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Comparative In Vitro Activities of Aztreonam, Ciprofloxacin, Norfloxacin, Ofloxacin, HR 810 (a New Cephalosporin), RU28965 (a New Macrolide), and Other Agents Against Enteropathogens

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The in vitro activity of drugs currently used in the treatment of diarrhea against 595 enteropathogens from worldwide sources was compared with that of six newly developed antibiotics, ciprofloxacin; norfloxacin; ofloxacin; aztreonam; HR810, an expanded-spectrum cephalosporin; and RU28965, a new macrolide. In contrast with ampicillin and chloramphenicol, trimethoprim-sulfamethoxazole showed an excellent activity against all of the enteropathogens tested, except *Campylobacter* species. Ciprofloxacin had the highest activity, with an overall 90% MIC of ≤0.097 μg/ml, except for *Campylobacter* species (0.39 μg/ml). Unlike other cephalosporins, HR810 showed a satisfactory activity against *Campylobacter* species (90% MIC of 3.12 μg/ml). RU28965 was three times less active than erythromycin against *Campylobacter* species.

Acute diarrhea is a common cause of morbidity and mortality throughout the world. The most severe, as well as the most frequently occurring, forms of this disease in developing countries are of bacterial origin (5). An increase in the frequency of antibiotic resistance of these organisms has been noted in developed as well as in developing countries (4, 8, 13). Therefore, continued concern about antimicrobial resistance is warranted, as are studies on the activity of new agents. For these reasons, we decided to perform a study on recently and randomly isolated enteropathogens from different parts of the world. A comparison was made between the in vitro activities of drugs currently used in the prevention and treatment of bacterial diarrhea with six newly developed preparations, three nalidixic acid analogs, ciprofloxacin, norfloxacin, and ofloxacin; HR810, a 3'-pyridinium-substituted expanded-spectrum cephalosporin; RU28965, a new macrolide, which is an ether oxine derivate of erythromycin; and aztreonam, a synthetic β-lactam antimicrobial agent belonging to the monobactam family.

MATERIALS AND METHODS

Antimicrobial agents. Ciprofloxacin was provided by Bayer; norfloxacin was provided by Merck, Sharp & Dohme; and ofloxacin was given by Hoechst AG. Aztreonam was provided by Squibb. HR810 was obtained from Hoechst AG. RU28965 was supplied by Roussel-UCLAF, 93230 Romainville, France. The remaining antimicrobial agents were kindly provided as follows: ampicillin and amoxicillin from Beecham; cefotaxime from Hoechst AG; gentamicin and netilmicin from Schering-Essex; tetracycline from C.E.R.T.A.; erythromycin from Abbott; chloramphenicol from Lepetit; colistin from Bellon; nifuroxazide from Robert & Carrière; nalidixic acid from Winthrop; sulfamethoxazole (SMX) and trimethoprim (TMP) from Roche; as well as cotrimoxazole, the 20:1 combination of SMX-TMP.

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Bacterial isolates. We examined the susceptibilities of 595 enteric pathogens that had recently been isolated from stool specimens in our laboratory or sent to us from different areas of the world as part of a survey of antibiotic resistance. We included in the study 42 enterotoxigenic *Escherichia coli* strains, isolated in Butare, Rwanda, since 1982 (22 strains were shown to produce a heat-stable toxin, 13 strains produced a heat-labile toxin, and 7 strains produced both, by previously reported methods [6, 10, 19, 22]). Other *E. coli* strains included were: 48 enteropathogenic *E. coli* strains from the same origin and 14 enteroinvasive *E. coli* strains, isolated in Bangladesh (5), the United States (4), and Hungary (5). The following *Salmonella* strains were tested: 96 *S. typhi* isolated in Peru between 1980 and 1982; and 52 non-*typhi Salmonella* strains isolated in Butare, Rwanda, between 1981 and 1982, including more than 20 different serotypes. A total of 52 *Yersinia enterocolitica* strains, were isolated in Brussels, Belgium; and 95 *Campylobacter* organisms (differentiated on the basis of hippurate hydrolysis [21] into 79 *C. jejuni* and 16 *C. coli* strains) were isolated in 1984 by a new selective medium (12). A total of 176 *Shigella* strains were tested, including 119 isolated in Butare, Rwanda, since 1983 (49 *S. dysenteriae*, 53 *S. flexneri*, 6 *S. boydii*, and 11 *S. sonnei*) and 57 isolated in Belgium since 1982 (10 *S. dysenteriae*, 32 *S. flexneri*, 10 *S. boydii*, and 5 *S. sonnei*). Finally, 20 *Aeromonas hydrophila* strains were included (8 isolated in India and 12 isolated in Thailand). All strains were stored at −70°C, and fresh subcultures were used for susceptibility testing.

