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NOTES

Comparative In Vitro Activities of Several New Fluoroquinolones and β-Lactam Antimicrobial Agents against Community Isolates of *Streptococcus pneumoniae*

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The in vitro susceptibilities of 551 community isolates of *Streptococcus pneumoniae* from the Canadian province of Ontario to several new fluoroquinolones and β-lactam antimicrobial agents were determined by a broth microdilution technique. Eight (1.5%) of these isolates were moderately susceptible (MICs, ≥0.12 and ≤1.0 μg/ml) to penicillin; none was resistant. Temafloxacin, ciprofloxacin, and ofloxacin (MICs for 90% of strains tested, between 1 and 2 μg/ml) were the most active fluoroquinolones tested, and BMY-28100 (MIC for 90% of strains tested, 0.25 μg/ml) was the most active of the new β-lactams tested.

Streptococcus pneumoniae remains a leading cause of upper and lower respiratory tract infections as well as a significant cause of meningitis, septicemia, and other serious infections (24). For years, penicillin has remained the treatment of choice. However, since 1967, when *S. pneumoniae* resistant to penicillin was first reported (7), further evidence has suggested that these isolates are occurring worldwide with greater frequency and that the penicillin MICs for these isolates are increasing (1, 11, 24; C. Thornsberry, J. M. Swenson, and R. R. Facklam, Antimicrob. Newsl. 5:23–24, 1988). Recently, several new, clinically important antimicrobial agents, particularly of the fluoroquinolone and β-lactam groups have been developed. These newer agents possess a relatively broad spectrum of activity and have excellent absorption after oral administration (10, 16). However, their activity against streptococci, particularly *S. pneumoniae*, has varied considerably from agent to agent (2, 3, 13, 15, 16, 19, 25), and this may limit their usefulness in treating pneumococcal infections. Therefore, we determined the prevalence of penicillin-resistant *S. pneumoniae* among community isolates in Ontario and determined their in vitro susceptibility to several new antimicrobial agents.

A total of 551 community outpatient isolates of *S. pneumoniae* were collected from across the Canadian province of Ontario between May 1988 to September 1988 from MDS Laboratories, which provide service to family physicians and nursing homes. All isolates were identified as *S. pneumoniae* based on colonial morphology, Gram stain characteristics, bile solubility, and optochin susceptibility. Reference strains of *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212 were used as control organisms for susceptibility testing. As well, three control strains of *S. pneumoniae* for which MICs were previously reported (12) were provided by Francois Lamothe, Department of Microbiology, Hôpital Saint-Luc, Montreal, Quebec, Canada. The isolates were frozen at −70°C, thawed, subcultured onto Columbia agar containing 5% sheep blood, and incubated at 37°C in 5% CO_2 for 24 h immediately before susceptibility testing.

All isolates were tested for in vitro susceptibility by a broth microdilution technique, following National Committee for Clinical Laboratory Standards M7-A guidelines (17). Cation-supplemented Mueller-Hinton broth (GIBCO Diagnostics, Madison, Wis.) with 5% lysed horse blood and an inoculum of 10^5 CFU/ml was used. MICs (defined as the lowest concentration that inhibits visible growth completely) were determined after overnight incubation at 37°C in 5% CO_2. Susceptibility to penicillin was also determined by measuring the zone of inhibition around a 1-μg oxacillin disk on cation-supplemented Mueller-Hinton agar supplemented with 5% lysed horse blood, according to National Committee for Clinical Laboratory Standards M2-A3 guidelines (18). The antimicrobial agents tested (SCH 39720 [ceftibuten], LY163892 [loracarbef], BMY-28100, ciprofloxacin, enoxacin, fleroxacin, ofloxacin, pefloxacin, lomefloxacin, temafloxacin, penicillin G, ampicillin, cefaclor, cefamandole, erythromycin, clindamycin, tetracycline, and trimethoprim-sulfamethoxazole) were supplied by the respective manufacturers.

