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Brief Reports

In-vitro activity of 21 β-lactam antibiotics against penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae*

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MICs of 21 β-lactams were determined by agar dilution against 283 penicillin-susceptible (pen-S), 122 intermediate (pen-I) and 23 fully penicillin-resistant (pen-R) pneumococci. MICs of all β-lactams increased with increasing MICs of penicillin. Clometocillin was the most active penicillin against pen-I or pen-R pneumococci. All oral cephalosporins except cefuroxime and cefpodoxime were less active than penicillin and none was satisfactory against pen-I or pen-R pneumococci. The parenteral third- and fourth-generation cephalosporins (except ceftazidime) were similar in activity to penicillin against pen-S isolates. Cefpirome showed the lowest mean MICs against pen-I and pen-R strains.

Introduction

Streptococcus pneumoniae remains an important bacterial pathogen in human infection. In Belgium the prevalence of penicillin resistance has increased significantly over the past three years, from 2.3% in 1993 to 10.5% in 1996. The aim of this study was to determine the MICs of old and new β-lactam antibiotics against penicillin-susceptible (pen-S), penicillin-intermediate (pen-I) and penicillin-resistant (pen-R) *S. pneumoniae*, recently isolated in Belgium from blood or other normally sterile sites.

Material and methods

Bacterial isolates

Isolates were collected by laboratories spread over the entire country and sent to this laboratory, which is the national reference centre for *S. pneumoniae.* The strains were from blood or pleural fluid (75%), cerebrospinal fluid (7.5%), middle ear aspirates (14%), or from various other puncture sites (3.5%). Capsular types were determined by phase-contrast microscopy using Neufeld's reaction with sera commercially available from the Statens Seruminstitut (Copenhagen, Denmark).

All pen-I and pen-R strains (145) isolated in 1995 and 1996 and almost twice as many pen-S-strains (283) selected at random over the same period were studied.

Antimicrobial agents

Stock solutions of the following 21 β-lactam antiotics were prepared from powders with known potency supplied by representatives of the respective companies in Belgium: penicillin G (Continental Pharma, Brussels), clometocillin (Menarini, Brussels), ampicillin and amoxycillin (SmithKline–Beecham, Genval), piperacillin (Wyeth-Lederle, Louvain-la-Neuve), imipenem (Merck, Sharp & Dohme, Brussels), meropenem (Zeneca, Destelbergen), cefotaxime, cefpirome, cefpodoxime and cefixime (Hoechst-Marion-Roussel, Brussels), ceftriaxone and cefetamet (Hoffman–LaRoche, Brussels), ceftazidime and cefuroxime (Glaxo–Wellcome, Brussels), cefepime, cefadroxil, cephradine and cefatrizine (Bristol–Myers Squibb, Brussels), cefaclor (Eli Lilly, Brussels), and ceftibuten (Schering–Plough, Brussels).

Susceptibility to penicillin was defined according to NCCLS criteria as follows: susceptible, MIC $\leqslant$ 0.06 mg/L; intermediate, MIC = 0.12–1 mg/L; resistant, MIC $\geqslant$ 2 mg/L.[1]

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Table I. Susceptibility of pneumococci to β-lactam antibiotics

Antibiotic	*n*	Cumulative % of strains inhibited at concentration (mg/L) of											Geometric mean MIC (mg/L)
		≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	
Penicillins													
penicillin G													0.052
pen-S	283	43.8	90.1	99.3	100								
pen-I	122					7.4	13.9	29.5	100				
pen-R	23									100			
clometocillin													0.049
pen-S	210	60.9	95.7	97.6	98.1	100							
pen-I	122				0.8	13.9	24.6	94.3	100				
pen-R	23							47.8	100				
ampicillin													0.135
pen-S	283	14.5	33.2	64	98.2	98.6	100						
pen-I	122				0.8	9	13.1	13.9	18.8	35.2	94.2	100	
pen-R	23										30.4	100	
amoxycillin													0.069
pen-S	283	17.3	77.7	98.2	98.5	99.6	100						
pen-I	122			1.6	8.2	12.3	17.2	25.4	49.2	94.3	100		
pen-R	23									52.2	100		
piperacillin													0.134
pen-S	283	4.2	21.9	89.4	97.2	97.9	99.3	100					
pen-I	122						6.6	11.5	18	41	98.4	100	
pen-R	23										91.3	100	
Carbapenems													
imipenem													0.021
pen-S	283	96.1	98.9	99.3	100								
pen-I	122	1.6	7.4	13.9	23.8	66.4	97.5	100					
pen-R	23					4.3	86.9	100					
meropenem													0.029
pen-S	283	79.5	98.2	99.6	99.6	100							
pen-I	122		4.1	9.8	13.1	16.4	62.3	95.1	100				
pen-R	23							100					
Oral cephalosporins													
cephradine													2.155
pen-S	283					2.8	5.6	17.3	95.4	98.9	100		
pen-I	122							0.8	4.1	8.2	10.7	16.4	
pen-R	23												
cefadroxil													1.594
pen-S	283					2.5	28.6	56.2	98.2	100			
pen-I	122								4.9	9	10.8	16.4	
pen-R	23												
cefaclor													0.783
pen-S	283					37.8	92.9	99.6	100				
pen-I	12							5.7	10.7	11.5	17.2	19.7	
pen-R	23												
cefatrizine													0.283
pen-S	283		2.8	12.4	77.4	96.8	98.6	100					
pen-I	122					0.8	8.2	13.9	17.2	23.8	52.5	86.9	
pen-R	23										8.7	47.8	
cefuroxime													0.083
pen-S	283	30.7	83	92.2	97.5	100							
pen-I	122				1.6	3.3	11.5	16.4	20.5	46.7	97.5	100	
pen-R	23										91.8	100	

