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In-vitro activity of 29 antimicrobial agents against penicillin-resistant and -intermediate isolates of *Streptococcus pneumoniae*

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Antibiotic resistance among isolates of *Streptococcus pneumoniae* is increasing worldwide. Optimal therapy, though unknown, should be guided by in-vitro susceptibility testing. Currently, vancomycin is the only approved antibiotic that is universally active against multi-resistant *S. pneumoniae.* In-vitro activities were determined for 29 antimicrobial agents against 22 penicillin-intermediate *S. pneumoniae* (PISP) and 16 penicillin-resistant *S. pneumoniae* (PRSP) isolates. MICs were determined in cation-adjusted Mueller–Hinton broth with 3% lysed horse blood in microtitre trays. Antimicrobial classes tested included cephalosporins, penicillin, aminopenicillins, macrolides, quinolones, carbapenems and other antimicrobial agents. Among the classes of antimicrobial agents tested, wide differences in susceptibility were demonstrated for both PISP and PRSP. Of the cephalosporins, ceftriaxone and cefotaxime demonstrated the best in-vitro activity for both PISP and PRSP. Of the quinolones, clinafloxacin and trovafloxacin showed the greatest in-vitro activity. Rifampicin and teicoplanin demonstrated excellent in-vitro activity. Promising in-vitro results of newer agents, such as quinupristin/dalfopristin, ramoplanin, teicoplanin and linezolid may justify further evaluation of these agents in clinical trials.

Introduction

Streptococcus pneumoniae is a common pathogen causing pneumonia, sinusitis, acute otitis media and meningitis.[1] The increase in *S. pneumoniae* resistant to penicillin and other antimicrobial agents is now recognized as a global threat.

Mutants of *S. pneumoniae* resistant to penicillin G were selected soon after the introduction of penicillin G yet clinical resistance was not reported until 20 years later. Hansman & Bullen were the first to report penicillin-resistant *S. pneumoniae*.[2] Their strain was isolated in Australia from the sputum of a patient with hypogammaglobulinaemia. After this initial report, penicillin resistance became problematic in South Africa in 1977 and within the next decade was reported from several continents in the world.[3] A recent report from a surveillance study conducted in the USA indicated that 33.5% of 9190 *S. pneumoniae* isolates from 1996 to 1997 were resistant to penicillin, emphasizing the magnitude of this problem.[4]

Based on the current guidelines for in-vitro testing, *S. pneumoniae* isolates can be divided into three categories: penicillin susceptible (PSSP; MICs $\leqslant$ 0.06 mg/L), penicillin intermediate (PISP; MICs = 0.12–1.0 mg/L) and penicillin resistant (PRSP; MICs $\geqslant$ 2.0 mg/L).[5] Penicillin resistance is associated with the presence of up to five low-affinity penicillin binding proteins (PBPs), including PBPs 1a, 1b, 2a, 2b and 2x. The emergence of low β-lactam affinity appears to have evolved not by mutation but by acquisition of foreign DNA.[6] Resistance to extended-spectrum cephalosporins is currently limited to changes in PBP1 and PBP2x, which are encoded by closely linked genes that can be transferred *en bloc* to a susceptible host.

With the increase in penicillin resistance, in-vitro susceptibility testing has become necessary to guide treatment. Vancomycin is currently the only approved antibiotic that is universally active against multiresistant *S. pneumoniae.* In order to help direct therapy of PRSP, we determined the in-vitro activities of 29 antimicrobial agents against 22 PISP and 16 PRSP. Comparative in-vitro activities of penicillin, aminopenicillins, carbapenems, cephalo-

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sporins, macrolides, quinolones, glycopeptides and other antimicrobial agents are reported in this article.

Materials and methods

Twenty-two PISP and 16 PRSP sequential isolates were recovered from various sources. The PISP isolates were from the following types of specimens: respiratory (14), eye (4), blood (3) and cerebrospinal fluid (1), while the PRSP isolates were from respiratory (9), eye (4), blood (1), wound (1) and urine (1) specimens. Isolates with penicillin MICs of ⩾2.0 mg/L were considered resistant and those with MICs of 0.12–1.0 mg/L were classified as intermediate.

MICs were determined by microbroth dilution. Standard powders provided by the manufacturers were used to prepare stock antibiotic dilutions as outlined in the NCCLS standards.[5] Two-fold antimicrobial dilutions were made in cation-adjusted Mueller–Hinton broth supplemented with 3% lysed horse blood (final concentration) (Cleveland Scientific, Cleveland, OH, USA). Antimicrobial concentrations were started at 64 mg/L and serial two-fold dilutions were made to 0.03 mg/L. The inocula were prepared from an 18 h pure cultures in saline, adjusted to a 0.5 McFarland standard. The final bacterial concentration was 5×10^5 cfu. *S. pneumoniae* ATCC 49619 was used as a control with each antibiotic. Plates were incubated at 35°C for 20–22 h. MBCs were performed following NCCLS guidelines and were determined at the dilution representing 99.9% kill.[5]

