

Antistreptococcal Activity of AR-709 Compared to That of Other Agents[∇]

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Against 300 strains of pneumococci and 100 group A streptococci of differing β -lactam, macrolide, and quinolone resistance phenotypes, AR-709 was very active, with all MICs being ≤ 2 $\mu\text{g/ml}$. Furthermore, AR-709 was active against strains that were both susceptible and resistant to trimethoprim-sulfamethoxazole.

Streptococcus pneumoniae strains that are resistant to penicillin G as well as other agents occur worldwide. Most macrolide-resistant pneumococci also are penicillin G resistant, and pneumococcal quinolone resistance also has been reported. Trimethoprim-sulfamethoxazole resistance in pneumococci is high, invalidating its clinical use in infections by these organisms (1, 2, 5, 8–10, 16, 19–22, 25). The problem of drug-resistant pneumococci is exacerbated by their tendency to spread from country to country and also from continent to continent (18). Macrolide resistance also has been reported for *Streptococcus pyogenes* (4, 6, 13, 22, 26).

AR-709 is an investigational diaminopyrimidine that inhibits microbial dihydrofolate reductase and is under development by Arpida AG for the therapy of community-acquired upper and lower respiratory tract infections (7, 11, 12, 14, 15, 24). We have compared the in vitro activity of AR-709 (a new diaminopyrimidine antibiotic) (Fig. 1) to those of trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, cefuroxime, azithromycin, ciprofloxacin, and levofloxacin against a spectrum of pneumococci and group A streptococci with differing resistance phenotypes.

Clinical strains of *S. pneumoniae* included the majority of known resistotypes, of which 101 were penicillin G susceptible (33.6%), 122 were penicillin G intermediate (40.6%), and 77 were fully penicillin G resistant (25.6%). Of these 300 strains, 158 (52.6%) were macrolide resistant [66 had *erm*(B), 59 *mef*(A), 1 *erm*(B) plus *mef*(A), 4 *erm*(A), 23 L4, and 4 23S rRNA ribosomal protein mutations]. Twenty-nine (~10%) pneumococcal strains were quinolone resistant, with defined mutations in the quinolone resistance-determining region. One hundred nine pneumococcal strains were susceptible to trimethoprim-sulfamethoxazole, and the trimethoprim-sulfamethoxazole MICs for 191 strains were intermediate (1 to 2 $\mu\text{g/ml}$) or resistant (≥ 4 $\mu\text{g/ml}$) (3). The 100 *S. pyogenes* isolates included 61 macrolide-resistant strains [20 had *erm*(B), 20 *mef*(A), and 1 L4 ribosomal protein mutations]. Organisms were identified by standard methodology and stored at -70°C in double-strength skim milk (Difco Laboratories, Detroit, MI) until use. With the exception of streptococci with ribosomal mutations, all strains were isolated within 8 years of the beginning of the study. Because an effort was

made to include as many phenotypes as possible, we decided to include older cultures as well. Regardless of the time of isolation, all phenotypes are similar to those from strains isolated during the past few years and, thus, are representative of strains likely to be encountered clinically at the present time.

AR-709 was obtained from Arpida AG, Reinach, Switzerland, and sulfamethoxazole was obtained from Sigma Laboratories, St. Louis, MO. Other drugs were obtained from their respective agents. MIC testing was done by agar dilution methodology using Mueller-Hinton agar (Difco Laboratories) with 5% lysed horse blood for AR-709 and trimethoprim-sulfamethoxazole. All other drugs were tested with 5% added sheep blood. Suspensions with turbidities that were equivalent to a 0.5 McFarland standard were scraped from blood plates and diluted to obtain a final inoculum of 10^4 CFU/spot. Plates were inoculated and incubated overnight in ambient air at 35°C . Quality control strains recommended by the Clinical Laboratory Standards Institute, including *S. pneumoniae* ATCC 49619, were included in each run (3).

