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In Vitro Activity of Ciprofloxacin (Bay o 9867)

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The in vitro activities of ciprofloxacin (Bay o 9867) and seven comparative antimicrobial agents against 664 aerobic and facultatively anaerobic bacterial isolates were studied. Minimal inhibitory concentrations (MICs) of ciprofloxacin were ≤2 μg/ml for *Enterobacteriaceae*, ≤8 μg/ml for nonfermentative gram-negative bacilli, ≤4 μg/ml for gram-positive cocci, ≤0.03 μg/ml for *Aeromonas hydrophila* and *Pasteurella multocida*, and ≤1 μg/ml for *Listeria monocytogenes*. MICs for multi-drug-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* were ≤4 μg/ml. Ciprofloxacin MICs were consistently 0 to 4 (usually 2 to 3) dilution steps lower than those of a related drug, norfloxacin ($P < 0.0001$). For most species, they were lower than MICs of cefotaxime, aztreonam, thienamycin, mezlocillin, trimethoprim-sulfamethoxazole, and amikacin. With all eight drugs, increasing the inoculum size by 100-fold had a variable effect on MICs which was species related. Ciprofloxacin is a potent broad-spectrum new antimicrobial agent.

Nalidixic acid and related quinoline derivatives have been available for years, primarily for the treatment of urinary tract infections caused by gram-negative enteric bacilli. Recently, structurally related derivatives with greater potencies and broader antibacterial spectrums have been developed; included are norfloxacin (3), ciprofloxacin (Bay o 9867) (4), and DL-8280 (1). In this study, the in vitro activities of ciprofloxacin and seven comparative antimicrobial agents against a variety of aerobic and facultatively anaerobic bacterial species were studied.

MATERIALS AND METHODS

Organisms. The organisms studied included 664 bacterial strains recently isolated by or referred to The Ohio State University Hospitals Microbiology Laboratories; included were 614 randomly selected isolates (Tables 1 to 3), of which 62% were isolated from blood cultures, and 50 were known to be multi-drug resistant. The multi-drug-resistant isolates included three to six strains each of *Escherichia coli, Klebsiella pneumoniae, Citrobacter diversus, Enterobacter cloacae, Enterobacter aerogenes, Serratia marcescens, Proteus vulgaris, Providencia stuartii, Morganella morganii*, and *Pseudomonas aeruginosa*. Minimal inhibitory concentrations (MICs) were known to be: ampicillin, >16 μg/ml; carbenicillin, >128 μg/ml; cephalothin, >8 μg/ml; gentamicin, >4 μg/ml.

Antimicrobial agents. Ciprofloxacin and mezlocillin were obtained from Miles Pharmaceuticals, West Haven, Conn. Norfloxacin and thienamycin formamidide (thienamycin) were obtained from Merck Sharp & Dohme Research Institute, West Point, Pa. Cefotaxime was obtained from Hoechst-Roussel Pharmaceuticals, Somerville, N.J. Aztreonam was obtained from the Squibb Institute, Princeton, N.J. Amikacin was obtained from Bristol Laboratories, Syracuse, N.Y. Trimethoprim (TMP)-sulfamethoxazole (SMZ) was obtained from Hoffmann-LaRoche Inc., Nutley, N.J. Laboratory standards were diluted according to the recommendations of the manufacturer and dispensed into microdilution plates by using a MIC-2000 Plus dispensing machine (Dynatech Laboratories, Alexandria, Va.) in $\log_2$ dilution steps within the range 0.004 to 128 μg/ml. TMP-SMZ was tested in a fixed ratio of 1:19. Plates were stored at −70°C until used.

Susceptibility tests. MICs were determined by a standardized microdilution method (2) in 0.1-ml volumes of cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.); 0.1 U of thymidine phosphorylase (Burroughs Wellcome Co., Research Triangle Park, N.C.) was added per ml for testing TMP-SMZ. Microdilution plates were inoculated with disposable inoculators (Dynatech) so that the final inoculum was approximately 5×10^5 CFU/ml. To determine the effects of various inoculum sizes, MICs of the eight antimicrobial agents were simultaneously determined with the standard inoculum and with inocula containing 100-fold higher and 100-fold lower bacterial concentrations.

