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NOTES

In Vitro Susceptibility of *Haemophilus influenzae* to Cefixime

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The in vitro activity of cefixime against 2,458 clinical isolates of *Haemophilus influenzae* was determined. All the strains were inhibited by ≤2 μg of cefixime per ml, and the modal MIC was 0.03 μg/ml. Activity was unaffected by the presence of β-lactamase produced by 157 isolates. Nineteen of the twenty-four isolates for which cefixime MICs were ≥0.5 μg/ml were β-lactamase negative but showed reduced susceptibility to ampicillin.

With the advent of β-lactamase-producing *Haemophilus influenzae* strains in the 1970s (13) and the rising prevalence of such strains in the United Kingdom over the last decade (4, 8, 9) (currently 5.8% in noncapsulate and 18% in type b isolates), there has been an increasing need to develop alternative oral antimicrobial agents with low toxicity for the treatment of noninvasive infections caused by this species.

In the early 1980s, a new cephem was developed which showed broad-spectrum anti-gram-negative-bacterial activity and which was active after oral administration in animal studies (5). Its activity in the presence of a wide range of β-lactamases, including TEM-1, was unimpaired, and in vitro testing showed high activity against a small number of *H. influenzae* isolates (7). This compound, cefixime, has now been tested against 2,458 clinical isolates of *H. influenzae* collected during 1986 from 23 laboratories in the United Kingdom.

Ampicillin (Beecham Pharmaceuticals, Brentford, United Kingdom) and cefixime (Cyanamid of Great Britain Ltd., Richmond, United Kingdom) were supplied as standard laboratory powders of known potencies.

Isolates were collected from 23 laboratories in the United Kingdom, and the identity of the isolates was confirmed by the criteria of XV dependence, a negative porphyrin production test (6), and CO_2 independence. Five-hour cultures of each isolate in 1 ml of nutrient broth (Oxoid Ltd., Basingstoke, United Kingdom) containing 5% (vol/vol) inactivated Fildes extract (Oxoid) were diluted 1/100 in peptone water. A Denley multipoint inoculator was used to deliver 0.003-ml volumes (i.e., approximately 10^4 CFU per spot) to the surface of DST (Oxoid) agar plates supplemented with 0.25% (vol/vol) lysed horse blood and 10 μg of NAD (BDH Chemicals, Poole, United Kingdom) per ml and containing doubling dilutions of the antibiotics from 256 to 0.008 μg of ampicillin and 16 to 0.008 μg of cefixime per ml. The MIC was defined as the lowest concentration of antibiotic necessary to prevent visible growth.

Ampicillin (2-μg) and cefixime (30-μg) disk testing was performed by swab inoculation (from the peptone water suspension) of the same supplemented agar without antibiotic. Isolates found to show zone diameters of ≥20 mm around 2-μg ampicillin disks were subjected to 2-μg/ml ampicillin plates only to screen for inhibition of growth by this concentration. The plates were incubated for 18 h at 37°C in 5% CO_2–95% air.

All strains that showed zones of <20 mm were tested for β-lactamase production by an acidometric method (Oxoid) and a cell suspension iodometric method (2).

To facilitate analysis of results, the strains were divided into four groups according to zone sizes around 2-μg ampicillin disks (12) and MICs of ampicillin. By the criteria adopted in three United Kingdom studies which used identical susceptibility testing methods (4, 8, 9), 2,201 isolates were considered ampicillin susceptible (2,169 showed zones of ≥20 mm and MICs of ≤2 μg/ml, and 32 showed zones of <20 mm and MICs of 0.25 to 0.5 μg/ml), 157 isolates were β-lactamase positive, and 100 isolates were β-lactamase negative and showed reduced zones (<20 mm); for 38 of the latter, ampicillin MICs were ≥4 μg/ml, and for 62 ampicillin MICs were 1 or 2 μg/ml. These 100 strains were considered to possess some degree of non-enzyme-mediated (i.e., intrinsic) resistance to ampicillin. The modal MIC of cefixime was 0.03 μg/ml, with a range of ≤0.008 to 2 μg/ml (Table 1). Ampicillin-susceptible and β-lactamase-positive, ampicillin-resistant isolates showed very similar in vitro susceptibilities to cefixime, with the same modal MICs.

When the proportions of strains in each group for which MICs were ≤0.25 or >0.25 μg/ml (i.e., more than three dilution steps above the modal MIC) were compared, a highly significant difference ($P < 0.001$) was found between the ampicillin-susceptible strains and both groups of strains with intrinsic resistance. Of the 24 strains for which cefixime MICs were ≥0.5 μg/ml, 19 showed nonenzymic reduced

TABLE 1. MICs of cefixime for four groups delineated by susceptibility to ampicillin

Group (no. of isolates)	MIC (μg/ml)[a]		
	Mode	50%	90%
Ampicillin susceptible (2,201)	0.03	0.03	0.12
β-Lactamase positive (157)	0.03	0.03	0.06
β-Lactamase negative (zone diam, <20 mm; MIC, 1–2 μg/ml) (62)	0.06	0.06	0.5
β-Lactamase negative (zone diam, <20 mm; MIC, ≥4 μg/ml) (38)	0.25	0.12	0.5

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

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susceptibility to ampicillin, 11 of them requiring ≥4 μg and 8 of them requiring 1 or 2 μg of ampicillin per ml for inhibition. The remaining five strains were β-lactamase negative and showed zones of <25 mm; for three of them ampicillin MICs were 2 μg/ml and for two of them ampicillin MICs were 0.5 μg/ml.

