Bacteroides distasonis
Clostridium perfringens

Other microorganisms:
Mycoplasma hominis
Ureaplasma urealyticum

C. Effects of Miscellaneous Factors on Activity

1. Effect of Medium

Studies were performed in order to evaluate the effects of growth medium on the MICs to trovafloxacin with several aerobic and anaerobic organisms. In one study which included isolates of *S. aureus*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *E. cloacae*, and *P. aeruginosa*, the MICs and MBCs generally differed no more than fourfold (two tube dilutions) when determined in brain heart infusion broth, trypticase soy broth, nutrient broth, or cation-supplemented Mueller-Hinton broth (11).

In another study, the effects of various growth media on the activity of trovafloxacin against anaerobes was investigated (66). Supplemented brain heart infusion agar demonstrated the lowest MIC values, with correlation coefficients for most anaerobes of <0.65 when compared with Wilkens Chalgren agar. Wilkens Chalgren and supplemented Brucella agar gave comparative susceptibility results (correlation coefficients ≥0.90).

2. Effect of Inoculum

Increasing the initial cell inoculum from had minimal effect on the MICs of trovafloxacin as determined in an agar dilution test (39).

3. Effect of pH

As with other fluoroquinolones, decreasing the pH of the test medium raised the MIC of most organisms (11,39). This effect was particularly noteworthy against *P. aeruginosa*, while little effect occurred with *S. aureus*.

Against anaerobes, the activity of trovafloxacin was twofold lower when agar media was adjusted to pH 6.0 in comparison to pH 7.0 or pH 7.5.

4. Effect of Divalent Cations

As with other fluoroquinolones, adding Mg^{+2} (9 mM) to Mueller-Hinton broth antagonized the *in vitro* activity of trovafloxacin, with MICs increasing fourfold over those obtained using unsupplemented medium (39).

5. Effect of Incubation Atmosphere

Incubation of the MIC test in aerobic or anaerobic conditions had little effect on the activity of trovafloxacin against two isolates each of *E. coli* and *S. aureus* (39).

6. Effect of Serum

The effect of serum protein binding on the antibacterial activity of trovafloxacin *in vitro* was studied by Child *et al.* (37). Protein binding values ranged between 70 and 88% (29,37), which is higher than values reported for most other fluoroquinolones (77). The addition of 20 or 70% human serum to susceptibility tests with Gram-positive and Gram-negative organisms indicated that MICs and MBCs increased by two to 32-fold (Table 8). The effect was most pronounced with two strains of *Klebsiella* sp.

Table 8. Effect of Human Serum (HS) on the *In Vitro* Activity of Trovafloxacin in Iso-Sensitest Broth (Iso) Against a Test Bacterial Inoculum of 10^5 CFU/mL

Organism	MIC Iso	MBC Iso	MIC Iso + 20% HS	MBC Iso + 20% HS	MIC Iso + 70% HS	MBC Iso + 70% HS
E. coli	0.03	0.03	0.06	0.06	0.06	0.125
E. coli	0.015	0.03	0.015	0.03	0.03	0.06
Klebsiella spp.	0.015	0.015	0.06	0.5	0.5	1.0
Klebsiella spp.	0.03	0.03	0.06	0.25	1.0	2.0
S. aureus	0.03	0.125	0.03	0.125	0.125	0.125
S. aureus	0.03	0.125	0.03	0.03	0.125	0.125
M. catarrhalis	0.008	0.008	0.015	0.015	0.03	0.03
M. catarrhalis	0.004	0.008	0.015	0.015	0.03	0.06

Data from (37)

D. Bactericidal Activity

The bactericidal activity of trovafloxacin has been assessed in two different types of assays. The first measures the minimum bactericidal concentration (MBC) in single time point susceptibility studies performed in broth. The MBC is defined as the lowest concentration of drug that kills 99.9% (>three $\log_{10}$) of the initial inoculum in 24 h. The second method, the Killing Curve studies, assessed bactericidal activity in broth over 24 h, sampling for viability at timed intervals.

One report describes the MBC values obtained for trovafloxacin for single isolates of *S. aureus, S. pneumoniae, E. coli, K. pneumoniae, E. cloacae*, and *P. aeruginosa* (11). MBC values were within one dilution of the MIC determined in cation-supplemented Mueller-Hinton broth or brain heart infusion broth. Another study also found that MICs and MBCs were within one tube

dilution when five strains each of *E. coli, K. pneumoniae, P. aeruginosa*, and MRSA were studied (39).

Visalli *et al.* (71) studied the bactericidal activity of trovafloxacin against 10 isolates of *S. pneumoniae*: 4 penicillin-susceptible, 2 penicillin-intermediate, and 4 penicillin-resistant isolates. As observed with other organisms, the MBCs were within one dilution of the MIC for seven strains and within two dilutions for the remaining three strains.

The bactericidal activity of trovafloxacin was also evaluated against six species of anaerobes tested in Wilkens-Chalgren broth (72). The MIC/MBC values for trovafloxacin are shown in Table 9. MBC values were within one dilution of the MIC.

The kinetics of killing by trovafloxacin were examined by Gootz *et al.* (11). In these tests trovafloxacin and ciprofloxacin were found to be bactericidal within 24 h at the MIC against quinolone-susceptible *E. coli* (Fig. 1a), however, complete killing did not take place until after the first 6 hours of incubation. Sparfloxacin did not accomplish complete killing of this strain at the same MIC (Fig. 1a). Trovafloxacin and sparfloxacin were bactericidal within 24 h at the MIC against quinolone/methicillin-susceptible *S. aureus* (Fig. 1b), however, complete killing did not take place until after the first 6 hours of incubation. Ciprofloxacin exhibited a bacteriostatic effect and was not highly bactericidal even at four times the MIC (Fig. 1b). Trovafloxacin produced complete killing by 6 h at the MIC (2.0 μg/mL) against a ciprofloxacin- and methicillin-resistant *S. aureus* strain (Fig. 1c). Incomplete killing over 24 h was observed with this strain by ciprofloxacin and sparfloxacin at the MIC (32 μg/mL). In test with a quinolone-susceptible *P. aeruginosa* (Fig. 1d), ciprofloxacin was the most bactericidal agent when tested at the MIC (1 μg/mL). At 6 h, ciprofloxacin and trovafloxacin produced complete killing at 4X the MIC which was maintained after 24 h. Sparfloxacin at 4X the MIC was bactericidal at 24 h.

Table 9. Bacteriostatic/Bactericidal Levels (μg/mL) at 48 h against anaerobes

Antibiotic	*Bacteroides fragilis*	*Bacteroides thetaiotaomicron*	*Prevotella melaninogenica*	*Fusobacterium mortiferum*	*Peptostreptococcus magnus*	*Clostridium perfringens*
Trovafloxacin	0.25/0.5	0.25/0.5	1.0/1.0	0.5/1.0	0.015/0.06	0.03/0.03
Ciprofloxacin	16.0/32.0	8.0/16.0	4.0/4.0	2.0/2.0	1.0/2.0	0.25/0.25
Sparfloxacin	2.0/8.0	0.5/1.0	2.0/4.0	0.5/1.0	1.0/1.0	0.06/0.06
Metronidazole	1.0/1.0	2.0/2.0	1.0/1.0	1.0/2.0	64.0/64.0	1.0/1.0
Cefoxitin	16.0/32.0	1.0/1.0	0.5/1.0	2.0/2.0	4.0/8.0	0.125/0.125
Piperacillin	64.0/64.0	16.0/32.0	8.0/8.0	1.0/1.0	0.25/0.5	0.125/0.125
Piperacillin/ tazobactam	2.8/8.0	2.0/4.0	0.125/0.25	1.0/1.0	0.25/0.5	0.06/0.125

From Spangler, Jacobs, and Appelbaum (72).

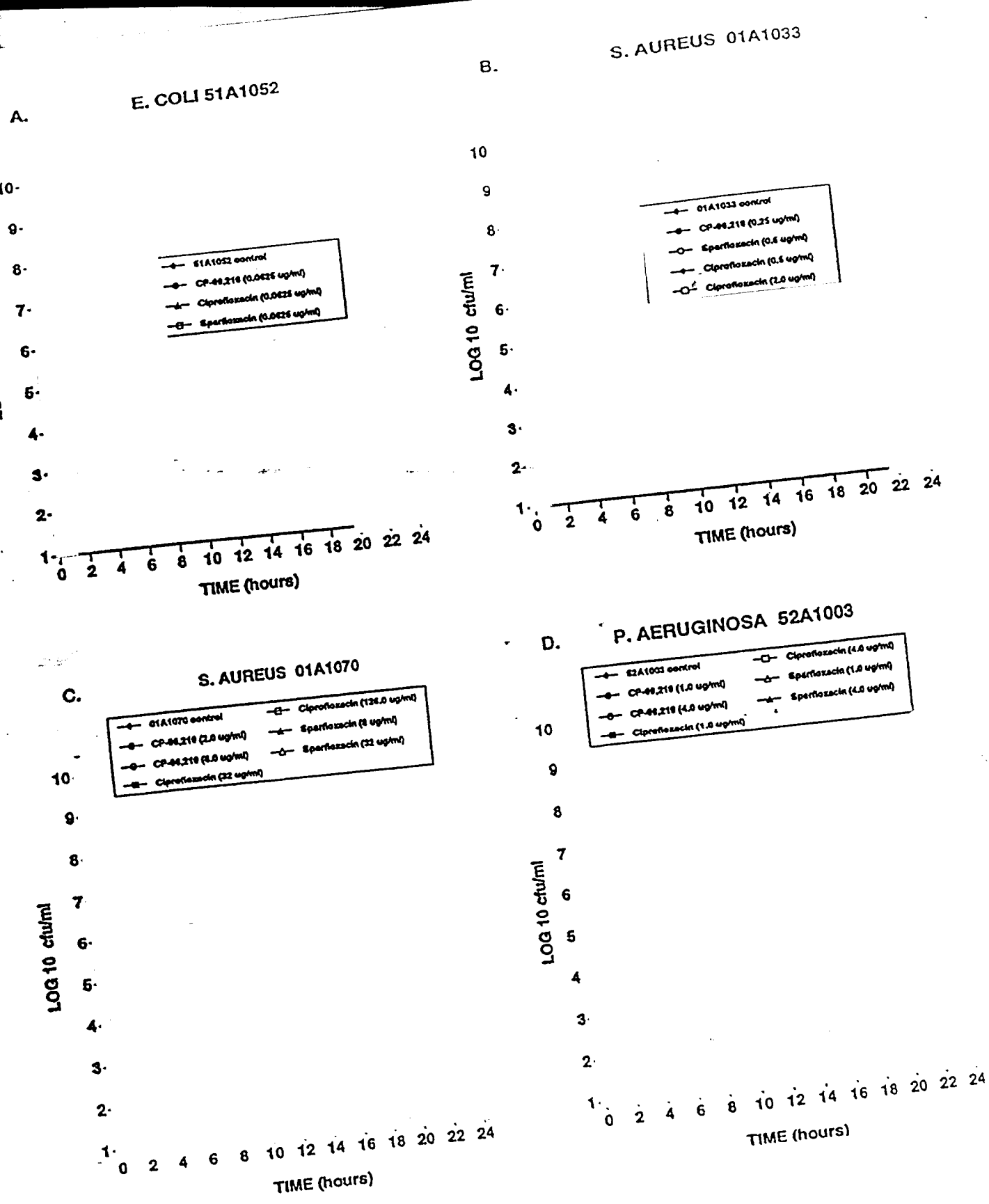


Figure 1. Kill curves performed in BHIB against *Escherichia coli* 51A1052 (a), *Staphylococcus aureus* 01A1033 (b), *S. aureus* 01A1070 (c), and *Pseudomonas aeruginosa* 52A1003 (d). Lowest drug concentrations tested equaled the respective minimum inhibitory concentration. CP-99,219 designation for trovafloxacin.