Bacterial inoculum. The inoculum was prepared as follows. The strains were plated on Mueller-Hinton agar (Bio Mérieux 5180) and incubated for 24 h at 37°C. Colonies from these plates were then suspended in Mueller-Hinton broth (Bio Mérieux 40702) and incubated overnight. The overnight cultures were adjusted to the opacity of the McFarland no. 1 standard. The cultures were then diluted 1:20 in Mueller-Hinton broth. A final inoculum of 1.5×10^5 CFU/ml was obtained on the microtiter plates. The inoculum for *Campylobacter* spp. was prepared as described by Vanhoof et al. (24).

TABLE 1. Comparative MICs between antibiotics currently used in the treatment of diarrhea and some newly developed drugs

Organisms (no. of strains)	Drug	MIC (μg/ml)		
		Range	50%	90%
Enteropathogenic *E. coli* (48)	Amoxicillin	0.78–100	3.12	25
	Aztreonam	≤0.097–0.78	≤0.097	0.195
	Cefotaxime	≤0.097–0.195	≤0.097	≤0.097
	HR810	≤0.097	≤0.097	≤0.097
	Ciprofloxacin	≤0.097	≤0.097	≤0.097
	Norfloxacin	≤0.097	≤0.097	≤0.097
	Ofloxacin	≤0.097–0.195	≤0.097	≤0.097
	Nifuroxazide	3.12–50	25	50
	TMP	≤0.097–>100	≤0.097	0.195
	TMP-SMX[a]	≤0.097–>100	≤0.097	0.195
Enterotoxigenic *E. coli* (42)	Amoxicillin	1.56–100	6.25	12.50
	Aztreonam	≤0.097–6.25	≤0.097	0.195
	Cefotaxime	≤0.097–0.195	≤0.097	≤0.097
	HR810	≤0.097	≤0.097	≤0.097
	Ciprofloxacin	≤0.097	≤0.097	≤0.097
	Norfloxacin	≤0.097	≤0.097	≤0.097
	Ofloxacin	≤0.097–0.195	≤0.097	≤0.097
	Nifuroxazide	0.195–50	12.50	25
	TMP	≤0.097–>100	≤0.097	0.195
	TMP-SMX	≤0.097–>100	≤0.097	0.195
Enteroinvasive *E. coli* (14)	Amoxicillin	6.25–>100	25	25
	Aztreonam	≤0.097–3.12	≤0.097	0.78
	Cefotaxime	≤0.097–1.56	≤0.097	≤0.097
	HR810	≤0.097–0.39	≤0.097	≤0.097
	Ciprofloxacin	≤0.097	≤0.097	≤0.097
	Norfloxacin	≤0.097	≤0.097	≤0.097
	Ofloxacin	≤0.097	≤0.097	≤0.097
	Nifuroxazide	6.25–12.50	12.50	12.50
	TMP	≤0.097–0.195	0.195	0.195
	TMP-SMX	≤0.097–0.195	≤0.097	0.195
Shigella spp. (127)	Ampicillin	0.78–>100	12.50	25
	Aztreonam	≤0.097–>100	≤0.097	0.39
	Cefotaxime	≤0.097–0.78	≤0.097	≤0.097
	HR810	≤0.097–0.39	≤0.097	≤0.097
	Chloramphenicol	0.195–>100	0.78	50
	Tetracycline	≤0.097–>100	1.56	100
	Ciprofloxacin	≤0.097–0.195	≤0.097	≤0.097
	Norfloxacin	≤0.097–0.39	≤0.097	≤0.097
	Ofloxacin	≤0.097–0.39	≤0.097	≤0.097
	Erythromycin	≤0.097–50	6.25	25
	TMP	≤0.097–>100	0.195	0.78
	TMP-SMX	0.195–>100	0.78	6.25
Y. enterocolitica (52)	Amoxicillin	3.12–25	12.50	25
	Aztreonam	≤0.097–3.12	0.78	1.56
	Cefotaxime	≤0.097	≤0.097	≤0.097
	HR810	≤0.097	≤0.097	≤0.097
	Chloramphenicol	0.78–6.25	1.56	3.12
	Gentamicin	0.39–1.56	0.78	1.56
	Netilmicin	≤0.097–1.56	0.78	1.56
	Ciprofloxacin	≤0.097–0.195	≤0.097	≤0.097
	Norfloxacin	≤0.097	≤0.097	≤0.097
	Ofloxacin	≤0.097	≤0.097	≤0.097
	Nifuroxazide	0.78–12.50	12.50	12.50
	TMP-SMX	0.78–6.25	1.56	3.12
A. hydrophila (20)	Amoxicillin	3.12–>100	12.50	25
	Aztreonam	≤0.097	≤0.097	≤0.097
	Cefotaxime	≤0.097–0.39	≤0.097	≤0.097
	HR810	≤0.097	≤0.097	≤0.097
	Chloramphenicol	0.195–3.12	0.39	0.78
	Ciprofloxacin	≤0.097–0.195	≤0.097	≤0.097
	Norfloxacin	≤0.097–0.195	≤0.097	≤0.097