RESULTS

The MICs of the eight study drugs for the randomly selected *Enterobacteriaceae* are shown in Table 1. All strains were inhibited by ≤2 μg of ciprofloxacin per ml; median MICs for

TABLE 1. Antibacterial activities of ciprofloxacin, norfloxacin, cefotaxime, aztreonam, thienamycin, mezlocillin, TMP-SMZ, and amikacin against *Enterobacteriaceae*

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Escherichia coli (25)	Ciprofloxacin	0.008–0.016	0.016	0.016
	Norfloxacin	0.03–0.13	0.06	0.06
	Cefotaxime	≤0.016–0.13	0.03	0.06
	Aztreonam	0.03–0.13	0.06	0.13
	Thienamycin	0.06–0.25	0.13	0.25
	Mezlocillin	1–>128	2	128
	TMP-SMZ	0.25–>128	1	8
	Amikacin	0.5–4	2	2
Klebsiella pneumoniae (25)	Ciprofloxacin	0.016–0.5	0.03	0.06
	Norfloxacin	0.06–2	0.13	0.25
	Cefotaxime	≤0.16–0.13	0.03	0.06
	Aztreonam	0.03–0.25	0.03	0.13
	Thienamycin	0.13–0.5	0.25	0.25
	Mezlocillin	4–>128	8	>128
	TMP-SMZ	0.25–>128	1	128
	Amikacin	1–4	1	2
Citrobacter diversus (25)	Ciprofloxacin	≤0.004–0.03	0.008	0.03
	Norfloxacin	0.03–0.13	0.03	0.13
	Cefotaxime	≤0.016–0.5	0.06	0.13
	Aztreonam	0.03–2	0.06	2
	Thienamycin	0.13–0.25	0.25	0.25
	Mezlocillin	2–32	8	8
	TMP-SMZ	0.25–2	1	2
	Amikacin	0.5–2	1	1
Citrobacter freundii (25)	Ciprofloxacin	≤0.004–0.13	0.016	0.06
	Norfloxacin	0.03–0.5	0.06	0.25
	Cefotaxime	0.03–>32	0.25	32
	Aztreonam	0.03–>32	0.25	32
	Thienamycin	0.13–0.5	0.25	0.5
	Mezlocillin	1–>128	4	128
	TMP-SMZ	0.5–>128	1	8
	Amikacin	0.5–4	1	4
Enterobacter cloacae (25)	Ciprofloxacin	≤0.004–0.13	0.016	0.03
	Norfloxacin	0.03–1	0.06	0.13
	Cefotaxime	0.06–>32	0.25	32
	Aztreonam	0.03–>32	0.06	32
	Thienamycin	0.13–1	0.25	0.5
	Mezlocillin	2–>128	4	>128
	TMP-SMZ	0.5–8	1	8
	Amikacin	0.5–4	1	2
Enterobacter aerogenes (25)	Ciprofloxacin	0.008–0.25	0.016	0.06
	Norfloxacin	0.03–2	0.13	0.25
	Cefotaxime	0.06–16	0.13	8
	Aztreonam	0.03–16	0.13	4
	Thienamycin	0.06–2	0.25	1
	Mezlocillin	2–>128	4	32
	TMP-SMZ	0.5–8	1	4
	Amikacin	0.5–8	1	4
Serratia marcescens (25)	Ciprofloxacin	0.016–1	0.06	0.13
	Norfloxacin	0.06–8	0.13	0.5
	Cefotaxime	0.13–16	0.5	2
	Aztreonam	0.06–4	0.13	1
	Thienamycin	0.25–2	0.5	1
	Mezlocillin	2–>128	4	128
	TMP-SMZ	2–>128	4	8
	Amikacin	1–8	2	4

TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Proteus mirabilis (25)	Ciprofloxacin	0.008–0.06	0.03	0.03
	Norfloxacin	0.03–0.13	0.06	0.13
	Cefotaxime	≤0.016–0.06	≤0.016	0.03
	Aztreonam	≤0.016	≤0.016	≤0.016
	Thienamycin	0.25–4	0.5	2
	Mezlocillin	0.13–2	0.5	1
	TMP-SMZ	0.5–8	1	2
	Amikacin	0.5–4	2	4
Proteus vulgaris (25)	Ciprofloxacin	0.016–0.13	0.03	0.06
	Norfloxacin	0.03–0.13	0.06	0.13
	Cefotaxime	≤0.016–1	0.03	0.25
	Aztreonam	≤0.016–0.06	≤0.016	0.03
	Thienamycin	0.25–4	1	2
	Mezlocillin	0.5–16	1	4
	TMP-SMZ	1–32	2	4
	Amikacin	0.5–4	1	2
Providencia stuartii (25)	Ciprofloxacin	0.008–2	0.06	1
	Norfloxacin	0.03–4	0.13	2
	Cefotaxime	≤0.016–4	0.06	1
	Aztreonam	≤0.016–0.5	≤0.016	0.25
	Thienamycin	0.25–2	1	2
	Mezlocillin	0.5–>128	4	>128
	TMP-SMZ	1–>128	4	128
	Amikacin	0.25–4	0.5	2
Morganella morganii (25)	Ciprofloxacin	0.008–0.016	0.016	0.016
	Norfloxacin	0.03–0.06	0.03	0.06
	Cefotaxime	≤0.016–8	0.06	2
	Aztreonam	≤0.016–1	≤0.016	0.5
	Thienamycin	0.5–2	2	2
	Mezlocillin	0.5–64	2	32
	TMP-SMZ	1–4	2	2
	Amikacin	0.5–4	2	2

[a] 50% and 90%, MIC required to inhibit 50 and 90% of the isolates, respectively.

all species were 0.008 to 0.06 μg/ml. All strains were also inhibited by ≤4 μg of thienamycin per ml and ≤8 μg of norfloxacin and amikacin per ml. Most strains were inhibited by ≤8 μg of cefotaxime and aztreonam per ml. The activities of mezlocillin and TMP-SMZ were more variable.

The MICs of the eight study drugs for other randomly selected gram-negative bacteria are shown in Table 2. All nonfermentative gram-negative bacilli (*Pseudomonas* sp. and *Acinetobacter calcoaceticus*) were inhibited by ≤8 μg of ciprofloxacin per ml; median MICs for all species were 0.13 to 2 μg/ml. MICs of the other drugs were usually higher and more variable. All *Aeromonas hydrophila* and *Pasteurella multocida* strains were inhibited by ≤0.03 μg of ciprofloxacin per ml. Most were also highly susceptible to the other drugs, but their MICs were higher than those of ciprofloxacin.

The MICs of the eight study drugs for the randomly selected gram-positive bacteria are shown in Table 3. All strains were inhibited by ≤4 μg of ciprofloxacin per ml; median MICs for all species were 0.25 to 2 μg/ml. Thienamycin was the most active drug tested; median MICs were ≤0.016 to 0.25 μg/ml. Norfloxacin, aztreonam, and amikacin MICs were consistently higher than those of ciprofloxacin. Aztreonam was inactive against all species and amikacin was inactive against streptococci. The activities of cefotaxime, mezlocillin, and TMP-SMZ varied with the species tested.

The MICs of the eight study drugs for the 50 multidrug-resistant gram-negative bacilli were higher than the respective MICs for randomly selected isolates of the same species. All were inhibited by ≤4 μg of ciprofloxacin per ml, ≤8 μg of norfloxacin per ml, ≤8 μg of thienamycin per ml, and ≤32 μg of amikacin per ml. Suscep-

TABLE 2. Antibacterial activities of ciprofloxacin, norfloxacin, cefotaxime, aztreonam, thienamycin, mezlocillin, TMP-SMZ, and amikacin against gram-negative bacteria