Strains were tested against the following antimicrobial agents: cefuroxime (Eli Lilly, Indianapolis, IN, USA), cefmetazole (Upjohn, Kalamazoo, MI, USA), cefotaxime (Hoechst Marion Roussel, Somerville, NJ, USA), ceftriaxone (Hoffman LaRoche, Nutley, NJ, USA), ceftizoxime (SmithKline Beecham, Philadelphia, PA, USA), ceftazidime (Glaxo–Welcome, Research Triangle Park, NC, USA), cefprozil (Bristol Myers Squibb, Princeton, NJ, USA), cefixime (Lederle, Wayne, NJ, USA), penicillin (Bristol Myers Squibb), ampicillin/sulbactam (Pfizer, New York, NY, USA), amoxycillin–clavulanic acid (SmithKline Beecham), imipenem (Merck, Rahway, NJ, USA), meropenem (Zeneca, Pearl City, NY, USA), erythromycin (Abbott, Abbott Park, IL, USA), tetracycline (Lederle), doxycycline (Pfizer, New York, NY, USA), ciprofloxacin (Miles, West Haven, CT, USA), clinafloxacin, sparfloxacin (Park Davis, Ann Arbor, MI, USA), trovafloxacin (Pfizer), clindamycin (Upjohn), vancomycin (Eli Lilly), rifampicin (Merrel Dow, Cincinnati, OH, USA), trimethoprim/sulphamethoxazole (TMP/SMX; Roche, Nutley, NJ, USA), ramoplanin (Merrel Dow), quinupristin/daltopristin (Rhône-Poulenc, Collegeville, PA, USA), linezolid (Upjohn) and teicoplanin (Merrel Dow).

MICs were summarized using the box-plot method[7,8] (Figures 1 and 2). A box plot displays summary statistics for the distribution of the data. The lower boundary of the box is the 25th percentile and the upper boundary is the 75th

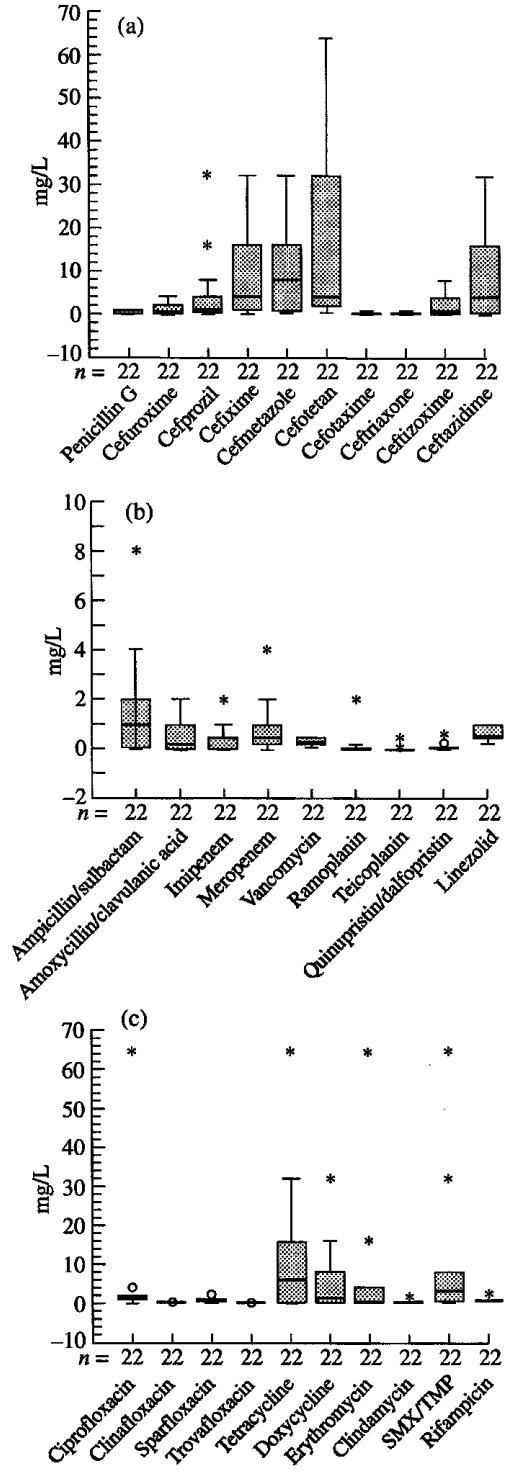

Figure 1. The activity of antimicrobial agents against penicillin-intermediate *S. pneumoniae.*

percentile. The horizontal line inside the box represents the median. Fifty percent of the cases have values within the box. The length of the box corresponds to the interquartile range, the difference between the 75th and 25th percentiles. Cases marked with an asterisk are more than three box-lengths from the upper or lower edge of the box