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TABLE 1—*Continued*

Organisms (no. of strains)	Drug	MIC (μg/ml)		
		Range	50%	90%
	Ofloxacin	≤0.097	≤0.097	≤0.097
	Nifuroxazide	0.39–12.50	3.12	12.50
	TMP-SMX	≤0.097–1.56	0.195	0.39
Salmonella non-*typhi* (52)	Amoxicillin	≤0.097–>100	>100	>100
	Aztreonam	≤0.097–0.195	≤0.097	0.195
	Cefotaxime	≤0.097–0.78	0.195	0.39
	HR810	≤0.097–0.39	≤0.097	0.195
	Chloramphenicol	0.78–>100	100	>100
	Ciprofloxacin	≤0.097	≤0.097	≤0.097
	Norfloxacin	≤0.097	≤0.097	≤0.097
	Ofloxacin	≤0.097	≤0.097	≤0.097
	Nifuroxazide	25–50	50	50
	TMP	≤0.097–100	≤0.097	50
	TMP-SMX	0.39–>100	0.78	100
Salmonella typhi (96)	Amoxicillin	0.195–>100	0.195	0.195
	Aztreonam	≤0.097–0.195	≤0.097	≤0.097
	Cefotaxime	≤0.097–0.195	≤0.097	≤0.097
	HR810	≤0.097–0.195	≤0.097	≤0.097
	Chloramphenicol	3.12–>100	3.12	>100
	Ciprofloxacin	≤0.097	≤0.097	≤0.097
	Norfloxacin	≤0.097	≤0.097	≤0.097
	Ofloxacin	≤0.097	≤0.097	≤0.097
	TMP	≤0.097–>100	≤0.097	0.195
	TMP-SMX	0.78–>100	0.78	6.25
C. jejuni (79)	Amoxicillin	0.39–100	6.25	12.50
	Cefotaxime	0.78–100	6.25	12.50
	HR810	0.39–100	0.78	3.12
	Chloramphenicol	0.78–12.50	1.56	6.25
	Gentamicin	≤0.097–100	0.39	0.78
	Ciprofloxacin	≤0.097–0.78	≤0.097	0.39
	Norfloxacin	≤0.097–3.12	0.39	1.56
	Ofloxacin	≤0.097–1.56	0.195	0.78
	Erythromycin	≤0.097–3.12	0.39	0.78
	RU28965	0.78–>100	3.12	6.25
	TMP-SMX	3.12–50	12.50	50
C. coli (16)	Amoxicillin	0.39–12.50	6.25	12.50
	Cefotaxime	0.78–12.50	6.25	6.25
	HR810	0.78–12.50	1.56	6.25
	Chloramphenicol	0.78–12.50	3.12	6.25
	Gentamicin	0.195–>100	0.78	0.39
	Ciprofloxacin	≤0.097–0.78	0.195	0.39
	Norfloxacin	0.195–1.56	0.39	0.78
	Ofloxacin	≤0.097–0.78	0.39	0.78
	Erythromycin	≤0.097–0.78	0.39	0.78
	RU28965	0.195–>100	3.12	6.25
	TMP-SMX	3.12–50	12.50	50

[a] TMP-SMX, 20:1.

Antibiotic susceptibility testing. MICs were determined for each antimicrobial agent in liquid medium, using a Cooke Dynatech 2000 dispenser and inoculator. The microtiter wells were filled with 100-μl samples of the antibiotic solution prepared in Mueller-Hinton broth except for cotrimoxazole and trimethroprim, for which the medium was Mueller-Hinton broth with 5% lysed horse blood. The volume of the inoculum was 1 μl. Control strains of *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were simultaneously tested to ensure the potency of each drug. The viability of the bacteria was verified in a control well without antibiotic. The microtiter plates were incubated overnight at 37°C, except for those with *Campylobacter* spp., which were incubated at 42°C for 48 h in a 5% CO_2 atmosphere. The trays were examined on a Cooke Dynatech viewing box. MICs were recorded as the lowest concentrations of antibiotic inhibiting visible growth.

