Original article

In vitro activity of some fluoroquinolones and other unrelated antimicrobial agents against bacterial enteric pathogens

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Abstract: The *in-vitro* activity of ofloxacin, lomefloxacin, ciprifloxacin, fleroxacin, DR-335, and PD-127391 which are all members of the fluoroquinolones and other unrelated (classical) antimicrobials that include, amoxycillin, sulphamethoxazole, trimethoprim, tetracycline and chloramphenicol have been compared against *Salmonella typhi*, *campylobacter jejuni*, *Yersinia enterocolitica*, *Vibrio cholerae*, *Aeromonas hydrophila*, and *Vibrio parahaemolyticus*. Minimum inhibitory concentrations (MICs) of the antimicrobial agents examined were determined. All members of the fluoroquinolones were highly active against all insolates examined and inhibited them at a concentration of <1mg/L. By contrast, many of the isolates examined were resistant to one or more of the classical antimicrobial agents. These results showed a potential clinical role for all the fluoroquinolone compounds tested in the treatment or prophylaxis of bacterial enteric infections where antimicrobial intervention is indicated.[*Ethiop. J. Health Dev.* 1998;12(2)111:116]

Introduction

On a global scale, diarrhoeal diseases contribute to the greatest single cause of morbidity and mortality, far exceeding that from heart disease, cancer, or strokes in many parts of the world (1). Severe diarrhoeal diseases are among the most prevalent cause of infant and child-hood mortality in developing countries (2). In Ethiopia diarrhoeal diseases are also the leading cause of death particularly among children under five years of age (3). The bacterial species most frequently isolated from patients suffering from enteric diseases are *Shigella spp*., *Salmonella spp*., Entro-pathogenic and Enterotoxigenic strains of *Escherichia coli*, *Campylobacter jejuni*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Yersinia entrocolitica*, and *Aeromonas hydophila* (4).

In the case of secretory diarrhoea, the oral rehydration solution (ORS) plays an important role and in life-severe diarrhoea cases these sugar electrolyte solutions alone will not be adequate and should be given together with a drug that reduces intestinal hyper-secretion (5). Although their use remains controversial, antimicrobial agents have been used therapeutically and prophylactically for the treatment and prevention of enteric diseases for many years. But, in many countries of the world, especially in the developing ones, the uncontrolled availability and use of orally active compounds such as ampicillin, chloramphenicol and tetracycline have resulted in widespread resistance amongst bacterial enteric pathogens (6) leaving clinicians of the developing countries no choice but to depend on more expensive antibiotics. These prevailing conditions have urged researchers to search for new antimicrobial agents which could be safe and easy to administer, cost effective to use, and, most importantly, carry minimal risk of developing resistant strains. Fluorinated 4-quinolones are the new synthetic, orally administered antimicrobial agents. They

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have a high antimicrobial activity against a wide range of pathogenic organisms and resistance to this group of compounds occurs at a very low frequency (7). Recent studies have indicated the emergence of resistant Gram

Table 1*: **Comparative MICs in mg/L of selected antimicrobial agents against *Salmonella typhi* (n=24)**

Antimicrobials	MIC_{50}	MIC_{90}	Range	
Amoxycillin	0.5	1	0.5	- >64
Sulphamethoxazole	32	64	16	- >256
Trimethoprim	0.12	0.25	0.6	- >64
Tetracycline	1	4	1	- >64
Chloramphenicol	4	8	4	- 32
Ofloxacin	0.3	0.06	0.03	- 0.12
Ciprofloxacin	≤0.008	0.015	≤0.008	- 0.015
Lomifloxacin	0.12	0.25	0.06	- 0.25
Fleroxacin	0.03	0.03	0.008	- 0.25
DR-3355	0.015	0.015	0.008	- 0.12
Temafloxacin	0.03	0.03	0.015	- 0.25
PD-127391	0.008	0.008	0.004	- 0.015

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations of the drug required to inhibit 50% and 90% of the bacterial isolates, respectively

positive and Gram-negative strains of pathogenic bacteria against the fluoroquinolones (8,9). However the emergence of resistance among enteric pathogens is low and mostly occurs in *E. coli* (10,11). In this study an attempt is made to compare the *in-vitro* antimicrobial activity of these fluorinated 4-quinolones with some classical antimicrobial agents against the selected enteric pathogens.