Table I. *Continued*

Antibiotic	*n*	Cumulative % of strains inhibited at concentration (mg/L) of ≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	Geometric mean MIC (mg/L)
cefpodoxime													0.087
pen-S	283	11.6	59.7	95.4	97.9	99.6	100						
pen-I	122				0.8	9.8	12.3	14.7	33.6	89.3	100		
pen-R	23									26.1	95.6	100	
cefetamet													1.168
pen-S	283					9.2	59.7	80.6	98.6	99.6	100		
pen-I	122							0.8	9	13.1	13.9	17.2	
pen-R	23												
cefixime													1.570
pen-S	215					31.1	37.2	64.6	91.1	98.6	100		
pen-I	117						0.8	0.8	6.8	11.1	12.8	23.1	
pen-R	23												
ceftibuten													7.131
pen-S	283								24.8	58.3	93.3	96.8	
pen-I	122												
pen-R	23												
Parenteral cephalosporins													
cefotaxime													0.045
pen-S	283	43.1	90.5	98.9	100								
pen-I	122			0.8	10.7	12.3	23.8	55.7	99.2	100			
pen-R	23							4.3	82.6	100			
ceftriaxone													0.041
pen-S	283	59.4	90.1	97.5	99.3	100							
pen-I	122			2.5	9.8	11.5	16.4	77.9	99.2	100			
pen-R	23						4.3	17.4	95.6	100			
cefpirome													0.046
pen-S	283	16.2	85.9	94.7	100								
pen-I	122			2.5	8.2	13.1	37.7	99.2	100				
pen-R	23							78.3	95.6	95.6	100		
cefepime													0.071
pen-S	283	4.6	55.1	90.5	98.6	99.6	100						
pen-I	122			0.8	5.7	9.8	14.7	41.8	98.3	100			
pen-R	23							82.6	100				
ceftazidime													1.024
pen-S	210			0.5	6.7	39.5	90.5	98.6	99	100			
pen-I	122						0.8	1.6	1.6	15.6	16.4	25.4	
pen-R	23												

Determination of MICs

MICs were determined by the agar dilution method in Mueller–Hinton agar (Difco, Detroit, MI, USA) supplemented with 5% defibrinated sheep blood. The antimicrobial agents were incorporated into the agar in two-fold serial dilutions (range 0.008–8 mg/L). Suspensions with turbidity equivalent to that of a 0.5 McFarland standard were prepared from overnight blood agar cultures in sterile water and further diluted 1 in 10 to obtain 10^7 cfu/mL. Plates were inoculated with a multi-point inoculator delivering approximately 10^4 cfu per spot. *S. pneumoniae* ATCC 49619 was used for quality control. The agar plates were incubated at 36°C for 18 h in 5% CO_2. The MIC was defined at the lowest concentration of the agent that produced complete inhibition. A slight greening without growth was ignored.

Results

Results of susceptibility testing are presented in Table I as the range of MICs and the geometric mean MIC. Of the 428 strains tested, 283 were susceptible to penicillin, 122

were intermediate and 23 were resistant, all with an MIC of 2 mg/L. Within the penicillins, clometocillin was the most active, including against pen-I and pen-R strains, and ampicillin or piperacillin the least. The activity of amoxycillin and penicillin was similar for pen-S isolates, but amoxycillin was marginally more active against pen-I isolates.

The carbapenems were less affected in their activity than other β-lactams against pen-I and pen-R isolates.

With the exception of cefuroxime and cefpodixime, all other oral cephalosporins were at least 5 to 200 times (ceftibuten) less active than penicillin against pen-S strains. The parenteral third- and fourth-generation cephalosporins (except ceftazidime) were similar in activity to penicillin against pen-S pneumococci and showed slightly lower geometric mean MICs against pen-I and pen-R strains; the lowest mean MIC was that of cefpirome.

