one *Dialister pneumosintes* strains were tested.
[g] One *C. bifermentans*, one *C. fallax*, three *C. ramosum*, two *C. sphenoides*, and six *Clostridium* sp. strains were tested.
[h] Four *E. alactolyticum*, one *E. biforme*, 13 *E. lentum*, and 2 *E. ventriosum* strains were tested.
[i] Ten *P. acnes* and one *P. granulosum* strains were tested.
[j] Two *P. anaerobius*, 3 *P. asachharolyticus*, 10 *P. magnus*, 2 *P. micros*, 3 *P. prevotii*, 1 *P. parvulus*, and 2 *R. gnavus* strains were tested.

ronidazole (MIC from 4 to 16 mg/liter) was observed for fewer than 5% of the *B. fragilis* group strains (5, 8).

Against gram-negative bacteria other than the *B. fragilis* group, nitazoxanide showed the same activity as metronidazole. Both compounds were more effective than the other antibiotics with the exception of imipenem, which was much more effective. Strains resistant to amoxicillin, piperacillin, and clindamycin were observed.

The sporulated gram-positive bacilli (*Clostridium* spp.) were very susceptible to both nitazoxanide and many of the other antibiotics. Against *C. difficile*, nitazoxanide was more effective than all reference products, with a MIC_{90} of 0.06 mg/liter.

According to their susceptibilities to metronidazole, the nonsporulated gram-positive bacilli could generally be divided into two groups. Two-thirds of the *Eubacterium* and *Bifidobacterium* strains were susceptible to 5-nitroimidazoles (MICs, ≤4 mg/liter). *Propionibacterium* spp., however, were naturally resistant to these antibiotics (MICs, ≥32 mg/liter). Nitazoxanide was more effective than metronidazole against *Bifidobacterium* spp. (MIC_{90}s, 4 versus 64 mg/liter, respectively) and Propionibacterium spp. (MIC_{90}s, 16 versus 64 mg/liter, respectively), but both compounds were equally effective against *Eubacterium* spp. (MIC_{90}, 1 mg/liter). The other antibiotics which do not belong to the 5-nitroimidazoles were generally very effective against these three species of anaerobes.

TABLE 2. Comparative in vitro activities of nitazoxanide, tizoxanide, and metronidazole against 34 clinical isolates of *S. aureus* in relation to type of incubation condition

Condition and antimicrobial agent	MIC (mg/liter)		
	50%	90%	Range
Aerobic			
Nitazoxanide	64	64	32–64
Tizoxanide	>128	>128	>128
Metronidazole	>128	>128	>128
Anaerobic			
Nitazoxanide	4	4	2–4
Tizoxanide	128	128	32–128
Metronidazole	>128	>128	>128

Gram-positive cocci are generally known to be susceptible to metronidazole; the resistance rate is below 10% in France (2, 8). Nitazoxanide and all reference drugs were very effective against *Peptostreptococcus* strains.

The MICs of nitazoxanide, its first metabolite tizoxanide, and the 50%–50% combination of nitazoxanide-tizoxanide are tabulated in Table 1. Tizoxanide was generally onefold dilution less effective than its parent compound, but no antagonism was recorded during the present study. On the contrary, the nitazoxanide-tizoxanide combination was often synergistic. Synergy was demonstrated against 38 of the 70 *B. fragilis* group strains.

It is well established that metronidazole is not effective against facultatively anaerobic bacteria when they are incubated anaerobically. Our present study confirms this fact. When the plates were incubated in an anaerobic chamber, *Pseudomonas* strains that are obligate aerobes did not grow; meanwhile, facultatively anaerobic species were only inhibited by high concentrations of metronidazole (≥128 mg/liter). Thus, all strains were resistant to metronidazole (data not shown).

The MICs of nitazoxanide obtained under anaerobic conditions were lower than those obtained under aerobic conditions. The strains were generally resistant, with one notable exception: one reference and two clinical strains of *Staphylococcus* were susceptible to nitazoxanide at concentrations of 2, 2, and 0.5 mg/liter, respectively.

Few drugs are potentially effective against methicillin-resistant strains of *Staphylococcus* spp. Consequently, we further investigated the susceptibilities of 34 clinical isolates of *S. aureus*, including 12 strains which were methicillin resistant, to metronidazole, nitazoxanide, and its main metabolite, tizoxanide. The test was conducted under both aerobic and anaerobic conditions. MICs of 2 to 4 mg/liter were recorded for nitazoxanide when the organisms were incubated under anaerobic conditions, while MICs of 32 to 64 mg/ml were recorded when the organisms were incubated under aerobic conditions (Table 2). Tizoxanide and metronidazole did not show any

significant level of activity under either aerobic or anaerobic conditions.

In conclusion, nitazoxanide was highly effective against anaerobic strains susceptible to metronidazole. For some strains with decreased susceptibility (*B. fragilis*, *Eubacterium* spp., and *Bifidobacterium* spp.) or resistance (*Propionibacterium* spp.) to metronidazole, nitazoxanide was more effective. Tizoxanide was generally not as effective as its parent compound, nitazoxanide, but no antagonism between the two chemicals was observed. Nitazoxanide was not effective against aerobic gram-positive or gram-negative bacteria with the exception of *S. aureus* when the organism was incubated under anaerobic conditions.

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