Zone diameters around the 30-μg cefixime disks ranged from 21 to 49 mm. The coefficient of correlation between zone size and MIC (Spearman's correlation) was very poor, at −0.13 overall. Correlation was not improved by determining the coefficient for each of the four groups in turn.

As predicted by previous studies of its β-lactamase stability (7), there were no significant differences in susceptibilities to cefixime between the ampicillin-susceptible and β-lactamase-positive groups. Despite the comparative reduction in susceptibility shown by a minority of isolates, mainly β-lactamase-negative strains with reduced susceptibility to ampicillin, all 2,458 strains were inhibited by ≤2 μg of cefixime per ml.

The poor predictive value of the 30-μg cefixime disk with respect to MIC agrees with the observations of Fuchs et al. (3), whose data suggest that a 5-μg disk provides better discrimination. These researchers proposed that MICs of ≤1 and ≥4 μg/ml should be taken as susceptible and resistant breakpoints, respectively. Using these criteria, 2,454 isolates would be susceptible and 4 would be in the intermediate susceptibility range.

Pharmacokinetic studies in humans with cefixime by Brittain et al. (1) showed that 200 mg orally produced a mean peak concentration in serum of 2.63 μg/ml and levels at 8 h still in excess of 1 μg/ml and that a 400-mg dose produced a mean peak of 3.85 μg/ml. In addition, cefixime differed from other oral cephalosporins in having a serum half-life of 3 h (more than three times that of cefaclor and cephalexin) (1). Animal studies have suggested that considerable tubular reabsorption and serum protein binding are responsible for the prolongation of half-life (11) and that the low urinary recovery rate of cefixime is due to poor intestinal absorption and not to metabolism (10).

On the basis of in vitro susceptibility testing and what is known about the pharmacokinetics of this agent, it appears that cefixime is likely to be suitable for the treatment of noninvasive *H. influenzae* infections whether or not the organism responsible produces β-lactamase. More information from clinical trials is needed before it can be confidently described as possessing in vivo activity superior to those of other β-lactams available for oral use despite its marked β-lactamase stability. Its efficacy in infections caused by organisms for which MICs are at the upper end of the observed range will be of particular interest. Most of these isolates show some degree of intrinsic resistance to ampicillin, and a proportion have reduced susceptibility to a wide range of β-lactams. The therapeutic importance of this phenomenon depends on the ability of these agents to achieve the necessary concentrations for appropriate lengths of time at the infected sites.

LITERATURE CITED

1. **Brittain, D. C., B. E. Scully, T. Hirose, and H. C. Neu.** 1985. The pharmacokinetic and bactericidal characteristics of oral cefixime. Clin. Pharmacol. Ther. **38**:590–594.
2. **Catlin, B. W.** 1975. Iodometric detection of *Haemophilus influenzae* beta-lactamase: rapid presumptive test for ampicillin resistance. Antimicrob. Agents Chemother. **7**:265–270.
3. **Fuchs, P. C., A. L. Barry, and R. N. Jones.** 1986. Cefixime disk susceptibility test criteria. J. Clin. Microbiol. **24**:647–649.
4. **Howard, A. J., C. J. Hince, and J. D. Williams.** 1978. Antibiotic resistance in *Streptococcus pneumoniae* and *Haemophilus influenzae*. Br. Med. J. **1**:1657–1660.
5. **Kamimura, T., H. Kojo, Y. Matsumoto, Y. Mine, S. Goto, and S. Kuwahara.** 1984. In vitro and in vivo antibacterial properties of FK 027, a new orally active cephem antibiotic. Antimicrob. Agents Chemother. **25**:98–104.
6. **Kilian, M.** 1974. A rapid method for the differentiation of Haemophilus strains. Acta Pathol. Microbiol. Scand. **82**:835–842.
7. **Neu, H. C., N.-X. Chin, and P. Labthavikul.** 1984. Comparative in vitro activity and β-lactamase stability of FR 17027, a new orally active cephalosporin. Antimicrob. Agents Chemother. **26**:174–180.
8. **Philpott-Howard, J., and J. D. Williams.** 1982. Increase in antibiotic resistance in *Haemophilus influenzae* in the United Kingdom since 1977: report of study group. Br. Med. J. **284**: 1597–1601.
9. **Powell, M., C. Koutsia-Carouzou, D. Voutsinas, A. Seymour, and J. D. Williams.** 1987. Resistance of clinical isolates of *Haemophilus influenzae* in United Kingdom 1986. Br. Med. J. **295**:176–179.
10. **Sakamoto, H., T. Hirose, and Y. Mine.** 1985. Pharmacokinetics of FK 027 in rats and dogs. J. Antibiot. **28**:496–504.
11. **Sakamoto, H., T. Hirose, S. Nakamoto, and Y. Mine.** 1985. Mechanism of renal excretion of FK 027 in dogs and rabbits. J. Antibiot. **28**:1088–1095.
12. **Williams, J. D., and S. Kattan.** 1978. Haemophilus species, p. 106–111. *In* D. S. Reeves, I. Phillips, J. D. Williams, and R. Wise (ed.), Laboratory methods in antimicrobial chemotherapy. Churchill Livingstone, Ltd., Edinburgh.
13. **Williams, J. D., S. Kattan, and P. Cavanagh.** 1974. Penicillinase production by *Haemophilus influenzae*. Lancet **ii**:103.