Of the original 551 isolates of *S. pneumoniae*, 8 (1.5%) were found to have reduced susceptibility to penicillin (MICs, 0.12 to 1.0 μg/ml). As well, for the three control strains of *S. pneumoniae* the reproducible MICs were 0.25, 0.5, and 2 μg/ml. This gave a total of 10 isolates that were moderately susceptible to penicillin and one strain that was truly resistant to penicillin (MIC, ≥2.0 μg/ml). All of these 11 isolates were also detected by the 1-μg oxacillin disk with zones of inhibition of <19 mm. A total of 29 (5.2%) of the 551 isolates showed reduced susceptibility to at least one antimicrobial agent tested, with some demonstrating reduced susceptibility to multiple agents. Resistance to trimethoprim-sulfamethoxazole was the most common, with MICs for 2.5% of the isolates of ≥4/76 μg/ml. Three isolates were resistant to erythromycin (MIC, ≥32.0 μg/ml), clindamycin

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TABLE 1. In vitro susceptibility of 551 clinical isolates and 3 control strains of *S. pneumoniae* to various antimicrobial agents

Antimicrobial agent	MIC (μg/ml)				
	Susceptible[a] isolates (n = 543)			Isolates with reduced susceptibility[a] (n = 11)	
	Range	50%	90%	Range	50%
Ciprofloxacin	0.25–2.0	0.5	1.0	0.25–2.0	1.0
Enoxacin	1.0–>32.0	8.0	16.0	4.0–16.0	8.0
Lomefloxacin	1.0–16.0	4.0	8.0	4.0–8.0	8.0
Temafloxacin	0.12–1.0	0.5	1.0	0.25–1.0	0.5
Fleroxacin	1.0–16.0	8.0	8.0	4.0–8.0	8.0
Ofloxacin	0.5–4.0	1.0	2.0	1.0–4.0	2.0
Pefloxacin	0.5–16.0	4.0	8.0	2.0–8.0	4.0
BMY-28100	0.12–0.25	0.25	0.25	0.12–4.0	0.12
Loracarbef (LY163892)	0.12–1.0	0.5	1.0	0.5–>32.0	2.0
Ceftibuten (SCH 39720)	0.5–32.0	1.0	2.0	8.0–>32.0	16.0
Penicillin G	≤0.03–0.06	≤0.03	≤0.03	0.12–2.0	0.25
Ampicillin	≤0.06	≤0.06	≤0.06	≤0.06–>4.0	0.12
Cefaclor	≤1.0	≤1.0	≤1.0	≤0.25–>32.0	1.0
Cefamandole	≤0.25–4.0	≤0.25	≤0.25	≤0.25–16.0	0.5
Erythromycin	≤0.25–>32.0	≤0.25	≤0.25	≤0.12–16.0	≤0.12
Clindamycin	≤0.25–>32.0	≤0.25	≤0.25	≤0.12–16.0	≤0.12
Tetracycline	≤1.0–32.0	≤1.0	≤1.0	≤1.0–16.0	<1.0
TMP-SMX[b]	≤0.25/4.75–16.0/304	0.25/4.75	1/19	≤0.25/4.75–8.0/152	1.0/19

[a] Refers to susceptibility to penicillin.
[b] TMP-SMX, Trimethoprim-sulfamethoxazole.

(MIC, ≥32.0 μg/ml), and tetracycline (MIC, 32.0 μg/ml). An additional isolate was resistant only to clindamycin and erythromycin, and three more were resistant to erythromycin alone.

The in vitro susceptibilities of the isolates to the antimicrobial agents tested are shown in Table 1. Of the fluoroquinolones tested, ciprofloxacin, temafloxacin, and ofloxacin appeared to be the most active against penicillin-susceptible *S. pneumoniae* (MICs for 90% of isolates tested [MIC_{90}], between 1 and 2 μg/ml). The other fluoroquinolones showed much higher MIC_{90}s, with enoxacin being the least active (MIC_{90}, 16.0 μg/ml). Of the three new β-lactam antibiotics, BMY-28100 appeared to have the greatest in vitro activity with an MIC_{90} of 0.25 μg/ml. Loracarbef and ceftibuten had four- to eightfold higher MIC_{90}s, even against penicillin-susceptible strains of *S. pneumoniae*. Against the 11 isolates with reduced susceptibility to penicillin, ciprofloxacin (MIC for 50% of isolates tested [MIC_{50}], 1.0 μg/ml) and temafloxacin (MIC_{50}, 0.5 μg/ml) remained the most active fluoroquinolones, whereas BMY-28100 (MIC_{50}, 0.12 μg/ml) was the most active of the newer β-lactam antibiotics. Ceftibuten appeared to be the least active agent against these isolates; for seven isolates the MICs were ≥16 μg/ml.