Methods

Organisms:- The bacterial isolates were obtained from clinical materials examined in the Department of Clinical Microbiology at the University College Hospital, London, supplemented with isolates from Southern Europe, Africa, the Middle East, South and South-East Asia and South America. The number of isolates delt with were *Salmonella typhi* (24), *Yersinia enterocolitica* (9), Campylobacter jejuni (30), Vibrio cholerae (24), *Vibrio parahaemolyticus* (25) and *Aeromonas hydrophia* (24). The control strains used were Staphylococcus aureus (NCTC 5671) and Escherichia coli (NCTC 10418).

Antimicrobial compounds:- Antibiotic reference powders obtained from various suppliers were tested over 512-0.002 mg/L range of concentration as indicated by the

manufacturers for each compound. The compounds were: amoxycillin, sulpha-methoxazole and tetracycline (Sigma Chemical Ltd.), trimethoprim (Welcome Foundation Ltd.), chloramphenicol (BDH Chemical Ltd.), ofloxacin, floxacin, CGD Searles Ltd.), Ciprofloxacin, (Bayer UK Ltd.), temafloxacin, (Abbott Laboratories), fleroxacin (Roche), DR-3355 (Daiichi Seiyaku) PD-127391 (Warner-Lambert).

Determination of antimicrobial susceptibility:- Minimum inhibitory concentrations (MICs) of the antimicrobial agents for the bacterial isolates examined were determined using an agar incorporation technique (12) in Muller-Hinton medium (Oxoid Ltd., England) supplemented with saponin-lysed horse blood to a final concentration of 5% v/v, where necessary. The inoculum was approximately 10^4-10^5 colony forming units (cfu) of each isolate, contained in 1µl Muller-Hinton broth, which was delivered to the surface of the agar plates using a multipoint inoculator (Denley-Tech Ltd.). Following inoculation, plates were incubated in air at 37^0c for 24 h, except for *C. jejuni* spp. which required microaerophilic condition with the addition of about 10% CO_2. MICs were determined as

the lowest concentration of the antimicrobials tested which completely inhibited surface visible growth of the inoculum.

Table 2*: **Comparative MICs in mg/L of selected antimicrobial agents against *Vibrio cholerae* (n=24)**

Antimicrobials	MIC_{50}	MIC_{90}	Range	
Amoxycillin	8	>64	4	- >64
Sulphamethoxazole	256	>256	2	- >256
Trimethoprim	0.5	>64	0.03	- >64
Tetracycline	0.5	64	0.5	- 64
Chloramphenicol	1	1	0.5	- 1
Ofloxacin	0.015	0.015	≤0.008	- 0.015
Ciprofloxacin	≤0.008	≤0.008	all at ≤0.008	
Lomifloxacin	0.015	0.015	≤0.008	- 0.015
Fleroxacin	0.015	0.015	0.008	- 0.015
DR-3355	0.008	0.008	0.004	- 0.03
Temafloxacin	0.015	0.015	0.004	- 0.015
PD-127391	0.004	0.008	≤0.002	- 0.008

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations of the drug required to inhibit 50% and 90% of the bacterial isolates, respectively

Result

The concentration of five classical antibiotics and seven 4-quinolones compounds required to inhibit 50% (MIC_{50}) as well as 90% (MIC_{90}) for all strains of *salmonella typhi*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *campylobacter jejuni*, *Aeromonas hydrophila* and the range of inhibition concentration, including *Yersinia enterocolitica*, were calculated and these are shown in Tables 16. All isolates tested were sensitive to the 4-quinolone compounds while mostly remaining resistant to the classical antibiotics.