In time kill curve studies by Visalli *et al.* (71) against penicillin-resistant pneumococci, trovafloxacin was bacteriostatic after 24 h at MIC (0.03 µg/mL) and 2X MIC. At 4X the MIC it was bactericidal after 24 h and at 8X the MIC it was bactericidal at 6 h.

In studies designed to determine the optimum bactericidal concentration (OBC) of trovafloxacin, it was found that trovafloxacin, like ciprofloxacin, was more efficient in killing *E. coli* KL16 than *S. aureus* E3T (18) while sparfloxacin killed both organisms equally well. Against *E. coli* KL16 the OBC of ciprofloxacin< sparfloxacin, ofloxacin< trovafloxacin. Against *S. aureus* E3T the OBC of sparfloxacin< trovafloxacin< ciprofloxacin and ofloxacin. In tests with *S. pneumoniae* C3LN4, the OBC for trovafloxacin< ciprofloxacin< ofloxacin. When *Enterococcus faecalis* was treated with trovafloxacin, weak bactericidal activity occurred after the first 3 h, however, when incubation extended to 6 and 24 h, greater bactericidal activity was apparent. This slow bactericidal activity against *E. faecalis* is a characteristic shared by the majority of quinolone(18). This study demonstrated that after 3 h incubation at OBC, trovafloxacin was bactericidal (at least a 3 log kill) only for *E. coli* KL16 and *S. aureus* E3T. The killing of *S. pneumoniae* C3LN4 under the same conditions was incomplete. Trovafloxacin was not able to kill *E. faecalis* even after 24 h incubation (18). The studies by this author demonstrated that against *E. coli* trovafloxacin works with in 3 h post incubation by inhibiting DNA gyrase and against *S. aureus* it works by both inhibiting DNA gyrase and topoisomerase IV. Against *E. facalis* trovafloxacin works by inhibiting DNA gyrase only after 24.h incubation. With *S. pneumoniae* complete inhibition of DNA gyrase did not take place within the 3 h post incubation.

Another study evaluated the bactericidal activity of trovafloxacin against six *E. faecalis* and five *E. faecium* isolates using a time kill method with a fixed drug concentration of 3 µg/mL (52). Trovafloxacin demonstrated bactericidal activity (at least a 3 log kill) at 24 h against all isolates, including *E. faecalis* resistant to aminoglycosides and/or producing beta-lactamase and one *E. faecalis* vancomycin-resistant isolate, except when the MICs of ciprofloxacin were ≥ 4 µg/mL and those of trovafloxacin were ≥ 2 µg/mL. Comparable degrees of cidality were obtained over 24 h whether the initial inoculum was 10^5 or 10^7 CFU/mL (52).

In another study, Zinner *et al.* (73) investigated the activity of 3 µg/mL trovafloxacin in time kill assays against five strains of *E. faecium* and two strains of *E. faecalis* with variable susceptibility to ampicillin, vancomycin and gentamicin as follows: *E. faecium*, Amp R, Van S; *E. faecium*, Amp R, VanB R; *E. faecium*, Amp R, VanA R; *E. faecium*, Amp R, Gent R; *E. faecium*, Strep R, Van R; *E. faecalis*, VanB R; and *E. faecalis*, Gent R. A ≥3 log decrease in the viability of enterococci was observed after 24 h incubation only for the following two strains: *E. faecium*, Amp R, Van S and *E. faecium*, Amp R, VanB R.

The overall conclusion of these bactericidal studies is that trovafloxacin's MBCs are generally 1-2 tube dilutions greater than the MICs when the MICs are less than 2 µg/mL. Trovafloxacin has a reduced bactericidal activity against *P. aeruginosa* , Enterococci, and penicillin- resistant *S. pneumoniae* as compared to its good bactericidal activity against *S. aureus* and *E. coli*. From studies by Ian Morrissey (18) one may conclude that the enhanced activity of trovafloxacin against *S. aureus* is a result of DNA gyrase, and topoisomerase IV both being targets for trovafloxacin.

E. Intracellular Accumulation and Bacterial Cell Killing

Fluoroquinolones are known to accumulate in phagocytic cells at levels significantly higher than extracellular blood levels. The intracellular accumulation and antibacterial activity of trovafloxacin has been studied in several systems.

One study measured the accumulation of trovafloxacin into guinea pig alveolar macrophages (56) after 1 h incubation. Intracellular levels of trovafloxacin were 23-fold above original extracellular test concentrations. One hour after addition of radiolabelled drug, intracellular trovafloxacin levels were 1.2 (±0.02) mg per 10^7 cells, which was similar to the intracellular level of erythromycin obtained in these experiments. Accumulation was reversible, since about 50% of the drug egressed from the cells after one hour incubation in antibiotic-free medium (56).

In the same report, trovafloxacin was significantly more inhibitory than ofloxacin or erythromycin against two strains of *L. pneumoaphila* grown inside the alveolar macrophages. Erythromycin (1.0 μg/mL) and ofloxacin (0.25 μg/mL) were only partially inhibitory, with rapid regrowth of the organisms occurring after drug washout. Ofloxacin (1.0 μg/mL) and trovafloxacin (0.25 μg/mL), in contrast, were bactericidal, with no measurable regrowth detected up to 4 days after drug washout. Trovafloxacin at 0.1 μg/mL was bactericidal but the organisms grew rapidly after removal of the drug (56).

Another study evaluated the penetration of trovafloxacin into human neutrophils (PMN), peritoneal macrophages (PM), and tissue cultured epithelial cells (McCoy) (78). The accumulation of trovafloxacin into human PMNs was rapid, reversible, nonsaturable (at extracellular concentration ranging from 0.5 to 25 μg/mL), not energy dependent, and significantly increased at pH 6. At extracellular concentrations of 2 μg/mL, the cellular to extracellular ratios (C/E ratios) of trovafloxacin in PMN. PM, and McCoy cells were 10.2 ± 2.0, 10.2 ± 2.0 and 9.6 ± 1.9 respectively (20 min; 37° C). Trovafloxacin uptake by PMNs was significantly higher at pH 6 (C/E= 16.5 ± 0.4, $p < 0.05$) than at pH 7.2 (11.3 ± 0.8). The phagocytosis of opsonized *S. aureus* did not affect the intracellular accumulation of trovafloxacin in PMNs (C/E ratios of 9.0 ± 1.7 and 8.2 ± 1.6, respectively). The phagocytosis of opsonized zymosan, however, significantly increased the accumulation of trovafloxacin by PMNs (C/E ratio of 13.9 ± 1.7 $p < 0.05$).

The available data indicate that like other fluoroquinolones, trovafloxacin reaches intracellular concentrates within phagocytic and non-phagocytic cells 10- to 23-fold above extracellular concentrations, while remaining active intracellularly.

F. Postantibiotic Effect

Postantibiotic effect (PAE) refers to the recovery period or persistent suppression of bacterial growth after short antimicrobial exposure. It is generally accepted that a prolonged PAE will extend the chemotherapeutic action of an antimicrobial drug beyond the time that the agent is available in inhibitory concentrations at the site of infection in the host. The PAE reflects the amount of time required for a cell culture to increase by one log of growth after removal of antimicrobial, with the value corrected for any delay seen in a drug-free control culture of the same organism. Previous studies with fluoroquinolones indicate that the PAE is both strain and

drug specific. Also, higher levels of fluoroquinolone above the MIC produce a more prolonged PAE (75). The length of time of the initial exposure of drug before its removal can also affect the length of the PAE. For staphylococci and *P. aeruginosa*, exposure to ciprofloxacin at for 2-3 h resulted in a PAE of approximately 2 h (75). Under similar conditions, PAE against *E. coli* was 4 h, but against *E. faecalis*, no measurable postantibiotic effect was found (75).

One study determined the PAE of trovafloxacin against one strain each of *E. coli*), *S. aureus* , and *P. aeruginosa*). After a one hour exposure to drug, the PAE's were between 0.3 and 2.5 h (74). Ciprofloxacin tested under the same conditions produced PAEs of 0 to 1.0 h.

In a study by Dubois J. et al. (76) trovafloxacin, sparfloxacin, ciprofloxacin, erythromycin and rifampicin were evaluated against *Legionella* spp. The PAE of trovafloxacin (3.85 h) and ciprofloxacin (2.86 h) was greater than PAE of rifampicin (0.90 h) and sparfloxacin (0.68 h) against erythromycin-resistant *L. pneumophila* strains. Against erythromycin-susceptible *L. pneumophila* strains the PAE of , ciprofloxacin (3.61 h) and rifampicin (2.86 h) were grater than the PAE of trovafloxacin (1.63 h). The PAE of trovafloxacin (2.73 h) was greater than PAE of ciprofloxacin (2.13 h) and sparfloxacin (0.72 h) against erythromycin-resistant *Legionella* other than pneumophila strains.

From the small number of PAE studies that were performed with trovafloxacin, it appears that the observed time for the effect is highly variable and similar to that expected for other fluoroquinolones.

G. Synergy Studies

Limited *in vitro* synergy studies have been performed with trovafloxacin. Ciprofloxacin in combination with an aminoglycoside or a beta-lactam is generally indifferent or additive and synergy is generally not demonstrated (Eliopoulos, *Am. J. Med.* **87**: Suppl. 5A, 17S-22S, 1989). In one study, trovafloxacin and ciprofloxacin were tested by a checkerboard MIC method for synergy with gentamicin or ampicillin/sulbactam (2:1) against *P. aeruginosa*, *E. aerogenes*, *S. marcescens*, and *Stenotrophomonas maltophilia* (53). The effect of trovafloxacin or ciprofloxacin in combination with either gentamicin, ampicillin/sulbactam (2:1), or vancomycin was tested with non multi-drug resistant *E. faecium* and *E. faecalis*, as well as a multi-drug (vancomycin and ampicillin-resistant with or without high-level gentamicin resistant) *E. faecium* and multi-drug resistant (ampicillin and high-level gentamicin-resistant) *E. faecalis*. Synergy was defined as a four-fold reduction in the MIC of the antibiotic combination compared to the MIC with each antibiotic alone. The results from these studies are shown in Table 10. From a total of 168 strains studied, synergy was seen for trovafloxacin in only 4 strains of enterococci (2 with gentamicin, 2 with ampicillin-sulbactam). An additive effect was shown for over 50% of the multi-drug resistant *E. faecium* isolates tested with the combination of trovafloxacin and ampicillin/sulbactam and for over 50% of the *E. faecalis* isolates tested with the combination of trovafloxacin and vancomycin (53).

