IN VITRO STUDIES OF SEVEN QUINOLINECARBOXYLIC ACID COMPOUNDS AGAINST CAUSATIVE ORGANISMS OF URINARY TRACT INFECTIONS

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The *in vitro* activities of newly developed quinolinecarboxylic acid compounds, norfloxacin, ofloxacin and enoxacin against urinary tract pathogens were compared with those of nalidixic acid, piromidic acid, cinoxacin and pipemidic acid. These new compounds inhibited the growth of 90% of strains of *Escherichia coli* (MIC_{90s}) at the concentration of 0.20 to 0.39 μg/ml, *Citrobacter* spp. at 0.78 to 1.56 μg/ml, *Klebsiella pneumoniae* at 0.39 to 1.56 μg/ml, *Enterobacter* spp. at 0.78 to 3.13 μg/ml, *Serratia marcescens* at 3.13 to 6.25 μg/ml, *Proteus mirabilis* at 0.10 to 0.78 μg/ml, indole-positive *Proteus* spp. at 0.39 to 0.78 μg/ml and *Pseudomonas aeruginosa* at 6.25 to 25 μg/ml. The MIC_{90s} of norfloxacin, ofloxacin and enoxacin against *Staphylococcus epidermidis* were 3.13, 1.56 and 3.13 μg/ml, respectively, and 3.13, 3.13 and 12.5 μg/ml, respectively against *Streptococcus faecalis*. Furthermore, the new drugs exhibited strong activities against nalidixic acid-resistant bacteria. No noticeable difference in antibacterial activity was seen among the new compounds. From these results, all these new compounds were suggested to be more useful in urinary tract infections than nalidixic acid, piromidic acid, cinoxacin and pipemidic acid.

INTRODUCTION

It can be said that recent research in the development of the quinolinecarboxylic acid compounds has produced a new generation of drug such as norfloxacin (NFLX), ofloxacin (OFLX) and enoxacin (ENX). These new compounds, unlike nalidixic acid (NA), were reported to exhibit a wide variety of gram-positive and gram-negative bacteria, including those of *P. aeruginosa* and *S. marcescens*[1~8]. In the present study, we examined the usefulness of the new compounds, comparing the *in vitro* antibacterial activities against bacteria commonly isolated from urinary tract infections with those of their predecessors NA, piromidic acid (PA), cinoxacin (CINX) and pipemidic acid (PPA).

MATERIALS AND METHODS

Organisms : All organisms tested were isolated from urine specimens obtained from patients at the Department of Urology, Gifu University School of Medicine, Gifu, Japan. All patients had underlying urinary tract disease. The urine of these patients were confirmed to contain both white blood cells more than five cells in a high power field microscopically and viable bacteria more than 10^4 bacteria per milliliter. The number of bacterial strains tested were as follows ; 80 strains of *E. coli*, 12 of *Citrobacter* spp., 65 of *K. pneumoniae*, 8 of *Enterobacter* spp., 29 of *S. marcescens*, 46 of *P. mirabilis*, 34 of indole-positive *Proteus* spp. (6 of *P. vulgaris*, 23 of *M. morganii*, 4 of *P. rettgeri*, 1 of *P. inconstans*), 51 of *P. aeruginosa*, 8 of *S. epidermidis*, 86 of *S. faecalis*.

