Comparison of the Epidemiology of Bacterial Resistance to Mecillinam and Ampicillin

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Mecillinam is a new type of β -lactam antibiotic (an amidinopenicillanic acid) that is particularly active against *Enterobacteriaceae* and is taken orally as in the form of an ester, pivmecillinam. Assessment of any new antibiotic should include a survey of levels of bacterial resistance and investigation of its capacity to select resistant organisms or harm the commensal flora. Antibiotic resistance patterns of 2,000 *Enterobacteriaceae* isolated from the urine of patients with significant urinary tract infections were therefore determined. Mecillinam-resistant *Enterobacteriaceae* were found to be much less common than ampicillin-amoxycillin-resistant organisms both in the community and in hospital patients. Most ampicillin-resistant *Enterobacteriaceae* from infected urines were susceptible to mecillinam, but the relatively rare mecillinam-resistant organisms were usually resistant to ampicillin and cephaloridine. The fecal flora of 26 healthy volunteers who served as controls or were given repeated courses of therapeutic doses of either ampicillin or pivmecillinam was studied. Pivmecillinam had only a transient effect on the aerobic fecal flora and in contrast to ampicillin did not increase populations of resistant *Enterobacteriaceae*, which would be a potential hazard to the patient and contaminate the environment.

Although the development of bacterial resistance has often reduced the benefits of chemotherapy (1, 11), relatively little information is available for clinicians on the effect of specific antibiotics or regimens on the selection of resistant commensals or pathogens. Gram-negative bacteria in the gut are of great importance, since they form a large reservoir from which resistant organisms arising by cross-infection, mutation, or antibiotic resistance (R) factor transfer may be disseminated. Resistant gut bacteria may be selected every time a patient is treated with an antibiotic. These resistant populations present a potential hazard to the patient and increase levels of resistance in the environment. The ideal antibiotic, while effective in curing infection, should not select resistant organisms or harm the commensal flora. There is, therefore, a need to identify effective antibiotics and antibiotic regimens which are least likely to do harm. We have studied the epidemiology of bacterial resistance to a novel antibiotic that may well be used widely in treating urinary tract infections (6, 7, 30, 31) employing antibiotic regimens suitable for these infections.

This drug (mecillinam, a 6 β -amidinopenicillanic acid derivative) is a penicillin analogue

that is particularly active against gram-negative bacteria and differs from other penicillins and the cephalosporins in that the β -lactam nucleus is linked to the side chain by an amidino instead of an amido group (17). For oral therapy it must be given as its pivavoyloxymethyl ester (pivmecillinam), which is well absorbed and, although lacking antibacterial activity, is rapidly hydrolyzed to mecillinam by blood esterases (23). Although mecillinam has not yet been introduced into general use, its structural resemblance to the cephalosporins and penicillins raises the possibility that these β -lactam antibiotics may have already selected a high level of resistance to mecillinam in bacteria. Furthermore, slow growing, mecillinam-resistant phenotypic variants may arise in vitro when *Enterobacteriaceae* are grown under certain conditions in the presence of mecillinam (12, 17, 18, 29). These variants lose their antibiotic resistance when subcultured in the absence of mecillinam but could be a potential cause of treatment failure in vivo.

MATERIALS AND METHODS

Determination of MICs of antibiotics. Cephaloridine was obtained in the anhydrous form from vials of Ceporin injection (Glaxo Laboratories Ltd.). Am-

picillin (as the trihydrate) was a gift from Beecham Research Laboratories Ltd. Mecillinam (as the hydrochloride dihydrate) was a gift from Leo Laboratories Ltd. Oxoid (Oxoid Ltd., Basingstoke, Hants) diagnostic susceptibility test agar (CM 261) was supplemented with 5% saponin-lysed oxalated horse blood for minimal inhibitory concentration (MIC) determinations.

Doubling dilutions of the antibiotics in lysed blood agar (2 to 1,024 $\mu\text{g/ml}$) were prepared by adding suitable volumes of stock solutions to agar cooled to 45°C. Plates were allowed to dry in a laminar air flow cabinet for 15 min and then inoculated with small volumes (0.002 ml) of a range of dilutions of saline suspensions of bacteria prepared from colonies taken from 18-h agar plate cultures. Bacterial suspensions that were sufficiently dense to form discrete colonies on antibiotic-free control media after incubation at 37°C for 18 h were selected for estimation of MICs.