In another study involving checkerboard titration techniques, trovafloxacin was tested alone and in combination with ceftazidime, imipenem, or amikacin against 120 nonfermenters (58). These

included 47 *P. aeruginosa*, 4 *P. fluorescens/putida*, 14 *S. maltophilia*, 15 *Burkholderia cepacia*, 15 *Acinetobacter*, 16 *Alcaligenes*, and 9 *Flavobacteria/Chryseobacteria*. MIC_{90}s of trovafloxacin, ceftazidime, amikacin, and imipenem were: *P. aeruginosa* (4, 128, 32, 128 μg/mL), *P. fluorescens/putida* (8, 32, 8, 8 μg/mL), *S. maltophilia* (4, 128, 32, 128 μg/mL), *B. cepacia* (64, 128, 32, 8 μg/mL), *Acinetobacter* (8, 128, 32, 1 μg/mL), *Alcaligenes* (32, 128, 32, 8 μg/mL), and *Flavobacteria/Chryseobacteria* (4, 128, 32, 128 μg/mL). The percent of strains showing synergy (fractional inhibitory concentration or FIC ≤0.5) in these studies was highly variable and strain-dependent. In most instances, less than 33% of the strains from any species showed synergy with any of the three drug combinations. The most frequent synergy was seen in the case of *P. aeruginosa*: trovafloxacin plus ceftazidime 28%, trovafloxacin plus imipenem 26%, and trovafloxacin plus amikacin 11%. Antagonism (FIC >1) was observed with trovafloxacin and imipenem with 60% of *B. cepacia* and 63% of *Alcaligenes*. The combination of trovafloxacin plus amikacin was antagonistic for 21% of *P. aeruginosa* and 73% of *B. cepacia*. If the antagonism was defined as FIC > 4.0, then no antagonism was observed.

Table 10. *In vitro* Studies of Antibiotic Combinations

Antibiotic Combination (No. of Isolates)	Synergy	Addition	Indifference
Multi-drug resistant *Enterococcus faecium* (20)			
Trovafloxacin + ampicillin / sulbactam	0	12	8
Trovafloxacin + gentamicin	2	3	15
Trovafloxacin + vancomycin	0	5	15
Ciprofloxacin + ampicillin / sulbactam	0	2	18
Ciprofloxacin + gentamicin	0	0	20
Ciprofloxacin + vancomycin	0	5	15
Non multi-drug resistant *Enterococcus faecium* (24)			
Trovafloxacin + ampicillin / sulbactam	0	8	16
Trovafloxacin + gentamicin	0	3	21
Trovafloxacin + vancomycin	0	5	19
Ciprofloxacin + ampicillin / sulbactam	0	7	17
Ciprofloxacin + gentamicin	0	1	23
Ciprofloxacin + vancomycin	0	4	20
Non multi-drug resistant *Enterococcus faecalis* (24)			
Trovafloxacin + ampicillin / sulbactam	2	10	12
Trovafloxacin + gentamicin	0	6	18
Trovafloxacin + vancomycin	0	18	6
Ciprofloxacin + ampicillin / sulbactam	0	0	24
Ciprofloxacin + gentamicin	0	0	24
Ciprofloxacin + vancomycin	0	4	20
***Pseudomonas aeruginosa* (25)**			

Antibiotic Combination (No. of Isolates)	Synergy	Addition	Indifference
Trovafloxacin + ampicillin / sulbactam	0	2	23
Trovafloxacin + gentamicin	0	3	22
Ciprofloxacin + ampicillin / sulbactam	0	0	25
Ciprofloxacin + gentamicin	0	3	22
***Enterobacter aerogenes* (25)**			
Trovafloxacin + ampicillin / sulbactam	0	0	25
Trovafloxacin + gentamicin	0	3	22
Ciprofloxacin + ampicillin / sulbactam	0	0	25
Ciprofloxacin + gentamicin	0	0	25
***Serratia marcescens* (25)**			
Trovafloxacin + ampicillin / sulbactam	0	2	23
Trovafloxacin + gentamicin	0	2	23
Ciprofloxacin + ampicillin / sulbactam	0	1	24
Ciprofloxacin + gentamicin	0	2	23
***Stenotrophomonas maltophilia* (25)**			
Trovafloxacin + ampicillin / sulbactam	0	3	22
Trovafloxacin + gentamicin	0	4	21
Ciprofloxacin + ampicillin / sulbactam	0	0	25
Ciprofloxacin + gentamicin	1	6	18

From Dembry and Andriole (53).

In order to rule out possible antagonistic interaction of trovafloxacin with a beta-lactam against organisms that can cause meningitis, kill curves were performed with trovafloxacin alone and in combination with ceftriaxone (74). Strains of *S. pneumoniae* (including a penicillin-resistant strain), *Haemophilus influenzae* (beta-lactamase positive and negative), and *Neisseria meningitidis* were tested in a timed kill curve assay where the degree of killing obtained at 24 h with each drug combination was compared to the amount of killing achieved at the same level of each drug alone. No cases of synergy or antagonism were found in any of these tests (74).

Quite different results were found in a study in which the *in vitro* combination of trovafloxacin and sulbactam/ampicillin was examined (105). Of the 110 gram negatives examined, synergy or partial synergy was demonstrable for 91 (83%). Included were 10 isolates of *P. aeruginosa*, 10 strains of *Acinetobacter baumannii*, and 10 isolates of *Stenotrophomonas maltophilia*. An additive interaction was observed with an additional 12 (11%) isolates. Antagonism was noted with 2 (1.8%) of the gram negatives tested. Synergy or partial synergy was likewise demonstrable for 9/10 (90%) enterococci and 12/30 (40%) *S. pneumoniae* tested (including penicillin-resistant strains). A lack of interaction (indifference) was noted with 13/30 (43%) of the pneumococci.

In another study (106) the combination of trovafloxacin and ceftazidime was tested against 459 Gram-negative non-fermentative organisms using an agar dilution MIC method. While synergy

with these antimicrobials was infrequently observed with this collection of non-fermenters, synergy was detected in 32/36 strains of *S. maltophilia* tested.

The overall results obtained with combinations of trovafloxacin with beta-lactams and aminoglycosides indicate that synergy is strain specific and not commonly encountered. This agrees with results obtained previously with other fluoroquinolones (Eliopoulos, *Am. J. Med.* **87**:Suppl. 5A, 17S-22S, 1989).

H. Assessment of Mechanisms of Resistance

Previous studies with fluoroquinolones indicate that selection of resistant mutants *in vitro* occurs at a relatively low frequency of 1×10^{-8} - 1×10^{-9} mutants per cell plated (91). Resistance to fluoroquinolones is not plasmid-encoded, nor inducible; single mutations in chromosomal genes have been shown to be responsible for step-wise selection of resistance (91). Such step-wise increases in resistance usually involve cumulative mutations at multiple genes in a given strain.

One of the first studies to assess the development of resistance to trovafloxacin *in vitro* involved a daily passage of strains in media containing increasing concentrations of drug (39). As observed previously with other fluoroquinolones, after 5 daily subcultures in the presence of trovafloxacin, MICs increased by 4 to 500-fold above those obtained before passage. The large increases in resistance did not occur with all of the *S. aureus*, *E. coli*, and *P. aeruginosa* strains tested. Such observations indicate that certain bacterial isolates possess the potential to become resistant to trovafloxacin under the appropriate selection conditions. The same study determined the frequency of mutation to trovafloxacin resistance in strains of *S. aureus*, *S. epidermidis*, *E. coli*, *Enterobacter* spp., and *P. aeruginosa*. The method used involved a direct plating technique and selection concentrations of 4X MIC. Resistance frequencies generally ranged from 10^{-7} or 10^{-8} to 10^{-10}. Such frequencies are typical for currently available fluoroquinolones.

One report compared the *in vitro* frequency of mutation observed with isolates of staphylococci using ciprofloxacin and trovafloxacin as the selecting agents (92). Rather than testing for selection at a standard multiple of the MIC, these investigators tested both drugs at the clinically relevant concentrations At 1 μg/mL, the frequency of resistance selection with trovafloxacin for two MSSA and two MSSE strains were consistently $< 8 \times 10^{-10}$. This level of trovafloxacin was 16-fold above the MIC of each susceptible parent organism. In contrast, at 1 μg of ciprofloxacin per mL, selection frequencies ranged . Studies with two MRSA and two MRSE gave similar results.

These studies may suggest that at drug concentrations at the breakpoint for ciprofloxacin (1 μg/mL), resistance mutations are selected less frequently (less than 1 mutant from 10^{10} cells plated) with trovafloxacin than with ciprofloxacin. However the data is very limited and one must confirm this observation

A detailed analysis of *in vitro* selection to fluoroquinolone resistance in *S. pneumoniae* was described in one report (13). In this study the mutation frequency to resistance was studied using ciprofloxacin or trovafloxacin at the clinically relevant selecting concentrations At the breakpoint for ciprofloxacin (1 μg/mL) mutation frequency for four pneumococcal strains ranged from At this level of trovafloxacin, mutation frequencies to

resistance were $\leq 8.9 \times 10^{-9}$. Trovafloxacin MICs to these five strains of pneumococci were generally 16-fold lower than those for ciprofloxacin, accounting for the lower frequency of mutation to resistance with trovafloxacin.

This study also elucidated the mechanism of resistance to fluoroquinolones in mutants obtained in a step-wise selection with ciprofloxacin. The exact changes occurring in the quinolone resistance determining region (QRDR) of the DNA gyrase and topoisomerase IV A subunits were determined by PCR analysis. First-step mutants selected by plating parent cells on medium containing 4X MIC ciprofloxacin occurred at a frequency of 1×10^{-9} and possessed changes in the A subunit of topoisomerase IV (GrlA) only, involving substitution of Phe or Tyr at Ser80 (13). It is noteworthy that while these mutants had MICs to ciprofloxacin of , they were still inhibited by 0.5 µg/mL of trovafloxacin (Table 11).