RESULTS

MIC ranges and MICs that inhibited 50 and 90% of the strains (MIC_{50} and MIC_{90}, respectively) are detailed in Table 1. The *S. dysenteriae* strains isolated in Rwanda were not included in Table 1 since they arose from a single outbreak due to a multiresistant strain (P. De Mol, personal communication). Nonetheless, among the 70 other Rwandese *Shigella* strains, 26 (37.1%) required ampicillin MICs of ≥25

μg/ml in comparison with 22 of the 57 (36.8%) Belgian *Shigella* strains. Resistance to chloramphenicol in these 70 Rwandese strains was 18.5%, in comparison with 8.7% of the 57 Belgian strains (breakpoint, ≥16 μg/ml [18]). However, of all the 176 *Shigella* strains tested, only 8 (4.6%) were resistant (MIC ≥32 μg/ml [7]) to the 20:1 combination of SMX-TMP. Resistance to tetracycline (MIC ≥16 μg/ml [18]) occurred in 6 of the 42 (14.1%) enterotoxigenic *E. coli* strains.

RU28965 was tested against *Campylobacter* strains only. The in vitro activity for this agent appeared to be markedly lower than the in vitro activity of erythromycin. Furthermore, none of the *Campylobacter* strains required MICs of erythromycin of ≥6.25 μg/ml, as opposed to 16 (16.9%) strains for RU28965. The in vitro activity of HR810 was, in general, superior to cefotaxime, especially for the *Campylobacter* strains; MIC_{90}s for cefotaxime and HR810 against the 95 *Campylobacter* organisms tested were 12.50 and 3.12 μg/ml, respectively.

DISCUSSION

The exact role of antimicrobial chemotherapy in gastrointestinal infections remains controversial, partly because of the self-limiting nature of the disease and the rapid emergence of resistant strains.

Ampicillin is used in the empirical treatment of acute diarrhea, whereas chloramphenicol is widely administered in enteric fever. However, this study, together with our previous studies (13, 14, 24), show that *Shigella* spp., non-*typhi Salmonella*, *A. hydrophila*, *Y. enterocolitica*, and *Campylobacter* spp. are moderately to highly resistant to ampicillin and that a significant percentage of *S. typhi* and non-*typhi* are resistant to chloramphenicol. TMP and TMP-SMX retain an excellent activity against all the enteropathogens tested; however, this study confirms their high MICs against *Campylobacter* (23, 24). TMP alone or in combination with SMX should therefore be considered as drugs of choice for the treatment of shigellosis and together with doxycycline in preventing and treating traveller's diarrhea.

We found no significant difference in antibiotic susceptibility of enterotoxigenic *E. coli* strains producing heat-labile, heat-stable, or both enterotoxins.

Previous studies (13, 14, 23) stimulated us to test new effective drugs on enteric organisms. Three quinolones, ciprofloxacin, norfloxacin, and ofloxacin; an expanded-spectrum cephalosporin, HR810; a macrolide, RU28965; and a synthetic β-lactam, aztreonam; were considered for this study. Our findings are in agreement with the results of Carlson et al. (3), Goodman et al. (11), and Shungu et al. (20), who demonstrated the excellent in vitro activity of several of the quinolones against common bacterial enteric pathogens. We found that of the 24 antimicrobial agents tested, ciprofloxacin showed the highest in vitro activity. Its broad-spectrum activity, together with the satisfactory fecal and serum concentrations after oral administration (2), makes ciprofloxacin, and perhaps other quinolones, a very promising drug for the treatment or prevention or both of diarrhea. Clinical trials should be initiated to evaluate this.

Except for *Campylobacter* spp., aztreonam and cefotaxime exhibited uniform and high activity against all strains tested, irrespective of their multiple resistance. Lepage et al. (17) showed clearly in a large clinical trial in Kigali, Rwanda, the effectiveness of cefotaxime for the treatment of severe infections due to multiresistant *Salmonella typhimurium*. In this study, we included an expanded-spectrum cephalosporin, HR810, whose chemical structure is close to that of cefotaxime. HR810 was found to have a higher in vitro activity than cefotaxime, and it may be the only cephalosporin with a satisfying in vitro activity against *Campylobacter* spp. (23, 24). Erythromycin has been the standard therapy for treatment of *Campylobacter* diarrhea. Although this drug retains an excellent in vitro activity against *Campylobacter* spp., we decided to test a new macrolide, RU28965, because of the pharmacological disadvantages of erythromycin (9). Our results show clearly that RU28965 is less active in vitro than is erythromycin against *Campylobacter* spp. This is in contrast to the study of Barlam and Neu (1), who found similar activity for both drugs; however, these authors tested only a small number (i.e., six) of isolates. Yet, because of the pharmacological advantages of RU28965 (A. Bryskier, Institut Roussel UCLAF, Paris, France), this drug may still become a candidate in the oral treatment of *Campylobacter* diarrhea. Clinical trials are the only method by which we may evaluate this.

In summary, an in vitro study on 595 randomly selected enteric organisms from worldwide sources showed, in contrast to ampicillin and chloramphenicol, an excellent activity for TMP alone or in combination with SMX. The quinolines, especially ciprofloxacin, and HR810 were highly active. RU28965 was found to be less active than erythromycin on *Campylobacter* spp.

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