The relatively low prevalence of *S. pneumoniae* with reduced susceptibility to penicillin (1.5%) found in our study in Ontario appears to be similar to the 1.3% prevalence recently reported in Quebec (12) and the 2.4% found in Western Canada in 1977 (4). These results suggest that in Canada the prevalence of *S. pneumoniae* with reduced susceptibility to penicillin appears to be uniform across the country and that it has remained stable over the past 10 to 12 years. In contrast, in the United States, the current overall incidence of *S. pneumoniae* with reduced susceptibility to penicillin is approximately 4%, although there is great geographic variability, ranging from 0 to 15% (1; Thornsberry et al., Antimicrob. Newsl.). As well, the prevalence in Canada of these strains is far below that reported from other countries such as Spain (51%) or South Africa (62.2%) (1, 11). Moreover, only *S. pneumoniae* strains that are moderately susceptible to penicillin (MICs, ≥0.12 and ≤1.0 μg/ml) and none with true resistance (MIC, ≥2.0 μg/ml) have been isolated in Canada.

Our susceptibility test results are in agreement with previous studies, which have shown the wide variability in activity against *S. pneumoniae* that members of the fluoroquinolone and newer β-lactam antimicrobial agents may possess (6, 9, 13–15, 20, 21, 25). Temafloxacin, ofloxacin, and ciprofloxacin of the fluoroquinolone group and BMY-28100 of the newer β-lactam group appear to possess the greatest in vitro activity against *S. pneumoniae*. The MIC_{90}s of temafloxacin (1.0 μg/ml), ofloxacin (2.0 μg/ml), and ciprofloxacin (1.0 μg/ml) are below the achievable mean peak levels of these agents in serum (8, 23). However, compared with peak levels in sputum (5), the achievable concentrations of these agents more closely approach their MIC_{90}s. This fact may limit the usefulness of these agents in treating common infections caused by *S. pneumoniae*, such as pneumonia, despite their in vitro activity. Although some clinical trials demonstrating the effectiveness of these agents against *S. pneumoniae* have been done (22), further studies are needed to support their clinical efficacy and before recommendations for their use can be made.

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LITERATURE CITED

1. **Appelbaum, P. C.** 1987. Worldwide development of antibiotic resistance in pneumococci. Eur. J. Clin. Microbiol. **6**:367–377.
2. **Appelbaum, P. C., S. K. Spangler, E. Crotty, and M. R. Jacobs.** 1989. Susceptibility of penicillin-sensitive and -resistant strains of *Streptococcus pneumoniae* to new antimicrobial agents, including daptomycin, teicoplanin, cefpodoxime and quinolones. J. Antimicrob. Chemother. **23**:509–516.
3. **Barry, A. L., and R. N. Jones.** 1989. In-vitro activities of temafloxacin, tosufloxacin (A-61827) and five other fluoroquinolone agents. J. Antimicrob. Chemother. **23**:527–535.
4. **Dixon, J. M. S., A. E. Lipinski, and M. E. P. Graham.** 1977. Detection and prevalence of pneumococci with increased resistance to penicillin. Can. Med. Assoc. J. **117**:1159–1161.
5. **Eggleston, M., and S. Park.** 1987. Review of the 4-quinolones.