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Pseudomonas aeruginosa (25)	Ciprofloxacin	0.06–1	0.25	0.5
	Norfloxacin	0.25–2	0.5	2
	Cefotaxime	8–>32	16	>32
	Aztreonam	2–32	4	16
	Thienamycin	0.25–2	1	2
	Mezlocillin	16–>128	32	128
	TMP-SMZ	64–>128	128	>128
	Amikacin	2–32	8	32
Pseudomonas maltophilia (25)	Ciprofloxacin	0.25–8	2	4
	Norfloxacin	2–>32	16	32
	Cefotaxime	8–>32	>32	>32
	Aztreonam	8–>32	>32	>32
	Thienamycin	4–>32	>32	>32
	Mezlocillin	8–>128	32	>128
	TMP-SMZ	1–>128	2	8
	Amikacin	8–>32	>32	>32
Pseudomonas cepacia (19)	Ciprofloxacin	0.25–8	2	8
	Norfloxacin	4–32	8	32
	Cefotaxime	4–>32	16	32
	Aztreonam	2–>32	16	>32
	Thienamycin	0.25–>32	16	32
	Mezlocillin	4–64	8	16
	TMP-SMZ	2–>128	16	128
	Amikacin	16–>32	>32	>32
Acinetobacter calcoaceticus subsp. *anitratum* (25)	Ciprofloxacin	0.13–4	0.25	1
	Norfloxacin	1–32	4	16
	Cefotaxime	4–32	16	32
	Aztreonam	4–>32	16	>32
	Thienamycin	0.06–1	0.25	0.5
	Mezlocillin	16–64	32	64
	TMP-SMZ	1–>128	4	8
	Amikacin	1–32	2	4
Acinetobacter calcoaceticus subsp. *lwoffii* (25)	Ciprofloxacin	0.03–1	0.13	0.25
	Norfloxacin	0.25–8	1	4
	Cefotaxime	1–32	2	16
	Aztreonam	2–>32	8	32
	Thienamycin	0.03–1	0.13	0.25
	Mezlocillin	4–64	16	64
	TMP-SMZ	0.5–128	2	16
	Amikacin	≤0.13–8	0.5	2
Aeromonas hydrophila (25)	Ciprofloxacin	≤0.004–0.016	≤0.004	0.008
	Norfloxacin	≤0.016–0.13	≤0.016	0.03
	Cefotaxime	≤0.016–4	0.06	0.5
	Aztreonam	≤0.016–0.13	≤0.016	0.03
	Thienamycin	0.06–1	0.5	0.5
	Mezlocillin	1–>128	4	8
	TMP-SMZ	1–4	2	2
	Amikacin	0.5–2	1	2
Pasteurella multocida (16)	Ciprofloxacin	≤0.004–0.03	0.008	0.016
	Norfloxacin	0.03–0.13	0.06	0.13
	Cefotaxime	≤0.016	≤0.016	≤0.016
	Aztreonam	≤0.016	≤0.016	≤0.016
	Thienamycin	0.06–0.5	0.13	0.25
	Mezlocillin	≤0.06	≤0.06	≤0.06
	TMP-SMZ	0.13–2	0.5	1
	Amikacin	4–16	8	16

[a] 50% and 90%, MIC required to inhibit 50 and 90% of the isolates, respectively.

TABLE 3. Antibacterial activities of ciprofloxacin, norfloxacin, cefotaxime, aztreonam, thienamycin, mezlocillin, TMP-SMZ, and amikacin against gram-positive bacteria