Results of MIC testing are listed in Tables 1 and 2. As can be seen, AR-709 was very active against all streptococci tested irrespective of resistance phenotypes, with a MIC range against pneumococci of ≤ 0.008 to 2 $\mu\text{g/ml}$ and of ≤ 0.008 to 0.125 $\mu\text{g/ml}$ against group A streptococci. In contrast, trimethoprim-sulfamethoxazole MICs against pneumococci ranged between 0.03 and >8 $\mu\text{g/ml}$. Of all pneumococci, 191 (63.7%) were intermediate or resistant to trimethoprim-sulfamethoxazole; the MIC range for AR-709 against trimethoprim-sulfamethoxazole-intermediate or -resistant strains was ≤ 0.008 to 2 $\mu\text{g/ml}$, and against trimethoprim-sulfamethoxazole-susceptible strains it was ≤ 0.008 to 0.5 $\mu\text{g/ml}$. Therefore, AR-709 was similarly active against pneumococci that either were susceptible or intermediate/resistant to trimethoprim-sulfamethoxazole. Ninety-three pneumococcal strains (31%) were multidrug resistant (i.e., resistant to three or more different classes of drugs); against these isolates the MIC range for AR-709 was 0.06 to 2 $\mu\text{g/ml}$. In terms of other comparator agents, the MICs of β -lactams and azithromycin against pneumococci rose with those of penicillin G. Quinolone-susceptible pneumococcal strains had levofloxacin MICs ranging between 0.5 and 2 $\mu\text{g/ml}$. Furthermore, all group A streptococci were susceptible to β -lactams, trimethoprim-sulfamethoxazole, and quinolones. Against macrolide-resistant group A streptococci, the MICs of AR-709 were within one dilution of those against macrolide-susceptible strains.

AR-709 is an experimental diaminopyrimidine that exhibits

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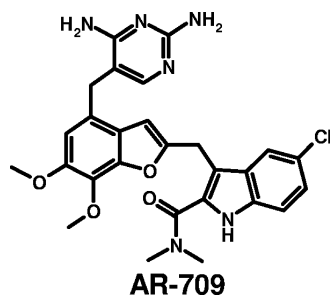


FIG. 1. Chemical structure of AR-709.

potent activity against pneumococci and other gram-positive pathogens by inhibiting bacterial dihydrofolate reductase (24). The drug has been shown to have postantibiotic effects for ≥ 1.1 h against pneumococci, with postantibiotic sub-MIC effects longer than those of postantibiotic + sub-MIC effects (7).

AR-709 concentrations remain high in all key compartments of the lung throughout a 12-h experimental period (17) and also exhibited excellent in vivo antipneumococcal activity in mice following intravenous, oral, and intranasal administration (14). Plasma pharmacokinetics suggest a once-daily dosing regimen (15).

In a recent paper (23), Pichichero and Casey reported the emergence in the United States of an otopathogenic strain of pneumococcus type 19A that is not included in the current pneumococcal 7-valent conjugate vaccine and that is resistant to all antibiotics currently approved by the FDA for treating acute otitis media in children. There is a pressing need for new drugs that will be active against such resistant phenotypes. The uniform susceptibility of all trimethoprim-sulfamethoxazole-nonsusceptible pneumococcal strains in the current study to AR-709, together with the pharmacokinetics and administration route of AR-709 men-