Survey of urinary *Enterobacteriaceae*. Populations of viable bacteria in all urines submitted to the hospital laboratory with a request for bacteriologic examination were determined by the "calibrated loop" technique on MacConkey agar (CM 7, Oxoid Ltd.). Organisms isolated from infected urines were generally classified by their colonial appearance and by simple biochemical reactions. All mecillinam-resistant organisms and a random selection of 100 other isolates were identified in greater detail (8).

Antibiotic susceptibility patterns of all organisms from significant urinary infections, i.e., catheter specimens containing $\geq 10^3$ organisms per ml and all other specimens containing $\geq 10^5$ cells per ml of a single organism, were determined by the antibiotic paper disk test (method A, reference 28) on Welcotest agar (Wellcome Foundation Ltd., Beckenham, Kent). Susceptibility to mecillinam was determined with disks provided by Leo Laboratories Ltd. and to other antibiotics with a suitable "polydisc" (Scientific Hospital Supplies Ltd., Liverpool 7). An amoxycillin disk on the polydisc used in our routine laboratory was, for convenience, used to determine the susceptibility of organisms to ampicillin. Beecham Pharmaceuticals Research Division, Brockham Park, Surrey (R. Sutherland, Senior Research Bacteriologist, private communication) recommends that amoxycillin 25- μg disks may be used in routine laboratories to determine the susceptibility of urinary pathogens to both amoxycillin and ampicillin. Furthermore, in an independent survey, susceptibility test results with ampicillin and amoxycillin were the same for 56 out of 57 urinary *Enterobacteriaceae* (5).

Investigation of the aerobic fecal flora. Eight healthy volunteers who served as controls were given no antibiotics. Thirteen others were given 400 mg of pivmecillinam four times daily for 5 days (as pivmecillinam hydrochloride capsules, 200 mg, equivalent to 68% anhydrous mecillinam; Leo Laboratories Ltd.). The majority of these individuals were given a second course of antibiotic and some a third course. Five more volunteers were each given two courses of 250 mg of ampicillin (as Penbritin capsules, Beecham Research Laboratories) four times daily for 5 days.

The aerobic fecal flora for all volunteers were studied quantitatively on MacConkey agar and on MacConkey agar containing either 10 μg of mecillinam per ml or 10 μg of ampicillin per ml (2). Coliforms (lactose-fermenting, bile-tolerant, gram-negative bacilli), non-lactose-fermenting *Enterobacteriaceae*, *Pseudomonas* spp., and fecal streptococci were enumerated separately. The antibiotic susceptibility patterns of a representative selection of resistant *Enterobacteriaceae* from each plate were confirmed by the antibiotic disk susceptibility test method (28) on Welcotest agar. The ability of the fecal strains of *Proteus* to transfer resistance was determined by a method similar to that used with other antibiotics (2).

RESULTS

Survey of urinary *Enterobacteriaceae*. Antibiotic disk susceptibility tests showed that mecillinam resistance was much less common than ampicillin-amoxycillin resistance both in the community and in hospital patients. Ampicillin-amoxycillin-resistant organisms were usually susceptible to mecillinam, but mecillinam-resistant organisms were usually resistant to these antibiotics and cephaloridine and frequently to other antibiotics (Table 1). Out of a random sample of 100 of the urinary *Enterobacteriaceae*, 85% were *Escherichia coli*, 7% were *Proteus* sp., 5% were *Klebsiella* sp., 3% were *Citrobacter* sp., and 2% were *Enterobacter* sp. In contrast, of the 37 mecillinam-resistant organisms, 59% were *Klebsiella* sp. (all but two isolates were a single serotype of *Klebsiella pneumoniae*), 28% were *Proteus* sp., and 16% were *E. coli*. The majority of mecillinam-resistant organisms had MICs of mecillinam and ampicillin on lysed blood agar medium of $>1,024$ $\mu\text{g/ml}$ and of between 128 and 256 $\mu\text{g/ml}$ for cephaloridine. The MICs of mecillinam-resistant urinary *Proteus* isolates varied widely on other media.

Effect of therapeutic doses of pivmecillinam upon the fecal flora. Out of 34 specimens of feces obtained from 13 healthy individuals over a period of 9 days, mecillinam-resistant *Enterobacteriaceae* were only found in two samples from a single individual. The fecal flora of these individuals was then monitored during and after a 5-day course of pivmecillinam. There was no significant increase in a mecillinam-resistant *Proteus rettgeri* which had been present before chemotherapy (about $3 \times 10^5/\text{g}$) in the feces of the single individual, and this organism disappeared within a week after completion of the course. No mecillinam-resistant coliforms or *Proteus* were detected in the feces of any of the remaining 12 individuals who had been treated with this antibiotic.