Table 1-1 Comparative MICs of 7 antimicrobial agents against urinary isolates

Organism (No. of isolates)	Antimicrobial agents[a]	MIC[b] (μg/ml)		
		Range	50%	90%
E. coli (80)	NA	0.39 - >100	3.13	12.5
	PA	0.39 - >100	25	50
	CINX	1.56 - 50	3.13	12.5
	PPA	0.78 - 50	1.56	6.25
	NFLX	≦0.10 - 0.78	≦0.10	0.20
	OFLX	≦0.10 - 1.56	≦0.10	0.39
	ENX	≦0.10 - 3.13	0.20	0.39
Citrobacter spp. (12)	NA	3.13 - >100	6.25	>100
	PA	25 - >100	50	>100
	CINX	6.25 - 100	6.25	100
	PPA	1.56 - 12.5	3.13	12.5
	NFLX	≦0.10 - 6.25	0.20	0.78
	OFLX	≦0.10 - 1.56	0.20	1.56
	ENX	≦0.10 - 1.56	0.39	1.56
K. pneumoniae (65)	NA	1.56 - >100	3.13	12.5
	PA	6.25 - >100	25	100
	CINX	1.56 - >100	6.25	50
	PPA	0.78 - 50	3.13	12.5
	NFLX	≦0.10 - 3.13	0.20	0.39
	OFLX	≦0.10 - 1.56	0.20	0.78
	ENX	≦0.10 - 3.13	0.39	1.56
Enterobacter spp. (8)	NA	3.13 - 25	12.5	25
	PA	6.25 - 100	50	100
	CINX	3.13 - >100	25	>100
	PPA	1.56 - 50	3.13	50
	NFLX	0.20 - 1.56	0.20	1.56
	OFLX	≦0.10 - 0.78	0.20	0.78
	ENX	0.20 - 3.13	0.39	3.13
S. marcescens (29)	NA	0.39 - >100	6.25	>100
	PA	6.25 - >100	100	>100
	CINX	6.25 - >100	50	>100
	PPA	0.78 - >100	3.13	50
	NFLX	≦0.10 - 25	0.39	3.13
	OFLX	≦0.10 - 100	1.56	6.25
	ENX	≦0.10 - 12.5	0.39	6.25
P. mirabilis (46)	NA	1.56 - 50	6.25	6.25
	PA	12.5 - >100	25	50
	CINX	0.20 - 12.5	6.25	6.25
	PPA	1.56 - 12.5	3.13	3.13
	NFLX	≦0.10 - 0.39	≦0.10	≦0.10
	OFLX	≦0.10 - 1.56	0.20	0.39
	ENX	0.20 - 3.13	0.39	0.78
Indole-positive *Proteus* spp. (34)	NA	1.56 - 100	3.13	12.5
	PA	6.25 - >100	25	50
	CINX	0.39 - 50	3.13	12.5
	PPA	0.78 - 25	1.56	3.13
	NFLX	≦0.10 - 6.25	≦0.10	0.39
	OFLX	≦0.10 - 1.56	0.20	0.78
	ENX	≦0.10 - 0.78	0.20	0.78

[a] NA : nalidixic acid, PA : piromidic acid, CINX : cinoxacin, PPA : pipemidic acid, NFLX : norfloxacin, OFLX : ofloxacin, ENX : enoxacin.

[b] 100-dilutions of overnight cultures were inoculated.

Table 1-2 Comparative MICs of 7 antimicrobial agents against urinary isolates

Organism (No. of isolates)	Antimicrobial agents[a]	MIC[b] (μg/ml)		
		Range	50%	90%
P. aeruginosa (51)	NA	3.13->100	>100	>100
	PA	0.78 >100	>100	>100
	CINX	6.25->100	>100	>100
	PPA	1.56->100	25	100
	NFLX	≦0.10- 12.5	1.56	6.25
	OFLX	0.20->100	3.13	25
	ENX	0.20- 50	1.56	6.25
S. epidermidis (8)	NA	50 ->100	100	>100
	PA	6.25- 100	12.5	100
	CINX	100 ->100	>100	>100
	PPA	25 ->100	50	>100
	NFLX	0.39- 3.13	1.56	3.13
	OFLX	0.39- 1.56	0.39	1.56
	ENX	0.78- 3.13	1.56	3.13
S. faecalis (86)	NA	>100	>100	>100
	PA	100 ->100	>100	>100
	CINX	50 ->100	>100	>100
	PPA	100 ->100	>100	>100
	NFLX	1.56- 6.25	3.13	3.13
	OFLX	1.56- 6.25	3.13	3.13
	ENX	3.13- 12.5	6.25	12.5

[a] NA : nalidixic acid, PA : piromidic acid, CINX : cinoxacin, PPA : pipemidic acid, NFLX : norfloxacin, OFLX : ofloxacin, ENX : enoxacin.

[b] 100-dilutions of overnight cultures were inoculated.

Minimum inhibitory concentrations (MICs) : The MICs were determined by the twofold agar dilution method[9]. 100-fold dilutions of overnight culture in Mueller-Hinton agar (Difco) were inoculated onto the Mueller-Hinton agar (Difco) containing twofold dilutions of the drugs. The final concentrations of the drug ranged from 0.10 to 100 μg/ml. After incubation for 18 to 20 hr at 37°C, the minimum inhibitory concentration (MIC) was determined.

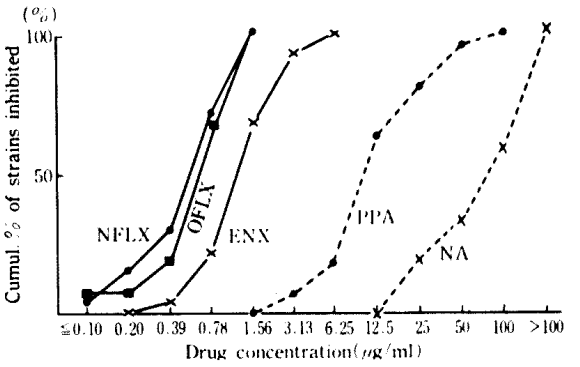
RESULTS

Susceptibility of gram-negative bacteria : Table 1 shows the results obtained from 419 isolates tested at an inoculum equivalent to a 100-fold dilution of overnight culture. The lowest concentration of the drug which inhibited the growth of fifty and ninety percent of strains in each species was expressed as MIC_{50} and MIC_{90}, respectively. The MIC_{90s} of the new drugs, NFLX, OFLX and ENX, against *E. coli*, *P. mirabilis*, and indole-positive *Proteus* spp. were all found to be 0.78 μg/ml. The MIC_{90s} of the new drugs against *Citrobacter* spp., *K. pneumoniae*, *Enterobacter* spp., and *S. marcescens* were 1.56, 1.56, 3.13, and 6.25 μg/ml, respectively. Against *P. aeruginosa*, the MIC_{90s} of NFLX, OFLX and ENX were revealed to be 6.25, 25, and 6.25 μg/ml, respectively, whereas those of NA, PA, CINX and PPA against *P. aeruginosa* were higher than 100 μg/ml.