Based on the geometric mean MICs, the activities of the tested cephalosporins were as follows: ceftriaxone > cefotaxime > cefpirome > cefepime > cefuroxime > cefpodoxime > cefatrizine > cefaclor > ceftazidime > cefetamet > cefixime > cefadroxil > cephradine > ceftibuten.

Table II shows the serotype/group distribution of the 428 pneumococci and their susceptibility to penicillin. Serogroups 14, 23 and 9 were in rank order the most frequent among the pen-I and pen-R isolates accounting for 85%, and serogroups 15, 6 and 9 represented a further 12%.

Discussion

S. pneumoniae no longer has predictable antibiotic susceptibility in Belgium, since strains with increased resistance to penicillin and other β-lactam antibiotics have become more common. The resistance to penicillin in *S. pneumoniae* is entirely due to the development of altered forms of the high molecular weight penicillin-binding proteins (PBPs) that have a reduced affinity for these antibiotics.[2–4] The pneumococcus has at least five PBPs (1A, 1B, 2A, 2B and 2X). The affinity of the different β-lactams for each of the pneumococcal PBPs varies.[5] The aim of the present study was to compare the in-vitro activity of a wide range of β-lactam antibiotics.

Our in-vitro data confirm that ceftriaxone and cefotaxime are at present the empirical treatment of choice for systemic infections. All pen-S isolates had MICs of ceftriaxone of ⩽012 mg/L and cefotaxime of ⩽0.06 mg/L. Resistance to third-generation cephalosporins has typically been observed with pen-I isolates.[6] In the current study five isolates were resistant to cefotaxime and two to ceftriaxone (MIC = 2 mg/L). All these were resistant to penicillin (MIC = 2 mg/L) except one (MIC = 0.5 mg/L). The three that were resistant to cefotaxime but not to ceftriaxone, had ceftriaxone MICs of 1 mg/L. It is unlikely that these slight differences of in-vitro activity are meaningful in clinical conditions. Ceftazidime was eight- to 16-fold less active than ceftriaxone and its clinical utility might be limited to pen-S isolates. Cefpirome was twice as potent as cefotaxime and ceftriaxone against pen-R isolates. MICs of carbapenems (imipenem and meropenem) increased with the penicillin MIC; however, all isolates had MICs of ⩽1 mg/L.

Against pen-S isolates, the MICs of the oral β-lactams ampicillin, amoxycillin, cefuroxime and cefpodoxime were very similar. Clometocillin, an older semisynthetic analogue of penicillin V,[7] was marginally more active against pen-S isolates, but more active against pen-I and pen-R pneumococci, and might be an oral antibiotic of choice.

Apart from penicillin, there are currently no NCCLS-approved breakpoints for oral β-lactams for pneumococci. It is probably difficult to define reliable breakpoints but we disagree with the NCCLS recommendations that pneumococcal isolates susceptible to penicillin should be

Table II. Serotype distribution of *S. pneumoniae* isolates

Serotype/group	No. of isolates	Pen-S	Pen-I + Pen-R
1	25	25	0
2	2	2	0
3	30	30	0
4	11	11	0
5	15	15	0
6	39	33	6
7	13	13	0
8	11	11	0
9	50	20	30
10	3	3	0
11	3	3	0
12	6	6	0
13	2	1	1
14	81	26	55
15	10	4	6
16	1	1	0
18	8	7	1
19	41	36	5
20	4	3	1
22	5	5	0
23	49	11	38
24	7	6	1
31	1	1	0
32	1	1	0
33	6	5	1
35	1	1	0
38	2	2	0
39	1	1	0

considered susceptible to all other β-lactams. Antibiotics such as cephradine, cefadroxil, cefetamet, cefixime or ceftibuten with a MIC_{90} of > 1 mg/L against pen-S pneumococci can hardly be trusted to be clinically effective.

In addition to MICs, human pharmacokinetic data such as bioavailability and the time for which serum concentrations of unbound antibiotic are above MICs, are important. Calculations from published pharmacokinetic data[8] indicate that only clometocillin, amoxycillin, cefatrizine, cefuroxime and cefpodoxime have serum concentrations above the respective MIC_{90} of susceptible pneumococci for 6 h. In the treatment of pharyngitis, sinusitis and acute exacerbations of chronic bronchitis, there was a progressive switch from oral penicillins to oral cephalosporins with reduced doses or extended dose intervals for compliance reasons. This results in serum concentrations being below MICs for long periods. This switch has probably contributed to the selection of resistant pneumococci. In conclusion, antimicrobial resistance in *S. pneumoniae* is a serious problem in Belgium. By analogy based on experiences in other countries of Europe, this problem is likely to grow in the future.

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