TABLE 1. MICs of AR-709 and other agents against pneumococci

Drug and strain susceptibility type ^a	MIC ($\mu\text{g/ml}$)			Drug and strain susceptibility type ^a	MIC ($\mu\text{g/ml}$)		
	Range	50%	90%		Range	50%	90%
AR-709				Azithromycin			
Penicillin S	≤ 0.008 –2	0.06	0.25	Penicillin S	0.03–>64	0.12	>64
Penicillin I	≤ 0.008 –1	0.25	0.5	Penicillin I	0.03–>64	0.12	>64
Penicillin R	0.016–2	0.25	1	Penicillin R	0.03–>64	8	>64
Macrolide S	≤ 0.008 –1	0.12	0.5	Macrolide S	0.03–0.25	0.12	0.12
Macrolide R	≤ 0.008 –2	0.25	0.5	Macrolide R	1–>64	>64	>64
Quinolone S	≤ 0.008 –2	0.12	0.5	Quinolone S	0.03–>64	4	>64
Quinolone R	0.016–2	0.25	1	Quinolone R	0.03–>64	0.12	>64
SXT S	≤ 0.008 –0.5	0.06	0.12	SXT S	0.03–>64	0.12	>64
SXT R	≤ 0.008 –2	0.25	0.5	SXT R	0.03–>64	8	>64
SXT				Ciprofloxacin			
Penicillin S	0.03–>8	0.25	4	Penicillin S	0.5–>32	2	16
Penicillin I	0.12–>8	4	8	Penicillin I	0.5–32	1	4
Penicillin R	0.12–>8	8	8	Penicillin R	0.5–>32	2	4
Macrolide S	0.03–>8	0.5	8	Macrolide S	0.5–>32	2	16
Macrolide R	0.12–>8	4	8	Macrolide R	0.5–>32	1	4
Quinolone S	0.06–>8	4	8	Quinolone S	0.5–32	1	2
Quinolone R	0.03–>8	4	8	Quinolone R	4–>32	32	>32
SXT S	0.03–0.5	0.25	0.5	SXT S	0.5–>32	2	8
SXT R	1–>8	8	8	SXT R	0.5–>32	1	4
Amoxicillin-clavulanate				Levofloxacin			
Penicillin S	≤ 0.016 –0.12	≤ 0.016	0.03	Penicillin S	0.5–16	1	8
Penicillin I	≤ 0.016 –1	0.12	1	Penicillin I	0.5–32	1	2
Penicillin R	0.25–8	2	4	Penicillin R	0.5–16	1	4
Macrolide S	≤ 0.016 –2	0.06	1	Macrolide S	0.5–16	1	8
Macrolide R	≤ 0.016 –8	0.12	4	Macrolide R	0.5–32	1	2
Quinolone S	≤ 0.016 –8	0.12	2	Quinolone S	0.5–2	1	1
Quinolone R	≤ 0.016 –4	0.5	2	Quinolone R	4–32	16	16
SXT S	≤ 0.016 –2	0.03	0.12	SXT S	0.5–16	1	2
SXT R	≤ 0.016 –8	0.5	4	SXT R	0.5–32	1	2
Cefuroxime				Penicillin G			
Penicillin S	0.016–0.5	0.03	0.12	Penicillin S	≤ 0.008 –0.06	0.016	0.06
Penicillin I	0.03–4	0.25	4	Penicillin I	0.12–1	0.25	1
Penicillin R	1–>64	4	16	Penicillin R	2–16	2	4
Macrolide S	0.016–8	0.25	4	Macrolide S	0.016–2	0.12	2
Macrolide R	0.016–>64	0.5	8	Macrolide R	≤ 0.008 –16	0.25	4
Quinolone S	0.016–>64	0.25	4	Quinolone S	0.12–16	0.12	2
Quinolone R	0.03–8	2	8	Quinolone R	0.016–4	0.5	2
SXT S	0.016–16	0.03	0.5	SXT S	≤ 0.008 –4	0.03	0.25
SXT R	0.016–>64	2	8	SXT R	0.016–16	1	4

^a S, susceptible; I, intermediate; R, resistant; and SXT, trimethoprim-sulfamethoxazole (1:19).

TABLE 2. MICs of AR-709 and other agents against group A streptococci

Drug and strain susceptibility type ^a	MIC (µg/ml)		
	MIC range	50%	90%
AR-709			
Macrolide S	≤0.008–0.12	0.06	0.06
Macrolide R	≤0.008–0.06	0.016	0.03
Trimethoprim-sulfamethoxazole (1:19)			
Macrolide S	0.06–0.5	0.12	0.25
Macrolide R	≤0.008–0.5	0.06	0.25
Amoxicillin-clavulanate			
Macrolide S	≤0.016–0.03	≤0.016	≤0.016
Macrolide R	≤0.016–0.03	≤0.016	≤0.016
Cefuroxime			
Macrolide S	0.016–0.016	0.016	0.016
Macrolide R	0.016–0.03	0.016	0.03
Azithromycin			
Macrolide S	0.06–0.25	0.12	0.25
Macrolide R	4–>64	>64	>64
Ciprofloxacin			
Macrolide S	0.5–4	0.5	1
Macrolide R	≤0.25–2	0.5	2
Levofloxacin			
Macrolide S	0.5–2	0.5	2
Macrolide R	0.25–2	0.5	1

^a S, susceptible; and R, resistant.

tioned above, makes it a possible alternative drug for the treatment of the above-described resistant pneumococcal infections.

The results of this study show that the MICs of AR-709 were low against all streptococci tested. Similarly, Ressler and co-workers (24) have reported AR-709 MIC ranges of ≤0.008 to 1 µg/ml, with MICs at which 50 and 90% of the isolates tested are inhibited of 0.25 and 0.5 µg/ml, respectively, when tested against 239 recent multiresistant North American isolates of invasive pneumococci. Furthermore, studies by other workers (11, 12) have shown the promising activity of AR-709 against multidrug-resistant pneumococci, including those of *erm*, *mef*, and *erm* plus *mef* phenotypes. The results for other compounds were similar to those reported in other studies (1, 5, 9, 10, 16, 19–22, 25). Pending human pharmacokinetic/pharmacodynamic, animal efficacy, and toxicology studies, AR-709 shows promise for the future therapy of pneumococcal infections, especially those caused in children by nonvaccine panresistant serotypes such as 19A.

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