Susceptibility of gram-positive cocci (*S. epidermidis* and *S. faecalis*) : The MIC_{90s} of NFLX, OFLX and ENX against urinary *S. epidermidis* and *S. faecalis* were as follows (Table 1). The MIC_{90s} of NFLX against *S. epidermidis* and *S. faecalis* : 3.13 and 3.13 μg/ml, OFLX : 1.56 and 3.13 μg/ml, ENX : 3.13 and 12.5 μg/ml, respectively. Contrarily, the MIC_{90s} of NA, PA, CINX and PPA against the same gram-positive cocci were higher than 100 μg/ml.

Susceptibility of NA-resistant gram-negative bacteria : The susceptibility of twenty-seven isolates resistant to NA (MIC≧25 μg/ml) was tested (Fig. 1). The growth of all strains was inhibited by NFLX, OFLX and ENX at the concentrations of 0.78, 0.78 and 6.25 μg/ml or lower, respectively, whereas those strains were moderately resistant

Fig. 1 Antibacterial activity against nalidixic acid-resistant strains(27 strains). Resistant MIC breakpoint≧25 μg/ml. Nalidixic acid-resistant strains include 13 ampicillin-resistant *E. coli*, 2 *Citrobacter* spp., 3 *Enterobacter* spp., 6 *K. pneumoniae*, 1 *P. mirabilis*, and 2 *Morganella morganii*.



NFLX : norfloxacin(●——●), OFLX : ofloxacin(■——■), ENX : enoxacin(×——×), PPA : pipemidic acid(●·····●), NA : nalidixic acid(×·····×)
100 dilutions of overnight cultures were inoculated.

to PPA. Only about twenty percent of the strains were inhibited by PPA at the concentration of 6.25 μg/ml.

DISCUSSION

Resistant strains to NA remained rare because of difficulty of mutation or in occurrence of plasmid-mediated resistance. However, recent isolation frequency of NA-resistant bacteria has been increasing in clinical field[10]. On the other hand, the incidence of infections due to gram-positive bacteria, especially due to *S. faecalis* has been increasing in proportion to recent increase in use of so-called the third generation cephems[11]. Against these gram-positive bacteria, the chemotherapy with preexisting quinolinecarboxylic acid compounds such as NA, PA, CINX and PPA is known to be ineffective. New quinolinecarboxylic acid compounds such as NFLX, OFLX and ENX, which appeared in recent years, have been reported to have strong activity not only against gram-negative bacteria but also against gram-positive bacteria.

In the present study, we evaluated the new drugs fundamentally aiming the clinical usefulness of them for urinary tract infections, and identified the remarkably strong activities against both gram-negative and gram-positive bacteria which were isolated from urinary tract infections and were resistant to the four predecessors (NA, PA, CINX and PPA). These new drugs also exhibited strong activities against NA-resistant strains. No marked difference in antibacterial activity is seen among the new drugs.

Conclusively, the new quinolinecarboxylic acid compounds, NFLX, OFLX, and ENX are considered to be the potentially useful therapeutic agents for treatment of complicated urinary tract infections.

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尿路から分離された細菌に対するキノリンカルボン酸系抗菌剤7薬剤の抗菌力

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尿路感染症由来株に対する新キノリンカルボン酸系抗菌剤 NFLX, OFLX, ENX および NA, PA, CINX, PPA の抗菌力を測定し，これらを比較検討した。新合成抗菌剤の *E. coli*, *Citrobacter* spp., *K. pneumoniae*, *Enterobacter* spp., *P. mirabilis*, indole-positive *Proteus* spp. の MIC_{90} は 0.20-3.13 μg/ml の間にあり，優れたものであったが，さらに *S. marcescens* は 3.13-6.25 μg/ml, *P. aeruginosa* は 6.25-25 μg/ml, *S. epidermidis* は 1.56-3.13 μg/ml, *S. faecalis* は 3.13-12.5 μg/ml であった。また，NA 耐性 (MIC≧25 μg/ml) グラム陰性桿菌に対しても極めて強い抗菌力を示した。以上の結果から，新合成抗菌剤 NFLX, OFLX, ENX は複雑性尿路感染症に対して極めて有効な薬剤であると思われた。