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus (25)	Ciprofloxacin	0.13–1	0.25	0.5
	Norfloxacin	0.25–2	1	1
	Cefotaxime	2–4	2	4
	Aztreonam	>32	>32	>32
	Thienamycin	0.03–0.06	0.03	0.03
	Mezlocillin	NA[b]	NA	NA
	TMP-SMZ	0.5–2	1	1
	Amikacin	1–8	2	4
Staphylococcus epidermidis (25)	Ciprofloxacin	0.25–0.5	0.25	0.5
	Norfloxacin	0.5–2	1	1
	Cefotaxime	0.5–16	4	16
	Aztreonam	>32	>32	>32
	Thienamycin	≤0.016–8	0.25	2
	Mezlocillin	0.5–64	4	32
	TMP-SMZ	0.25–>128	2	>128
	Amikacin	≤0.13–8	4	8
Streptococcus pneumoniae (25)	Ciprofloxacin	0.5–4	1	2
	Norfloxacin	4–32	8	16
	Cefotaxime	≤0.016–0.03	≤0.016	≤0.016
	Aztreonam	4–>32	>32	>32
	Thienamycin	≤0.016	≤0.016	≤0.016
	Mezlocillin	≤0.06	≤0.06	≤0.06
	TMP-SMZ	1–8	2	4
	Amikacin	4–>32	>32	>32
Streptococcus pyogenes (25)	Ciprofloxacin	0.25–4	0.5	2
	Norfloxacin	1–32	2	16
	Cefotaxime	≤0.016	≤0.016	≤0.016
	Aztreonam	4–16	16	16
	Thienamycin	≤0.016	≤0.016	≤0.016
	Mezlocillin	≤0.06	≤0.06	≤0.06
	TMP-SMZ	1–128	8	32
	Amikacin	≥32	>32	>32
Streptococcus agalactiae (25)	Ciprofloxacin	0.5–2	1	1
	Norfloxacin	2–8	4	8
	Cefotaxime	≤0.016–0.06	0.03	0.06
	Aztreonam	≥32	>32	>32
	Thienamycin	≤0.016	≤0.016	≤0.016
	Mezlocillin	≤0.06–0.13	≤0.06	0.13
	TMP-SMZ	0.5–8	2	4
	Amikacin	≥32	>32	>32
Viridans streptococci (25)	Ciprofloxacin	0.5–4	1	4
	Norfloxacin	2–>32	8	32
	Cefotaxime	≤0.016–8	0.13	2
	Aztreonam	16–>32	32	>32
	Thienamycin	≤0.016–4	0.03	0.13
	Mezlocillin	≤0.06–8	0.13	2
	TMP-SMZ	0.25–64	4	32
	Amikacin	4–>32	32	>32
Streptococcus faecalis (25)	Ciprofloxacin	0.5–2	2	2
	Norfloxacin	2–8	4	8
	Cefotaxime	>32	>32	>32
	Aztreonam	>32	>32	>32
	Thienamycin	0.5–2	1	2
	Mezlocillin	1–2	2	2
	TMP-SMZ	0.13–1	0.5	1
	Amikacin	>32	>32	>32

TABLE 3—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)[a]		
		Range	50%	90%
Listeria monocytogenes (4)	Ciprofloxacin	0.5–1	1	1
	Norfloxacin	2–4	4	4
	Cefotaxime	≥32	>32	>32
	Aztreonam	>32	>32	>32
	Thienamycin	0.06–0.13	0.13	0.13
	Mezlocillin	1–4	2	4
	TMP-SMZ	0.25	0.25	0.25
	Amikacin	1–2	2	2

[a] 50% and 90%, MIC required to inhibit 50 and 90% of isolates, respectively.
[b] NA, Not applicable; 92% produced penicillinase and were considered resistant.

tibilities to cefotaxime, aztreonam, mezlocillin, and TMP-SMZ were more variable.

The only pair of study drugs that had parallel MICs was ciprofloxacin and norfloxacin (Fig. 1). By linear regression analysis (where $y = Ax + B$; $x = \log_2$ MIC norfloxacin, $y = \log_2$ MIC ciprofloxacin, A = slope, B = y intercept), the line of best fit for all $\log_2$ MIC pairs was highly significant ($P < 0.0001$); the slope was close to unity (0.91), and the correlation coefficient was 0.96. For *Enterobacteriaceae* and gram-positive cocci, the mean ciprofloxacin MIC was approximately 2 (range 0 to 4) dilution steps lower than the mean norfloxacin MIC. For nonfermenters, *Aeromonas hydrophila* and *Pasteurella multocida*, the difference in mean MICs was approximately 3 (range, 1 to 4) dilution steps.