Table 11. Activity of Trovafloxacin Against Topoisomerase IV Mutants of *Streptococcus pneumoniae*

	MIC (µg/mL)			PCR Results for *grlA* and *gyrA*[a]	
Strain	**Trovafloxacin**	**Ciprofloxacin**	**Tetracycline**	**Topoisomerase IV**	**Gyrase**
S. pneumoniae 1016 parent	0.125	1.0	0.125	–[b]	–
S. pneumoniae 1056 parent	0.125	1.0	0.25	–	
S. pneumoniae 1016-30	0.5	4.0	0.125	Ser^{80} to Phe	–
S. pneumoniae 1016-36	0.5	4.0	0.125	Ser^{80} to Tyr	–
S. pneumoniae 1056-79	0.5	4.0	0.25	Ser^{80} to Phe	–
S. pneumoniae 1056-100	0.5	8.0	0.25	Ser^{80} to Phe	–

[a] Both topoisomerase IV *grlA* and DNA gyrase *gyrA* genes were amplified and sequenced using specific primers to the quinolone resistance determining region (QRDR) of each gene (Gootz *et al.*, 1996).
[b] (–) denotes wild-type gene sequence found.

Second-step mutants were derived from *S. pneumoniae* mutants altered in *grlA* by plating concentrated cell suspensions on agar medium containing 4X MIC of ciprofloxacin (32 µg/mL). Second-step mutants were similarly obtained at the low frequency of 1×10^{-9} (13).

PCR analysis of the QRDR of *grlA* and *gyrA* of these mutants revealed that they had a second change in the DNA gyrase A subunit corresponding to a substitution in Ser84 to Tyr or Phe, or in Glu88 to Lys (13). The second-step mutants had MICs to ciprofloxacin of and had trovafloxacin MICs . While such double mutants could be selected in two distinct steps in the laboratory, such *S. pneumoniae* strains have been rarely isolated from clinical samples (94).

These observations in *S. pneumoniae* appear to parallel resistance selection in other Gram-positives. The first step to fluoroquinolone resistance in *S. aureus* also occurs in topoisomerase IV (9). Second-step mutants with high-level resistance to ciprofloxacin contain an additional mutation in *gyrA* of DNA gyrase. While such double mutants have been documented in *S. aureus* (9), they have not been shown to date to occur in other Gram-positive organisms.

The activity of trovafloxacin against a collection of genetically defined strains of *S. aureus* and *E. coli* is summarized in Table 12 (95). The activity observed against *S. aureus* mutants parallels that observed in the mutants of *S. pneumoniae* just described above. In *S. aureus* mutants with changes in topoisomerase IV (MT111-MT5224C4) MICs to trovafloxacin were increased 8-fold over those of the parent, but remained at 0.25 μg/mL. A mutant with a change in both DNA gyrase and topoisomerase IV (EN1252a) had a trovafloxacin MIC of 4.0 μg/mL, representing a 128-fold increase over the parent (95). Interestingly, no effect was observed in a mutant (MT23142) overexpressing the NorA efflux protein, suggesting that trovafloxacin is not a substrate for this efflux transporter. This was also the case in *S. aureus* MT1222 which contained resistance mutations in *gyrA* and *grlA* in addition to having an up-regulated NorA. Such up-regulation of drug efflux in *S. aureus* is known to markedly affect susceptibility to ciprofloxacin (95).

The impact of resistance mutations is more complex in Gram-negative organisms. Here, single mutations in the DNA gyrase A subunit increase the MIC to trovafloxacin 32-fold over that for the wild type. Combinations of *gyrA* mutations and those in the outer membrane, such as in *E. coli* EN226, lead to higher levels of resistance (Table 12). Such Gram-negative strains with multiple mechanisms of fluoroquinolone resistance have been isolated from clinical material (95).

Table 12. Activity of Trovafloxacin Against Genetically Defined Strains of *Staphylococcus aureus* and *Escherichia coli*[a]

Strain	Genotype	MIC (µg/mL)	Fold Change
Staphylococcus aureus			
ISP794	wild type	0.0312	1
ISP86	nov		
MT5224c4	*grlA*(Ser80Phe)	0.25	8
MT5222	*grlA*(Ser80Phe)	0.25	8
MT111	*grlA*(Ala116Glu)	0.25	8
EN1252a	*grlA*(Ser80Phe) *gyrA*(Ser84Leu)		
MT23142	*flqB* (↑ NorA)[b]	0.0312	1
MT1222	*grlA*(Ala116Glu) *gyrA*(Ser84Leu) *flqB*	2.0	64
Escherichia coli			
KL16	wild type	0.032	1
KF130	*gyrA*	1.0	32
EN226-3	*gyrA*	1.0	32
EN226-8	*marR*	0.512	16
EN226	*gyrA* *marR* others	>2	>64

[a] Data from reference (95).
[b] Mutant with increased expression of NorA efflux protein.

Observations with groups of clinical isolates that are resistant to ciprofloxacin indicate that a substantial degree of cross resistance to trovafloxacin occurs. In one study (96) of such resistant *E. coli* isolates with an MIC_{90} to ciprofloxacin of 64 µg/mL, the MIC_{90} to trovafloxacin was 128 µg/mL. Similarly, no potency advantage with trovafloxacin was observed against ciprofloxacin-resistant strains of *Klebsiella* spp., *E. cloacae*, *P. aeruginosa*, *Acinetobacter* spp., and *B. cepacia*.

The available data indicate that trovafloxacin possesses greater potency than ciprofloxacin against many gram-positive organisms. Single mutations in genes which confer resistance to ciprofloxacin (*grlA*) also decrease susceptibility to trovafloxacin but usually to a lesser degree. In terms of gram-negative clinical isolates, trovafloxacin appears to have no consistent advantage over strains which demonstrate high-level resistance to ciprofloxacin.

III. PRECLINICAL EFFICACY (*IN VIVO*)

A. Pharmacokinetics and Bioavailability

1. Oral Administration: Bioavailability

The sponsor states that trovafloxacin is readily absorbed in humans after oral administration with peak blood levels of 1.1, 2.2, and 3.1 µg/mL observed following single doses of 100, 200, and 300 mg, respectively. Absorption is rapid, with a time to peak of approximately 1.1 h. Bioavailability has been measured to be 88%. Peak blood levels are dose-proportional through 1,000 mg, the top dose studied. The terminal elimination half-life of trovafloxacin is approximately 11 h.

In multiple dosing experiments at 100 and 300 mg once daily, consecutive dosing resulted in mean AUC_{0-24} values from 9.4 µg-h/mL on day 1 to 11.8 µg-h/mL on day 17 for the 100 mg dose and from 26.2 µg-h/mL on day 1 to 34.6 µg-h/mL on day 17 for the 300 mg dose (Table 13). This resulted in an accumulation factor of 1.25 for the 100 mg dose and of 1.32 for the 300 mg dose. Inspection of the predose trovafloxacin concentrations on days 6, 10, 14, and 17 indicated that at both dose levels steady state had been obtained by the third daily dose. Mean renal clearance values observed in this study ranged , suggesting that repetitive daily dosing did not affect renal clearance (21).

Table 13. Pharmacokinetic Parameters (mean ± SD) for Trovafloxacin Following Oral Administration at Single and Multiple Doses of 100 and 300 mg to Healthy Male Volunteers (data from ref. 21)

	Trovafloxacin dose (mg)				
	100		300		
Pharmacokinetic parameters	Day 1 (n = 8)	Day 17 (n = 8)	Day 1 (n = 8)	Day 17 (n = 6)	Mean[a]
C_{max} (µg/mL)	1.0 ± 0.3	1.1 ± 0.2	2.9 ± 0.4	3.3 ± 0.5	
T_{max} (h)	0.9 ± 0.4	1.0 ± 0.5	1.2 ± 0.6	1.3 ± 0.7	1.1 ± 0.6
AUC_{0-24} (µg-h/mL)	9.4 ± 1.5	11.8 ± 1.8	26.2 ± 4.5	34.6 ± 6.5	
$AUC_{0-\infty}$ (µg-h mL)	11.2 ± 2.2		33.3 ± 5.5		
CLr (L/h)	0.44 ± 0.08	0.47 ± 0.11	0.44 ± 0.08	0.35 ± 0.05	0.43 ± 0.09
fe (%)	5.3 ± 0.6	5.5 ± 1.2	5.1 ± 1.6	2.9 ± 0.7[b]	5.3 ± 1.1[c]
fu (%)	20.3 ± 7.4	22 ± 4.3	25.6 ± 3.7	28.3 ± 6.0	23.8 ± 6.1
$T_{1/2}$ (h)	9.2 ± 1.2	10.5 ± 0.7	10.5 ± 1.4	12.2 ± 1.9	10.5 ± 1.9

[a] Includes all subjects in all dose groups.
[b] 12-24 h urine samples were lost.
[c] Excluded the day 17 data.

2. Intravenous Administration

The pharmacokinetics following a single dose of the prodrug alatrofloxacin are shown in Table 14. The infusate concentration was 5 μg/mL for the 30-mg dose and the first three subjects in the 100-mg dose group, and it was 1 μg/mL for all subsequent dose groups. Alatrofloxacin prodrug was not detected in plasma at any time point following the end of the infusion. Urine concentrations of alatrofloxacin were not detected at the 300-mg dose level. Consequently, urine samples from the lower-dose were not analyzed for alatrofloxacin content.

Table 14. Mean Pharmacokinetic Values of Trovafloxacin Following Single Dose Alatrofloxacin Infusion in Man During Phase I Studies

Dose[a]	Day	n	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (μg·h/mL)	CL (mL/min)	V_{dss} (L)	$T_{1/2}$ (h)	CLr (mL/min)
30	1	4	0.4	0.9	--	--	--	--	15.1
100	1	4	1.8	1.0	16.4	104.9	89.1	10.4	12.0
200	1	3	2.3	1.0	31.2	107.7	110.2	12.3	9.8
300	1	4	4.3	1.0	43.4	116.0	99.2	10.8	13.1

[a] Infused over 1 h, data from Trovafloxacin Investigator's Brochure

Multiple doses of alatrofloxacin showed an accumulation factor of 1.3 between days 1 and 10 for the 300 mg dose group (22). Following once-daily doses of 300 mg infused over one hour, mean peak blood levels of 3.6 and 4.4 μg/mL were obtained on days 1 and 10, respectively. On day 10, a mean of 0.8 μg/mL of trovafloxacin was present in serum 24 h after the infusion (22). It seems that the prodrug is immediately converted to trovafloxacin and the pharmacokinetics of both formulations are similar.

Table 15 summarizes the pharmacokinetic parameters for the proposed oral doses of 100 and 200-mg QD and IV 200 and 300 QD. It is not clear to this reviewer as to why some of the parameters presented in Table 16 differ from the parameters presented in table 15 above for similar doses.

Table 15. Trovafloxacin Pharmacokinetic Parameters.