TABLE 1. Antibiotic resistance pattern of 2,000 *Enterobacteriaceae* isolated from patients with significant^a urinary tract infections in the York Health District

Source of urine specimens	No. of organisms examined	% Organisms susceptible to all antibiotics tested	% Organisms resistant by the disk antibiotic susceptibility test with disks containing:							
			Mecil- linam (10 µg)	Amoxycillin (for ampicillin, 25 µg)	Cepha- loridine (25 µg)	Sulpha- triad (300 µg)	Trimeth- oprim (2.5 µg)	Nitro- furantoin (200 µg)	Colistin (10 µg)	Genta- micin (10 µg)
General practice and antenatal patients	705	63.0	0.4	17.7	-	17.3	9.9	5.2	-	0.1
Hospital outpatients	227	53.3	0.4	26.9	-	26.0	9.7	4.0	-	0
Hospital inpatients	1,068	38.4	3.1	37.5	-	27.9	23.9	13.7	-	0.2
All mecillinam-resistant isolates	37	0	100	91.9	78.4	70.3	67.6	35.1	29.7	0

^a See Materials and Methods for definition of significance.

Eleven of the 13 persons, including the one who had harbored the resistant *Proteus*, were given a second course of pivmecillinam about 3 weeks after completion of the first course, and two were given a third course. Repeated courses of pivmecillinam were given to increase the chances of detecting resistant organisms arising during antibiotic treatment with a regimen intended for urinary infections. No mecillinam-resistant *Enterobacteriaceae* were found in any of the individuals given two or more courses of pivmecillinam. Thus, out of a total of 206 fecal samples from individuals who had received no antibiotics, 100 samples from individuals given ampicillin in the trial described below, and 254 samples from 13 persons given one or more courses of pivmecillinam, the only mecillinam-resistant enterobacteria found was the *P. rettgeri* previously discussed. This organism was sensitive to the common urinary antibiotics other than colistin, and its mecillinam resistance could not be transferred in vitro to a laboratory strain of *E. coli*.

In some individuals, fecal coliform populations fell during treatment with pivmecillinam and then returned to normal levels (Table 2, columns 3 to 5). Although large individual variations in the fecal flora preclude a firm conclusion, pivmecillinam did not appear to select ampicillin-resistant coliforms (Table 2, column 6), *Proteus* sp. or *Pseudomonas* sp., but did transiently increase populations of fecal streptococci, which were resistant to mecillinam (MIC $\geq$ 256 µg/ml).

Changes in the fecal flora in 33 specimens obtained from two individuals given a third course of pivmecillinam resembled the pattern found during the first two courses of treatment with this antibiotic.

Effect of therapeutic doses of ampicillin upon the fecal flora. Small numbers of ampicillin-resistant organisms were found in the feces of five individuals prior to treatment with this agent (Table 2, column 8). The fecal flora of these individuals was then monitored during and after two separate courses of ampicillin in therapeutic amounts. In contrast to pivmecillinam, this antibiotic exerted a strong selection pressure for ampicillin-resistant organisms. These two courses of ampicillin did not appear to have any marked effect upon total populations of coliforms, *Pseudomonas* sp., *Proteus* sp., or fecal streptococci.

Side effects of antibiotics. No adverse effects were noted in any of the five individuals given ampicillin. One individual receiving pivmecillinam complained of persistent mild dyspepsia and declined to take a second course of treatment. A second person complained of

TABLE 2. Effect of two courses of therapeutic doses of pivmecillinam or ampicillin upon the fecal flora of healthy volunteers

Time of sampling	Volunteers given pivmecillinam				Volunteers given ampicillin	
	No. of specimens of feces examined	% of specimens with counts of <i>Enterobacteriaceae</i> (per g of feces) of:			No. of specimens of feces examined	Mean % of ampicillin-resistant <i>Enterobacteriaceae</i>
		<10 ⁴	10 ⁴ -10 ⁶	>10 ⁶		
Days 1-9	34	0	9	91	12	<0.1
During first 5-day course of treatment	37	16	51	32	16	55
Days 15-19	32	9	28	63	5	56
Days 20-24	20	0	30	70	11	12
Days 25-29	16	0	13	87	6	0.2
Days 30-34	22	0	9	91	8	<0.1
During second 5-day course of treatment	30	43	17	40	15	51
Days 40-44	20	40	45	15	16	72
Days 45-49	19	10	16	74	5	0.2
Days 50-54	15	0	20	80	6	0.7
Days 55-59	10	0	20	80	12	2.5