Infect. Control 8:119–125.
6. **Gombert, M. E., and T. M. Aulicino.** 1984. Susceptibility of multiply antibiotic-resistant pneumococci to the new quinolone antibiotics, nalidixic acid, coumermycin, and novobiocin. Antimicrob. Agents Chemother. **26**:933–934.
7. **Hansman, D., and M. M. Bullen.** 1967. A resistant pneumococcus. Lancet **I**:264–265.
8. **Hardy, D. J., R. N. Swanson, D. M. Hensey, N. R. Ramer, R. R. Bower, C. W. Hanson, D. T. Chu, and P. B. Fernandes.** 1987. Comparative antibacterial activities of temafloxacin hydrochloride (A-62254) and two reference fluoroquinolones. Antimicrob. Agents Chemother. **31**:1768–1774.
9. **Heifetz, C. L., P. A. Bien, M. A. Cohen, M. E. Dombrowski, T. J. Griffin, T. E. Malta, M. A. Shapiro, and S. A. Wold.** 1988. Enoxacin: in-vitro and animal evaluation as a parenteral and oral agent against hospital bacterial isolates. J. Antimicrob. Chemother. **21**(Suppl. B):29–42.
10. **Hooper, D. C., and J. S. Wolfson.** 1985. The fluoroquinolones: pharmacology, clinical uses, and toxicities in humans. Antimicrob. Agents Chemother. **28**:716–721.
11. **Jacobs, M. R., H. J. Koornhof, R. M. Robins-Browne, C. M. Stevenson, Z. A. Vermaak, I. Freiman, G. B. Miller, M. A. Witcomb, M. Isaacson, J. I. Ward, and R. Austrian.** 1978. Emergence of multiply resistant pneumococci. N. Engl. J. Med. **299**:735–740.
12. **Jetté, L. P., F. Lamothe, and the Pneumococcus Study Group.** 1989. Surveillance of invasive *Streptococcus pneumoniae* infection in Quebec, Canada, from 1984 to 1986: serotype distribution, antimicrobial susceptibility, and clinical characteristics. J. Clin. Microbiol. **27**:1–5.
13. **Jones, R. N., and A. L. Barry.** 1988. Antimicrobial activity, spectrum, and recommendations for disk diffusion susceptibility testing of ceftibuten (7432-S; SCH 39720), a new orally administered cephalosporin. Antimicrob. Agents Chemother. **32**: 1576–1582.
14. **Jorgensen, J. H., J. S. Redding, and L. A. Maher.** 1988. Influence of storage and susceptibility test conditions on stability and activity of LY 163892 and four other cephalosporins. Antimicrob. Agents Chemother. **32**:1477–1480.
15. **Knapp, C. C., and J. A. Washington II.** 1988. In vitro activity of LY 163892, cefaclor, and cefuroxime. Antimicrob. Agents Chemother. **32**:131–133.
16. **Leitner, F., T. A. Pursiano, R. E. Buck, Y. H. Tsai, D. R. Chisholm, M. Misiek, J. V. Desiderio, and R. E. Kessler.** 1987. BMY 28100, a new oral cephalosporin. Antimicrob. Agents Chemother. **31**:238–243.
17. **National Committee for Clinical Laboratory Standards.** 1985. Approved standard M7-A. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **National Committee for Clinical Laboratory Standards.** 1984. Approved standard M2-A3. Performance standards for antimicrobic disk susceptibility tests, 3rd ed. National Committee for Clinical Laboratory Standards. Villanova, Pa.
19. **Phillips, I., and A. King.** 1988. Comparative activity of the 4-quinolones. Rev. Infect. Dis. **10**(Suppl. 1):S70–S76.
20. **Phillips, I., A. King, and K. Shannon.** 1988. In vitro properties of the quinolones, p. 83–113. *In* V. T. Andriole (ed.), The quinolones. Academic Press, Inc., London.
21. **Van der Auwera, P., P. Grenier, Y. Glupczynski, and D. Pierard.** 1989. In vitro activity of lomefloxacin in comparison with pefloxacin and ofloxacin. J. Antimicrob. Chemother. **23**:209–219.
22. **Vellend, H.** 1989. Role of fluoroquinolones in lower respiratory tract infections. Clin. Invest. Med. **12**:39–43.
23. **Walker, R. C., and A. J. Wright.** 1987. The quinolones. Mayo Clin. Proc. **62**:1007–1012.
24. **Ward, J.** 1981. Antibiotic-resistant *Streptococcus pneumoniae*: clinical and epidemiologic aspects. Rev. Infect. Dis. **3**:254–266.
25. **Wolfson, J. S., and D. C. Hooper.** 1985. The fluoroquinolones: structures, mechanisms of action and resistance and spectrum of activity in vitro. Antimicrob. Agents Chemother. **28**:581–586.