Discussion

The two major limitations that exist for available antibacterial agents in the treatment of bacterial diarrhoea are the development of antimicrobial resistance among enteric pathogens and failure to obtain a satisfactory *in-vivo* clinical response to therapy in certain enteric infections despite the *invitro* susceptibility of the pathogens to available drugs (13).

As many studies indicated most of the enteric pathogens especially in developing countries, are increasingly becoming resistant

Antimicrobials	MIC_{50}	MIC_{90}	Range	
Amoxycillin	64	>64	16	- >64
Sulphamethoxazole	128	>256	8	- >256
Trimethoprim	2	4	0.25	- 4
Tetracycline	1	1	0.5	- 2
Chloramphenicol	1	1	0.5	- 2
Ofloxacin	0.25	0.25	≤0.008	- 0.25
Ciprofloxacin	0.06	0.12	≤0.008	- 0.12
Lomifloxacin	0.25	0.25	0.015	- 0.5
Fleroxacin	0.12	0.25	0.008	- 0.25
DR-3355	0.12	0.12	0.004	- 0.12

Temafloxacin	0.25	0.25	0.03	- 0.5
PD-127391	0.06	0.06	0.004	- 0.06

Table 3*: **Comparative MICs in mg/L of selected antimicrobial agents against *Vibrio parahaemolyticus* (n =25)**

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations of the drug required to inhibit 50% and 90% of the bacterial isolates, respectively

Table 4*: **Comparative MICs in mg/L of selected antimicrobial agents against *Campylobacter jejuni* (n=30)**

Antimicrobials	MIC_{50}	MIC_{90}	Range	
Amoxycillin	8	32	2	- >64
Sulphamethoxazole	128	256	16	- 256
Trimethoprim	>64	>64	all at	- >64
Tetracycline	0.5	>64	0.25	- >64
Chloramphenicol	4	4	2	- >64
Ofloxacin	0.25	0.5	0.12	- 1
Ciprofloxacin	0.12	0.25	0.06	- 0.5
Lomifloxacin	0.5	0.5	0.25	-1
Fleroxacin	0.25	0.5	0.25	-1
DR-3355	0.12	0.12	0.06	- 0.5
Temafloxacin	0.06	0.06	0.03	- 0.12
PD-127391	0.03	0.03	0.015	-0.12

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations of the drug required to inhibit 50% and 90% of the bacterial isolates, respectively

to the commonly available antimicrobial chemotherapy (14,15). Similarly, in the present study isolates obtained from different parts of the world were resistant to one or more of the other unrelated (classical) antimicrobial agents. Most of the isolates show MIC_{90} > 64 and >512. Few isolates such as *Aeromonas hydrophila* and *Salmonella typhi* were sensitive to most of the classical antibiotics. While *Vibrio cholera* was sensitive to only chloramphenicol, *Vibrio parahaemolyticus* was sensitive to trimethoprim, tetracycline and chloramphenicol with MIC_{90} from 4mg/L to 1mg/L. Aeromonas hydrophila is similar to *Vibrio parahaemolyticus* but with an even lower MIC_{90} for all the three drugs indicated. It had MIC_{90} of 1mg/L. Although some of the isolates appeared to be sensitive within the strain, there were quite a number of organisms which were resistant. Therefore resistance was on an increase amongst enteric pathogens world wide.

The emergence of antimicrobial resistance among prevalent enteropathogens and the failure of certain bacterial enteric infections to respond to unrelated antimicrobial chemotherapy make the development and testing of new drugs advisable. Thus the following 4-quinolone compounds which

include ofloxacin, Ciprofloxacin, lomefloxacin, fleroxacin, DR-3355, temafloxacin, and PD-127391 are some of these synthetically produced new quinolone compounds. The

Table 5*: **Comparative MICs in mg/L of selected antimicrobial agents against *Aeromonas hydrophila* (n=24)**