	C_{max} (μg/mL)	T_{max} (h)	AUC[a] (μg·h/mL)	$T_{1/2}$ (h)
Trovafloxacin 100 mg				
Single dose	1.0 ± 0.3	0.9 ± 0.4	9.4 ± 1.5	9.2
Multiple dose	1.1 ± 0.2	1.0 ± 0.5	11.8 ± 1.8	10.5
Trovafloxacin 200 mg				
Single dose	2.1 ± 0.5	1.8 ± 0.9	26.7 ± 7.5	9.6
Multiple dose	3.1 ± 1.0	1.2 ± 0.5	34.4 ± 5.7	12.2
Alatrofloxacin 200 mg[b]				

Single dose	2.7 ± 0.4	1.0 ± 0	28.1 ± 5.1	9.4
Multiple dose	3.1 ± 0.6	1.0 ± 0	32.2 ± 7.3	11.7
Alatrofloxacin 300 mg[b]				
Single dose	3.6 ± 0.6	1.3 ± 0.4	46.1 ± 5.2	11.2
Multiple dose	4.4 ± 0.6	1.2 ± 0.2	46.3 ± 3.9	12.7

[a] Single dose: AUC_{0-24}, Multiple dose: $AUC_{0-\infty}$
[b] trovafloxacin equivalents

These data indicates that for comparable doses, no dose adjustment is necessary when switching from parenteral to oral administration.

3. Tissue Distribution

The sponsor states that the volume of distribution of trovafloxacin in humans following intravenous administration of 100, 200, or 300 mg alatrofloxacin ranged from 89.1 to 110.2 L. This suggests that trovafloxacin distributes extensively into body tissues and results in significantly higher trovafloxacin concentrations in most target tissues than in plasma or serum. Table 16 summarizes trovafloxacin levels in various tissues post administration.

Table 16. Trovafloxacin distribution into tissues.

Fluid or Tissue	Tissue-Fluid/Serum Ratio	μg/mL or μg/g	Time/Interval Post Dose, (hr)
bronchial macrophages	24.1	34.3	6
lung mucosa	1.1	1.7	6
lung epithelial lining fluid			
single dose	2.3		6
multiple dose	5.8	10.2	6
lung	2.1	2.0	2-25
brain	0.3		2-8
cerebrospinal fluid			
(CSF, adults)	0.25	0.91	5-24
bowel	0.5		2-8
heart	1.8		2-8
colonic tissue	0.7	2.8	1-15
kidney	2.0		2-8
liver	5.2		2-8
bile	14.9	18-38	0-24
skin	1.0	1-2	2-27
subcutaneous tissue	0.4	1-2	2-25
skin blister fluid	0.7-0.9		4-24
	(blister/plasma)	1.14	
peritoneal fluid	0.4		0-15
skeletal muscle	1.5	1-2	2-27
bone	1.0	1-2	2-27
prostatic tissue	1.0	>1.0	3-10
cervix (single dose)	0.5	1.4	3-29
(multiple dose)	0.6		3-16
ovary	1.6	3.8	3-11
fallopian tube	0.7	1.72	3-29
myometrium (single dose)	0.5	1.0	5-13
(multiple dose)	0.6		7-16
uterus	0.6	1.3	3-29
vaginal fluid(single dose)	1.8		2-23
(multiple dose)	4.7	2-3	2-24

The penetration of trovafloxacin into the inflammatory fluid of cantharides-indused blister was assessed in eight humans given a single 200 mg oral dose(26). The mean peak concentration in plasma of 2.9 μg/mL was obtained at a mean time of 0.75 h post dose. The mean peak concentration in inflammatory fluid of 1.2 μg/mL (± 0.19) was obtained at 4.0 h. At 12 h the mean inflammatory fluid concentrations were 0.5 and 0.2 μg/mL, respectively. The overall penetration into inflammatory fluid was 64%.

The penetration of trovafloxacin into human bronchial tissues has also been studied. Following a single 200 mg dose the mean concentrations of trovafloxacin 1-6 h post dose were 2.2 μg/mL in

plasma and 6.1 μg/mL in cells obtained by bronchiolar lavage(27). At 18-24 h after dosing, mean trovafloxacin concentrations were 1.1 μg/mL and 5.2 mg/g in serum and cells, respectively.

In another study, nine patients given once daily 200 mg doses of trovafloxacin for four days were evaluated for drug levels in lung tissue. Six hours after the last dose, trovafloxacin levels in serum, alveolar macrophages, epithelial lining fluid, and bronchiolar mucosa were 1.5 μg/mL, 34.3 μg/mL, 10.2 μg/mL, and 1.7 μg/mL, respectively (100). These data suggests that extensive concentration of trovafloxacin occurs in cells present in the lung.

4. Excretion and Metabolism

Following an oral dose of 200 mg (≈118 mCi) of [^{14}C]trovafloxacin, the mean percentage of radioactivity in urine and feces of normal volunteers was approximately 23% and 63%, respectively (102). Mass spectrometric analyses of fecal extracts indicated that the major fecal drug-related components were unchanged trovafloxacin and the N-acetyl conjugate (37% and 14% of administered dose, respectively); the N-sulfamate conjugate and the diacid metabolite were minor fecal metabolites. The acyl glucuronides of both trovafloxacin and the N-acetyl conjugate were absent in the feces, which was attributed to their hydrolysis by gut microflora. In urine, the major drug-related components were the acyl glucuronide conjugate and unchanged trovafloxacin (11% and 7% of administered dose, respectively); the N-acetyl and N-acetyl/acyl glucuronide conjugates and diacid and hydroxy carboxylic acid metabolites were minor urinary metabolites. In human serum, circulating radioactivity consisted of 52% unchanged trovafloxacin and 22% acyl glucuronide; the N-acetyl and N-acetyl/acyl glucuronide conjugates were minor circulating metabolites in man. Thus, this biotransformation study in man revealed that phase II metabolism (glucuronidation, N-acetylation, and N-sulfoconjugation) plays a major role in the elimination of trovafloxacin. A few oxidative metabolites such as the diacid, hydroxy carboxylic acid, and pyrroline were detected in man, but their percentage in the biological matrices was insignificant. The trovafloxacin metabolites identified in man had been previously identified in the rat and/or dog (28).

5. Protein Binding

Over the clinically relevant serum/plasma trovafloxacin concentration range of 1 to 5 μg/mL, the protein binding of trovafloxacin is 70% as measured by the equilibrium dialysis method (29). A second study by Wise et al. measured serum protein binding of trovafloxacin by the same methodology and calculated a value of 88% at 1 μg/mL (26).

6. Food Effects and Drug Interactions

The pharmacokinetics of the suspension/solution and tablet formulations of trovafloxacin were examined in male and female subjects under fasted and fed conditions. The mean relative bioavailability estimate of trovafloxacin tablets, relative to the solution in a fasted state, were 91.9% and 95.7% in fed and fasted states, respectively. The mean relative bioavailability of the fed tablet treatment, compared with the

fasted tablet treatment was 98.0% indicating that food did not influence the bioavailability of trovafloxacin (23).

Studies have indicated that coadministration of Maalox reduces the bioavailability of trovafloxacin by 66% (24). Administration of 40 mg of omeprazole 2 h before trovafloxacin slightly reduced the bioavailability of the quinolone (23).

Trovafloxacin does not interfere with the metabolism of theophylline (25).

7. Effects of Age and Gender

There were only minor differences in the pharmacokinetics of trovafloxacin in elderly subjects and between males and females (30).

8. Renal Impairment

Since trovafloxacin is predominantly excreted by nonrenal routes of elimination, renal impairment has a limited effect on the pharmacokinetics of this fluoronaphthyridone.

IV. CLINICAL EFFICACY (CLINICAL MICROBIOLOGY)

A. Clinical Laboratory Susceptibility Test Methods

The information summarized in this section is to define the standardized methods to be used by clinical microbiology laboratories to determine the susceptibility of clinical isolates to trovafloxacin.

1. Disk Content Studies

In a study by Fuchs et al. (51) several disk concentrations ranging from 5- to 60-mg trovafloxacin were tested and the 10-μg trovafloxacin disk gave satisfactory results. The sponsor has proposed the use of this disk in Disk Diffusion method for performing *in vitro* susceptibility testing.

The 10-μg disk may be used in performing Dish Diffusion test.

2. Quality Control

Studies by Fuchs et al. and Barry et al. (49,51,65) have been performed in order to establish quality control ranges for trovafloxacin. All studies were conducted according to the standard procedures and guidelines established by the National Committee for Clinical Laboratory Standards. Due to the disk diffusion characteristics of trovafloxacin, a 10-μg disk content has

been chosen for all disk susceptibility procedures. The quality control ranges that have been proposed for trovafloxacin are shown in Table 17.

These quality control ranges may stand as proposed by the sponsor.

Table 17. Quality Control Ranges for Trovafloxacin

Organism (Strain)	MIC (μg/mL)	Zone diameter[a] (mm)
E. coli (ATCC 25922)	0.004-0.016	29-36
S. aureus (ATCC 29213)	0.008-0.03	ND[b]
S. aureus(ATCC 25923)	ND	29-35
P. aeruginosa (ATCC 27853)	0.25-2.0	21-27
E. faecalis (ATCC 29212)	0.06-0.25	ND
H. influenzae (ATCC 49247)	0.004-0.016	32-39
S. pneumoniae (ATCC 49619)	0.06-0.25	25-32
N. gonorrhoeae (ATCC 49226)	0.004-0.016	42-55
B. fragilis (ATCC 25285)		ND
B. thetaiotaomicron (ATCC 29741)		ND
E. lentum (ATCC 43055)		ND

[a] All zone diameter studies performed with a 1μg trovafloxacin disk
[b] ND = not determined

3. Susceptibility Interpretive Breakpoints for Dilution and Diffusion Tests

In two studies by Fuchs et al. (49,51) the authors state that MIC breakpoints of ≤1.0 μg/mL and ≤ 2.0 μg/mL were chosen based on the assumption that the oral dose of trovafloxacin would be 100 to 300 mg, which would result in a mean C_{max} of 1.5 μg/mL for the 100 mg dose and 4.4 μg/mL for the 300 mg dose. The error rate-bound method was used to calculate the zone diameter criteria corresponding to these two MIC breakpoints. For MIC breakpoints of ≤ 2.0 μg/mL (susceptible) and ≥ 8.0 μg/mL (resistant), the corresponding zone diameter breakpoints for the 10-μg disk were ≥ 14 mm (susceptible) and ≤ 10 mm (resistant). If MIC breakpoints of ≤1.0 μg/mL and ≥ 4.0 μg/mL were chosen, the corresponding zone diameter breakpoints for the 10-μg disk were ≥ 16 mm (susceptible) and ≤ 12 mm (resistant).