In determining MICs, a reduction in inoculum size by 100-fold rarely affected results by more than 1 dilution step except with mezlocillin against penicillinase-producing strains of *Staphylococcus aureus*. The effects of increasing the inoculum size by 100-fold with 40 bacterial strains are shown in Table 4. With *Enterobacteriaceae*, the greatest inoculum effect (increase in MICs with an increase in inoculum size) was observed with aztreonam and mezlocillin, and the least was observed with amikacin; the inoculum effect was intermediate or variable with the other drugs. With *Pseudomonas aeruginosa*, the greatest inoculum effect was with aztreonam, mezlocillin, and cefotaxime; there was little or none with ciprofloxacin, norfloxacin, thienamycin, and amikacin. With *S. aureus*, the greatest inoculum effect was with ciprofloxacin, norfloxacin, and amikacin; there was little or none with

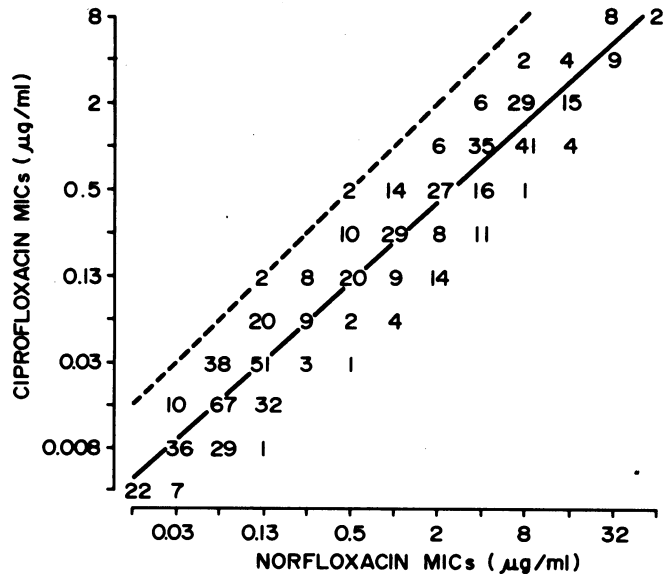

FIG. 1. Scatter diagram showing the relationship of ciprofloxacin MICs and norfloxacin MICs. Each number on the diagram indicates the number of strains which had the MIC shown. Solid line is the line of best fit. Dashed line is the line of identity.

TABLE 4. Effects of increasing the inoculum size by 100-fold on the MICs of study drugs

Organism[a]	Increase in MIC (no. of dilution steps) of:							
	Ciprofloxacin	Norfloxacin	Cefotaxime	Aztreonam	Thienamycin	Mezlocillin	TMP-SMZ	Amikacin
E. coli	2–7	1–5	4–8[b]	8	3–5	8	8	0–2
K. pneumoniae	1–5	0–5	0–6	2–8	2–8	3–8	1–8	0–2
E. cloacae	0–4	0–4	5–8	8	2–4	8	1–8	1–2
E. aerogenes	2–8	0–6	1–8	1–8	2–4	3–8	1–8	0–3
S. marcescens	2–6	1–3	8	8	6–8	8	2–4	1–2
P. aeruginosa	0–1	0–1	8	8	0–1	8	R[c]	0–1
S. aureus	2–8	2–8	0–2	R	0	R	0–8	8
S. faecalis	4–8	3–8	R	R	0	0–1	1–8	R

[a] Five strains of each species.
[b] 8, Increase by ≥8 dilution steps or to greater than the highest concentration tested.
[c] R, Resistant; differences could not be determined.

thienamycin and cefotaxime. With *Streptococcus faecalis*, the greatest inoculum effect was with ciprofloxacin, norfloxacin, and TMP-SMZ; there was little or none with thienamycin and mezlocillin.

DISCUSSION

In this study, ciprofloxacin had a broad spectrum of activity against a variety of aerobic and facultatively anaerobic bacteria. All 664 isolates studied, including 50 multi-drug-resistant strains, were inhibited by ≤8 μg/ml, 96% were inhibited by ≤2 μg/ml. *Enterobacteriaceae*, *A. hydrophila*, and *P. multocida* were more susceptible than nonfermenters, gram-positive cocci, and *Listeria monocytogenes*. MICs of ciprofloxacin paralleled those of the related drug, norfloxacin, but were always 0 to 4 dilution steps lower. Other antimocrobial agents, including a variety of β-lactams, amikacin, and TMP-SMZ, were usually less active than ciprofloxacin. Because the MIC breakpoint for defining susceptibility of ciprofloxacin has not yet been determined, a comparison of percentages of organisms susceptible to the various drugs tested was not possible.

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