Antimicrobials	MIC_{50}	MIC_{90}	Range	
Amoxycillin	>64	>64	64	- >64
Sulphamethoxazole	128	128	8	- 256
Trimethoprim	1	1	0.25	- 2
Tetracycline	0.5	1	0.5	- 32
Chloramphenicol	1	1	0.5	- 64
Ofloxacin	0.015	0.015	≤0.008	- 0.12
Ciprofloxacin	≤0.008	≤0.008	≤0.008	- 0.12
Lomifloxacin	0.015	0.03	0.015	- 1
Fleroxacin	0.015	0.015	0.008	- 1
DR-3355	0.008	0.008	0.004	- 0.5
Temafloxacin	0.03	0.06	≤0.008	- 0.5
PD-127391	0.004	0.008	≤0.002	-0.12

*MIC_{50} and MIC_{90} are minimum inhibitory concentrations of the drug required to inhibit 50% and 90% of the bacterial isolates, respectively

4-quinolone group of compounds displayed very high potency of activity against all the isolates examined and inhibited them at a concentration of ≤1mg/L (Tables).

In the present study PD-127391 showed the highest activity compared with the other 4-quinolones tested together against the enteric pathogens. The MIC_{90} of PD-127391 for *Salmonella typhi*, *Vibrio cholerae* and *Aeromonas hydrophila* was 0.008mg/L. For *Vibrio parahaemolyticus* it was 0.06mg/L whereas *Campylobacter jejuni* showed high sensitivity compared with the other groups of 4quinolones. Its MIC_{90} was only 0.03mg/L.

Table 6: **Comparative MICs in mg/L of selected antimicrobial agents against *Yersinia enterocolitica* (n=9)**

Antimicrobials	MIC_{50}	MIC_{90}	Range		
Amoxycillin	-	-	1	-	>64
Sulphamethoxazole	-	-	8	-	>512
Trimethoprim	-	-	0.25	-	4
Tetracycline	-	-	1	-	4
Chloramphenicol	-	-	1	-	8
Ofloxacin	-	-	≤0.008	-	0.12
Ciprofloxacin	-	-	≤0.008	-	0.015
Lomifloxacin	-	-	0.015	-	0.12
Fleroxacin	-	-	0.03	-	0.12
DR-3355	-	-	0.008	-	0.06
Temafloxacin	-	-	0.004	-	0.06
PD-127391	-	-	≤0.002	-	0.015

The report of other investigations also showed that this compound is one of the promising compounds with high activity against a wide range of organisms of medical importance (16). The second most active compound against the enteric pathogens tested in our study was Ciprofloxacin.

The MIC_{90} of Ciprofloxacin for *salmonella typhi, Vibrio cholerae, Aeromonas hydrophila* and *Vibrio parahaemolyticus* was much lower than that for *campylobacter jejuni* (Table). On the other hand all species of *Yersinia enterocolitica* were susceptible to all the 4-quinolone compounds. The most active compound of all still remained PD-127391.

The other members of 4-quinolone compounds investigated in this study were less active than PD-127391 and Ciprofloxacin. When compared with the classical anti-microbial agents, however, they were still very active.

From the results presented and other earlier studies (7,16) the new synthesized fluoroquinolones, which are now on the market and those which are still under experimental investigations, were found to be highly effective compounds against enteric pathogens *in vitro* in very low concentrations compared with the classical antimicrobial agents. Thus this group of compounds has potential as alternative treatment in most countries of the world, particularly in the developing ones where the problem of resistance to the classical antimicrobial agents is becoming increasingly very high. A similar study is suggested to see the effect of these antimicrobials against Ethiopian strains.

Fluoroquinolones have little or no effect on the normal gut flora compared with those antimicrobial agents classically used to treat enteric pathogens (17). This characteristic enhances the potential of this group of compounds in clinical practice. However, a recent study has shown that fluoroquinolones were found to cause cartilage damage as well as arthropathy in young animals (18,19,20). As a result of these findings fluoroquinolones are not advisable for use in pre-pubertal children and in pregnant or nursing women. The fluoroquinolones which are undergoing clinical trails and those which are at various stages of experimental development must therefore be well evaluated before they are released for routine use in clinical practice for the treatment and prevention of enteric pathogens and other infectious diseases.

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