The authors suggested that these tentative breakpoints should be used for interpretation of *in vitro* susceptibility tests for aerobic (including fastidious) and anaerobic organisms until supporting clinical data are available to confirm the utility of these criteria.

The sponsor has proposed the following breakpoints (Table 18) for interpretation of *in vitro* susceptibility tests for aerobic (including fastidious except for *N. gonorrhoeae*) and anaerobic organisms.

Table 18. Tentative Breakpoints and Interpretive Criteria for Trovafloxacin (10 μg disk) for Organisms Other than *Neisseria gonorrhoeae*

	Zone size (mm) (10 μg disk)	MIC (μg/mL)
Susceptible	≥ 14	≤ 2.0
Intermediate	11-13	4.0
Resistant	≤ 10	≥ 8.0

As it was state in Table 15 of section III.A.2. of this review, the steady state $AUC_{0-\infty}$ for 100 mg oral QD dose was 11.8 ± 1.8 mg.h/mL and for the 200 mg oral QD dose was 34.4 ± 5.7 mg.h/mL. The steady state $AUC_{0-\infty}$ for 200 mg I.V. QD dose was 32.2 ± 7.3 mg.h/mL and for the 300 mg I.V. QD dose was 46.3 ± 3.9 μg.h/mL. Since trovafloxacin is a fluoroquinolone and it acts in a concentration dependent manner, the parameter that most closely relates to its efficacy is the AUC:MIC ratio.

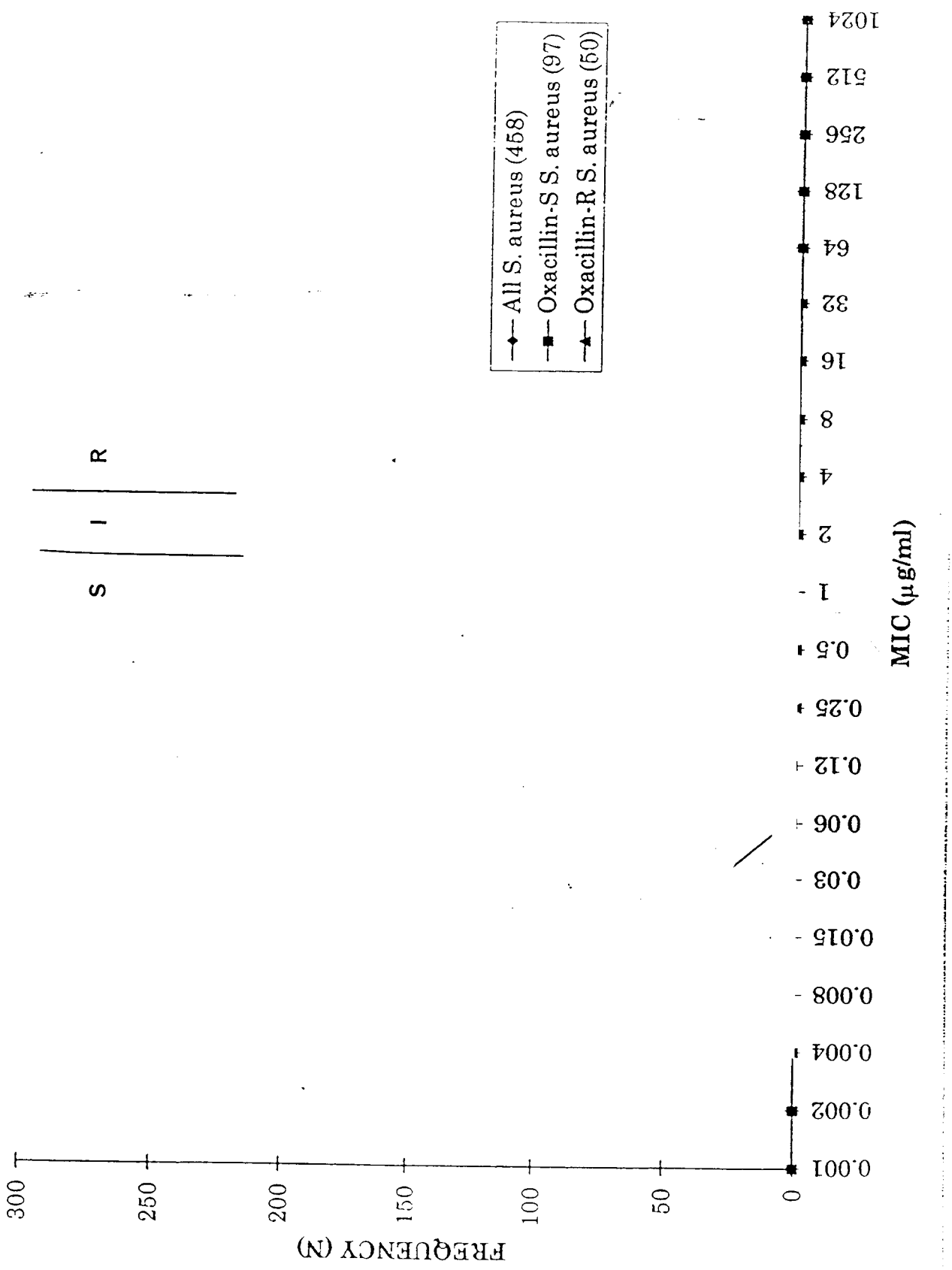
A comparison of the pharmacokinetic parameters for the common fluoroquinolones is presented in Table 19. The susceptible breakpoint (MIC B.P.) and the AUC:MIC ratio is included in this table for comparison. Historically, the MIC breakpoints have been set so that at the proposed dose the AUC:MIC ratio would be between 11 and 30. In the case of trovafloxacin there are three proposed dose of 100, 200, and 300 mg QD. As one can see from Table 19 MIC susceptible breakpoint of 1 μg/mL would result in AUC:MIC ratio of 11.8 which is within the target range of 11-30. If one accepts the sponsor proposed MIC breakpoint of 2 μg/mL, then the AUC:MIC ratio for the 100 mg dose would be 5.9 which is way below the target range

Table 19. Comparison of Pharmacokinetic Parameters for Fluoroquinolones

Drug	Dose (mg)	C_{max} (μg/mL)	AUC_{0-24} (μg.h/mL)	T_{max} (h)	$T_{1/2}$ (h)	MIC B.P. (μg/mL)	AUC_{0-24}:MIC ratio
Ciprofloxacin	500	2.4	11.6	1-2	4	1	11.6
Ofloxacin	400	4.6	61.0	1-2	4-5	2	30.5
Lomefloxacin	400	3.2	26.1	0.8-1.4	~8	2	13.1
Enoxacin	400	2.0	---	1-3	3-6	2	---
Norfloxacin	400	1.5	---	~1	3-4	4	---
Sparfloxacin	200	1.1	18.7			1	18.7
	400	1.3	20.6	3-4	18-20	1	20.6
Levofloxacin	250	2.8	27.2			2	13.6
	500	5.7	47.5	1-2	6.8	2	23.8
Grepafloxacin	400	1.35	14.08	2	7-12	1	14.1
	600	2.25	27.51	2	7-12	1	27.5
Trovafloxacin	100	1.0	9.4	0.5-1.5	9.2	1	9.4
	200	2.1	26.7	0.9-2.7	9.6	1	26.7
	300	3.6	46.1	0.9-1.7	11.2	1	46.1

In addition when one looks at the frequency distribution curves presented in Figures 2 - 8 it becomes evident that with the exception of anaerobes and *Neisseria gonorrhea* the susceptible breakpoint of 1 μg/mL nicely separates the susceptible from resistant populations and would allow for detection of resistance, should it develop, efficiently. For the anaerobes the breakpoint of 2 μg/mL is reasonable because the proposed dose is either 200 or 300 mg QD, which will put the AUC:MIC ratio For *Neisseria gonorrhea* the sponsor has proposed a single 100 mg dose with the mean AUC of 9.4 μg.h/mL. Since the MIC_{90} of these isolates is < 0.03 μg/mL, an MIC susceptible breakpoint of 0.25 μg/mL will give a satisfactory AUC:MIC ratio and would allow for rapid detection of resistance should it develop.

Figure 2. TROVAFLOXACIN *vs Staphylococcus aureus*
(N = 458)



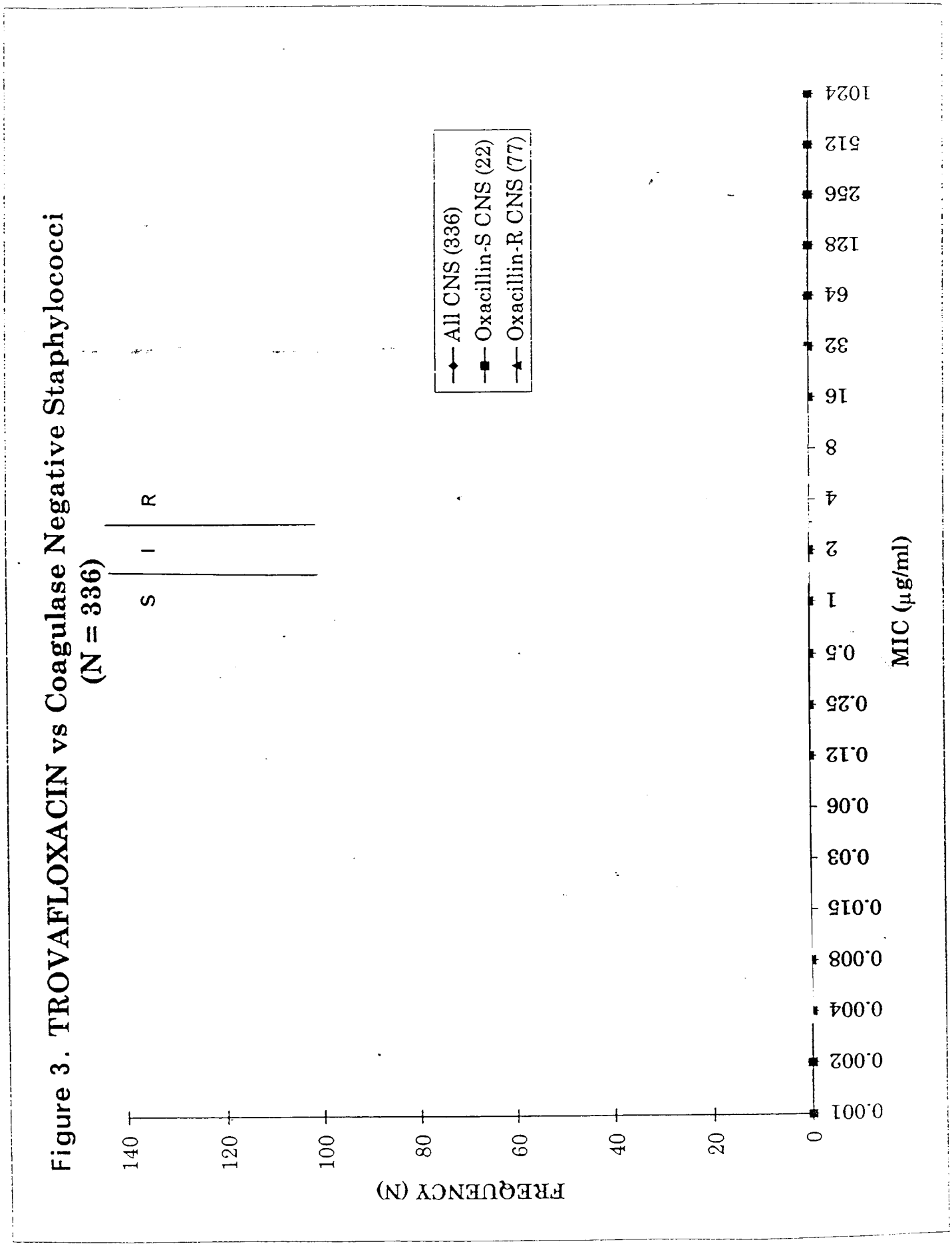


Figure 3. TROVAFLOXACIN vs Coagulase Negative Staphylococci
(N = 336)
S
I
R
All CNS (336)
Oxacillin-S CNS (22)
Oxacillin-R CNS (77)
FREQUENCY (N)
0
20
40
60
80
100
120
140
MIC (µg/ml)
0.001
0.002
0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
256
512
1024