occasional slight dyspepsia during the first course of treatment but discontinued treatment on day 1 of the second course because of dyspepsia and nausea. A third individual who had suffered with dyspepsia during previous treatment with ampicillin or tetracycline complained of mild occasional dyspepsia during the first course of treatment, but none during the second course when the tablets had been taken with milk or food. Alcoholic drinks may have exacerbated or caused some of the incidents of dyspepsia. No other side effects were noted, and all individuals were questioned to ensure that they had not suffered from rashes or diarrhea and had taken the capsules as directed.

DISCUSSION

Levels of antibiotic resistance in the community and in hospitals influence the primary choice of antibiotic and may determine the outcome of treatment. Populations of resistant organisms tend to increase with antibiotic use (1, 11, 15) and to fall when antibiotics are withdrawn (3, 15, 16, 20, 25). Available evidence indicates that antibiotics differ in their capacity to select resistant organisms, e.g., ampicillin (2, 9, 13, 25) and tetracycline (2, 9, 10, 13, 24, 25), which tend to increase numbers of resistant organisms in the feces, while co-trimoxazole (19, 21, 27) or amoxycillin (25) do not.

In contrast to ampicillin and some other antibiotics, mecillinam resistance was uncommon in fecal and urinary *Enterobacteriaceae* in the present study. Mecillinam-resistant phenotypic variants of susceptible organisms which may be formed under certain conditions in the labora-

tory (12, 17, 18, 29) were not found in this or in another (14) study of the fecal flora. However, other regimens might have a different effect upon the gut flora, and mecillinam-resistant *Enterobacteriaceae* were found in the feces of 7 out of 12 chronic salmonella carriers who had been given one or more 28-day courses of pivmecillinam. Some of this resistance was multiple and some was R-factor mediated, but all mecillinam-resistant organisms disappeared from the feces within 1 to 4 weeks of completion of treatment (14). Similarly, mecillinam-resistant *Enterobacteriaceae* were found during and after treatment in two out of six patients with acute salmonellosis who had been given pivmecillinam for 14 days (14). These studies with salmonella infections may not be representative of conditions found in the treatment of other infections, and no resistant organisms were found in the urine when pivmecillinam was used in four clinical trials of the treatment of urinary tract infections (6, 7, 30, 31).

The broad spectrum of mecillinam is consistent with experimental evidence that it has a different mode of action (18, 29) and greater resistance to β -lactamases than ampicillin (22, 29).

Although the outcome of therapy depends on factors other than the results of laboratory susceptibility tests, the survey summarized in Table 1 suggests that one may expect pivmecillinam to be effective against the majority of urinary tract infections caused by *Enterobacteriaceae*. Results of these disk susceptibility tests with urinary pathogens are in general agreement with a recent detailed survey of MIC and cidal concentrations of mecillinam for *En-*

terobacteriaceae isolated from hospital patients (22). All mecillinam-resistant *Klebsiella* infections occurred in hospital patients and most took place over a short period of time in catheterized patients in two surgical wards. Most of the resistant organisms had the same antibiogram, and there was other evidence that this relatively high level of resistance in a small number of patients was due to cross-infection by a single strain. As with many other hospital infections, control of cross-infection would appear to be more relevant than chemotherapy.

Therapeutic doses of pivmecillinam appeared to have only a transient effect upon the aerobic fecal flora, which generally returned to normal levels within a week of stopping treatment. No attempts were made to monitor the effect of pivmecillinam upon the anaerobic fecal flora. Although *Bacteroides fragilis* may inhibit antibiotic resistance transfer between coliforms in the laboratory (4), there is no evidence that anaerobes play a direct role in such transfer in the gut. Furthermore, *B. fragilis* has been shown to be resistant to mecillinam (22).

Upper gastrointestinal tract symptoms noted in three individuals in this relatively small investigation have only been reported in one other trial (6) but obviously deserve further consideration.

One cannot exclude the possibility that there will be a slow buildup of resistance when pivmecillinam is used on a larger scale. However, the evidence available so far indicates that if pivmecillinam is used for treating urinary infection, it would be unlikely to produce long-term changes in the fecal flora or promote the development of resistant organisms which might effect the patient or the environment.

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