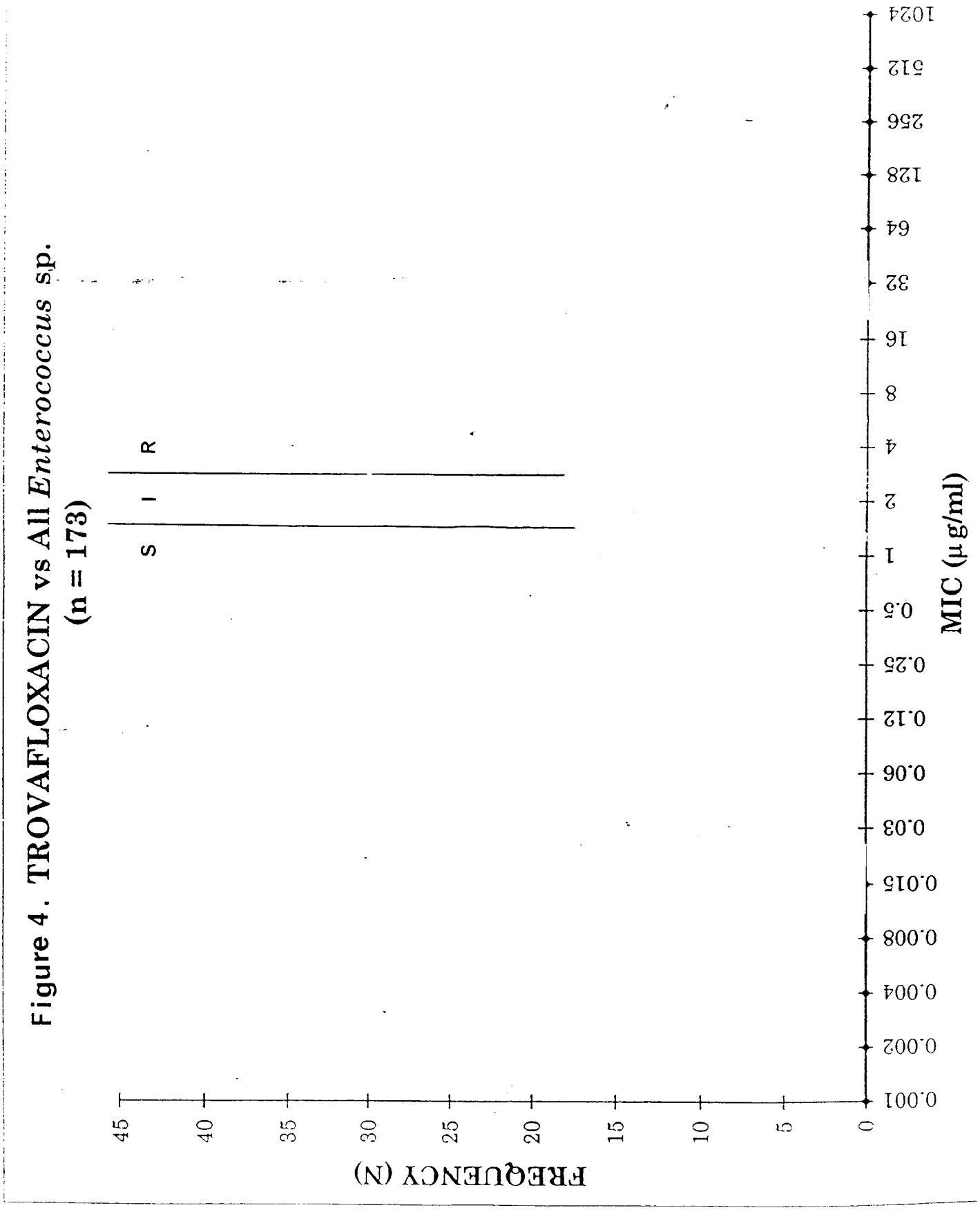
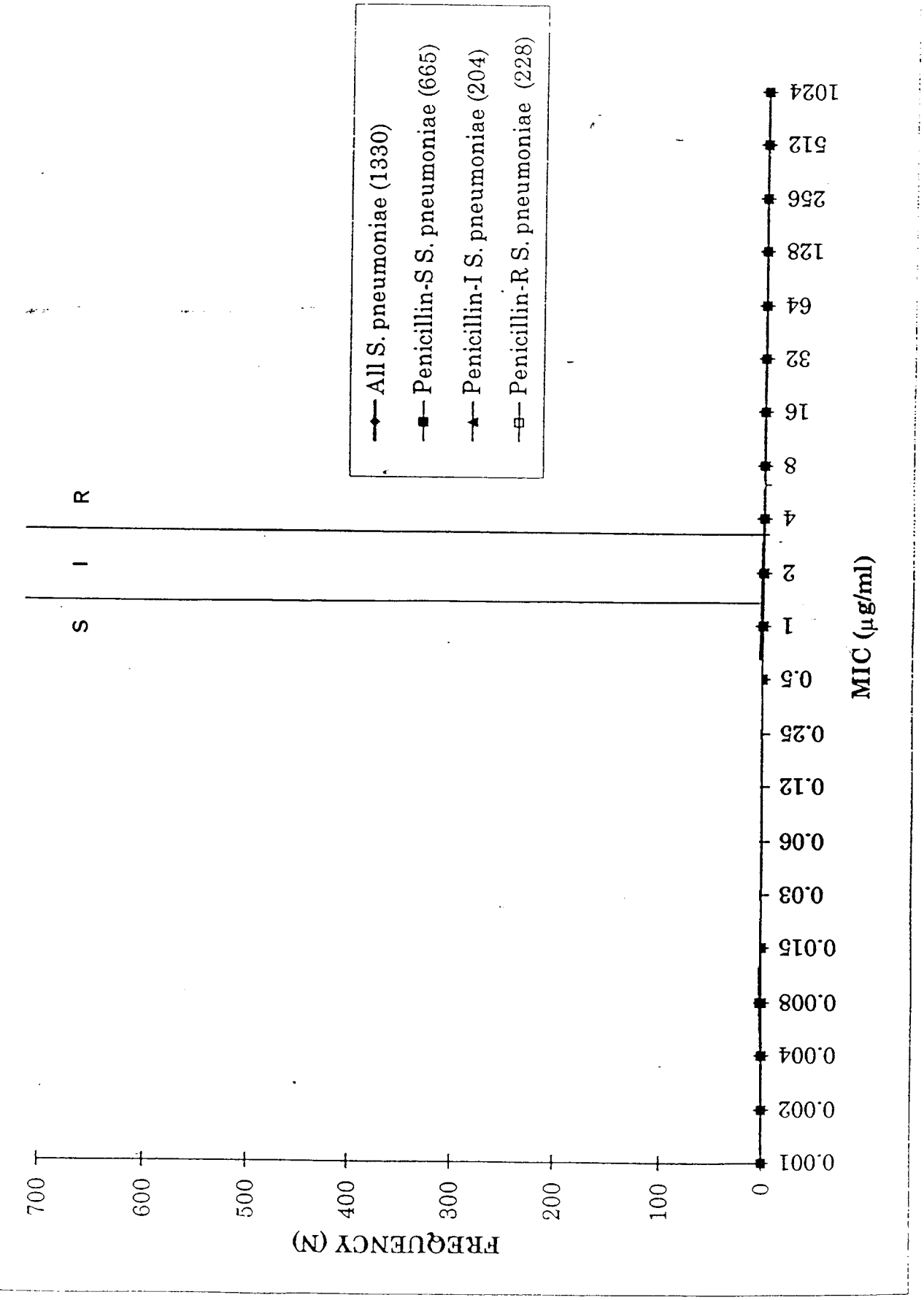


Figure 4. TROVAFLOXACIN vs All *Enterococcus* sp.
(n = 173)

Figure 5. TROVAFLOXACIN vs *Streptococcus pneumoniae* (N = 1330)



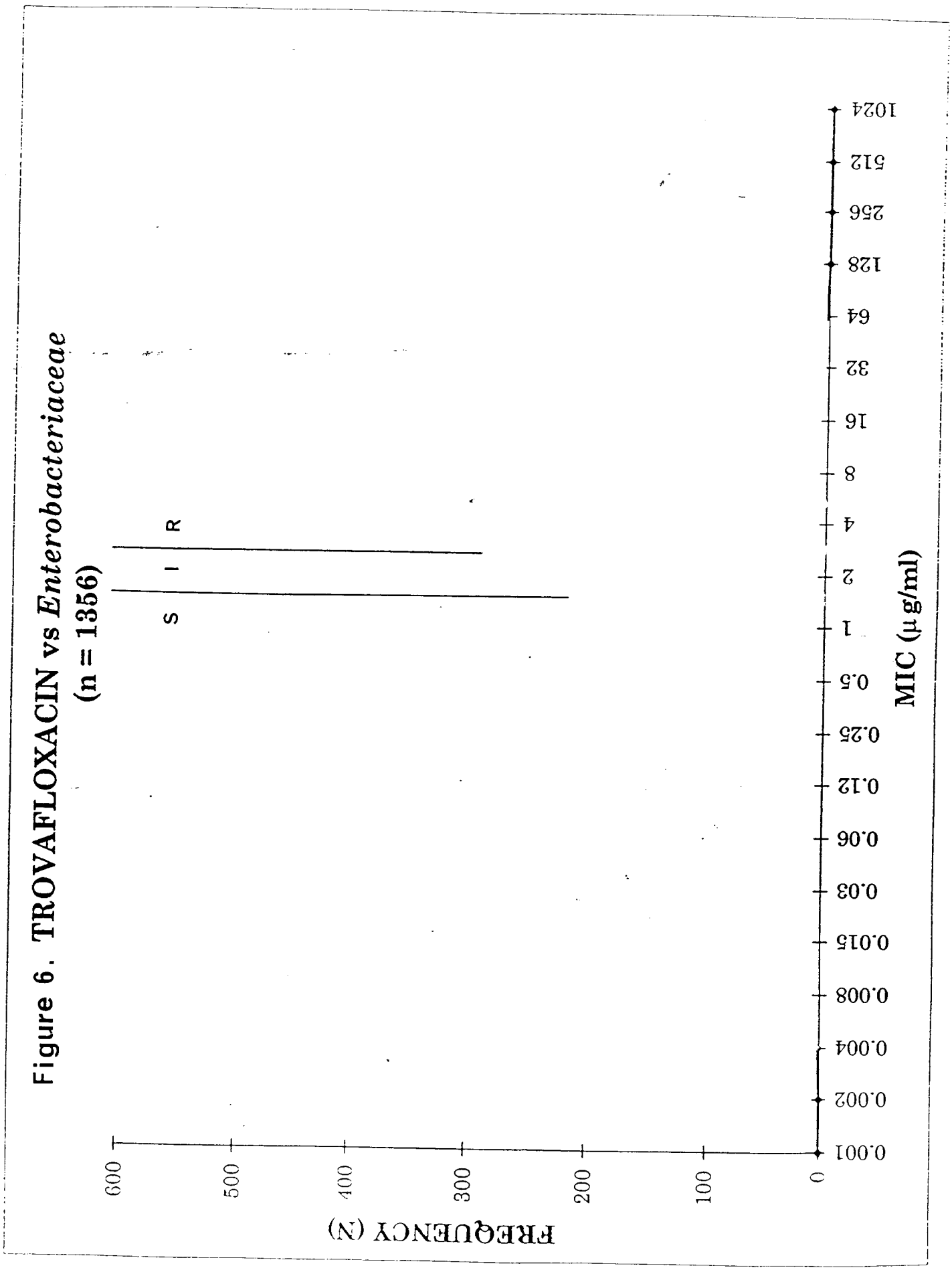


Figure 6. TROVAFLOXACIN vs Enterobacteriaceae
(n = 1356)
S
I
R
FREQUENCY (N)
0
100
200
300
400
500
600
MIC (µg/ml)
0.001
0.002
0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
256
512
1024

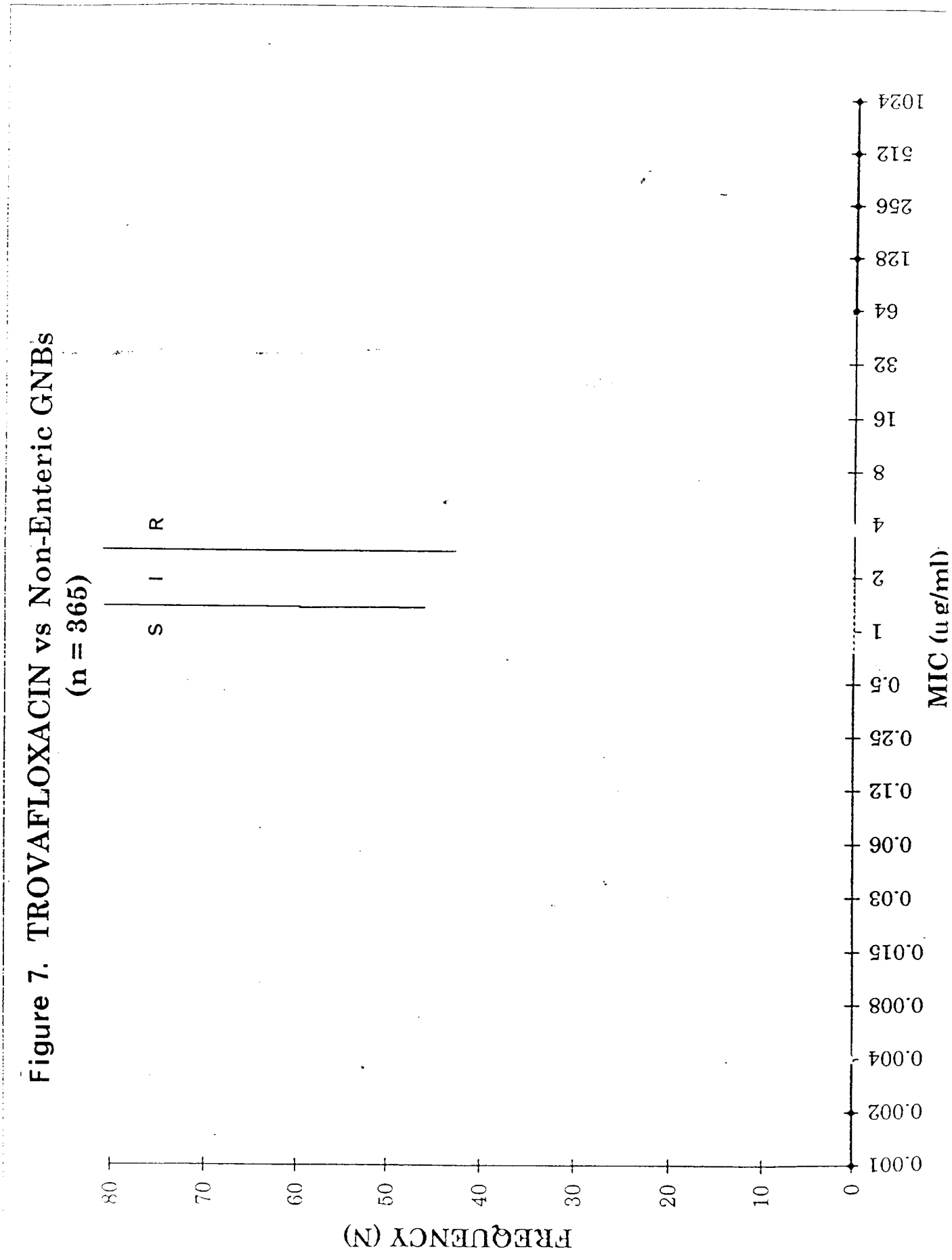


Figure 7. TROVAFLOXACIN vs Non-Enteric GNBs
(n = 365)
S
I
R
FREQUENCY (N)
0
10
20
30
40
50
60
70
80
MIC (µg/ml)
0.001
0.002
0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
256
512
1024

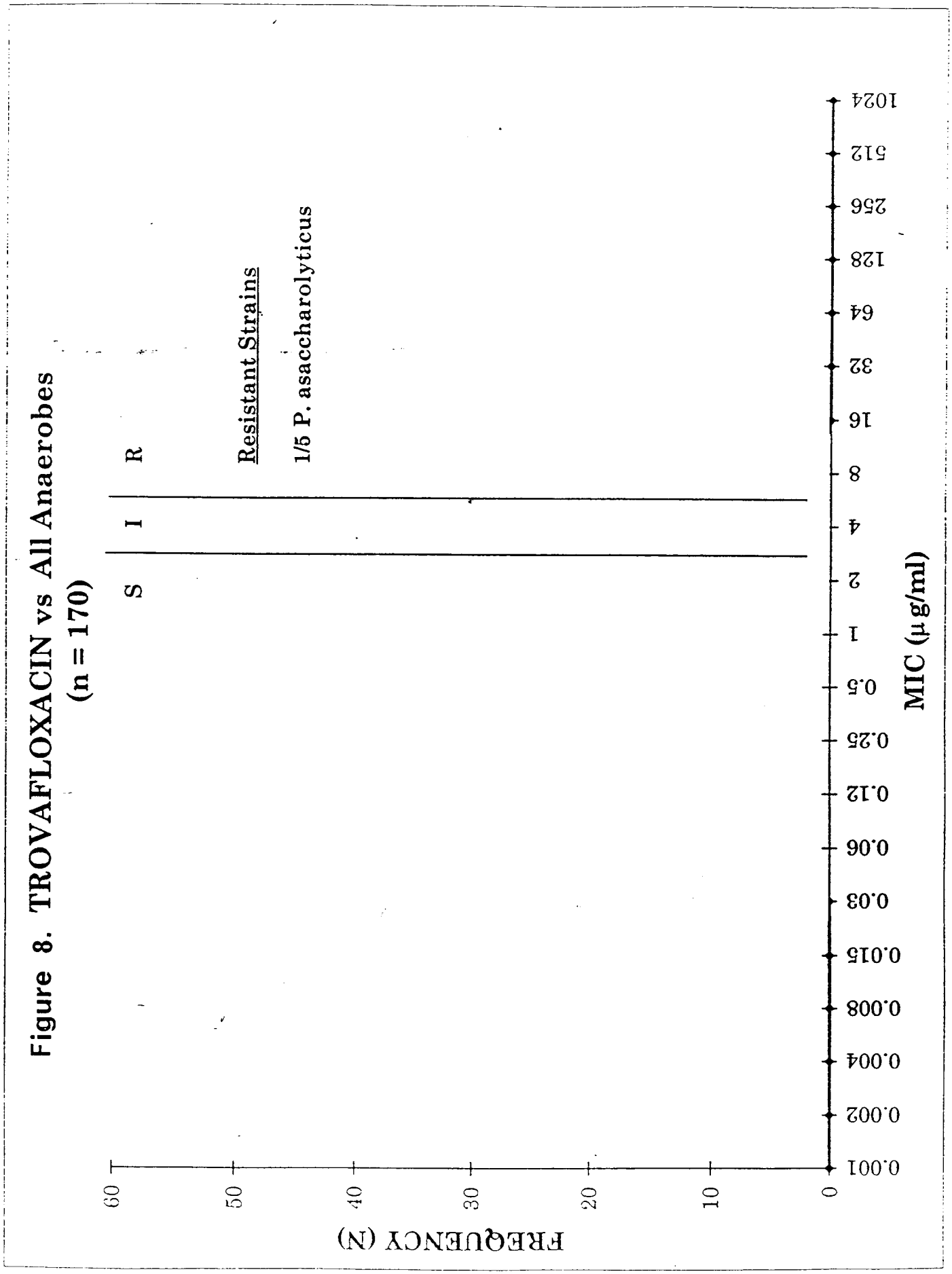


Figure 8. TROVAFLOXACIN vs All Anaerobes
(n = 170)
S
I
R
Resistant Strains
1/5 P. asaccharolyticus
FREQUENCY (N)
60
50
40
30
20
10
0
MIC (µg/ml)
0.001
0.002
0.004
0.008
0.015
0.03
0.06
0.12
0.25
0.5
1
2
4
8
16
32
64
128
256
512
1024