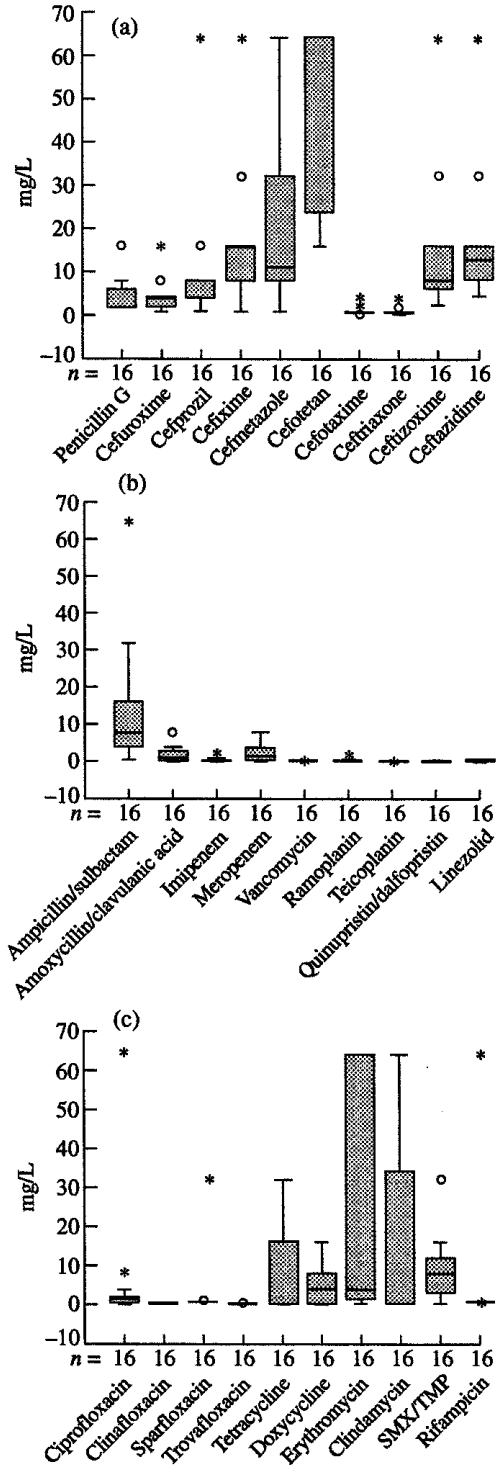

Figure 2. The activity of antimicrobial agents against penicillin-resistant *S. pneumoniae.*

and are called extreme values. An open circle indicates values that are between 1.5 and 3 box-lengths from the upper or lower edge of the box and are referred to as outliers. Lines drawn from the ends of the box represent the largest and smallest values that are not outliers.

Therapeutic indices were calculated using published achievable peak serum levels.[9–11] The peak serum level was divided by the MIC_{90} to obtain the therapeutic index.

Results

Among the classes of antimicrobials that were tested, wide differences in susceptibility were demonstrated for both PISP and PRSP (Figures 1 and 2). For the cephalosporins, based on MIC_{90}s, ceftriaxone and cefotaxime demonstrated the best in-vitro activity for both PISP and PRSP. There was a 32- to 64-fold difference between the most active cephalosporins (ceftriaxone and cefotaxime) and the least active (cefotetan).

Based on MIC_{90}s, the order of activity against PISP was: ceftriaxone, cefotaxime > ceftizoxime, cefuroxime > cefprozil, cefmetazole > cefixime, ceftazidime > cefotetan. The activity against PRSP according to MIC_{90}s was: ceftriaxone > cefotaxime > cefuroxime > cefprozil > cefixime, cefmetazole, ceftizoxime, ceftazidime > cefotetan (Figure 2a).

Of the quinolones, based on MIC_{90}s, clinafloxacin and trovafloxacin showed the greatest in-vitro activity. The order of activity for both PISP and PRSP was clinafloxacin > trovafloxacin > sparfloxacin > ciprofloxacin (Figures 1c and 2c). There was a 32- to 64-fold difference between the most active (clinafloxacin) and the least active (ciprofloxacin) quinolone. MBCs were within one tube of the MIC for all antimicrobials tested.

A summary of the therapeutic indices is found in the Table for all antimicrobials tested. Among the cephalosporins, the MIC_{90}s of cefprozil and cefixime were both above the achievable peak serum levels. Other antimicrobials for which the MIC_{90}s were above the achievable peak serum levels for both PISP and PRSP included co-trimoxazole, doxycycline, tetracycline and erythromycin.

When analysing antimicrobials with published NCCLS breakpoints (Table), the order of percent susceptibility for PISP was as follows: vancomycin > rifampicin > ceftriaxone > cefotaxime > clindamycin > amoxycillin–clavulanic acid > cefuroxime > erythromycin > tetracycline > imipenem > co-trimoxazole[5]. For PRSP the order of percent susceptibility was: vancomycin > rifampicin > cefotaxime > clindamycin > ceftriaxone > imipenem > tetracycline > amoxycillin–clavulanic acid > co-trimoxazole > erythromycin > cefuroxime. As judged from their MIC_{90}s, ceftriaxone and cefotaxime had similar activities against PRSP (MIC_{90}s = 1 and 2 mg/L, respectively). However, when they were compared based on published *S. pneumoniae* NCCLS breakpoints for PRSP isolates, 87% were susceptible to cefotaxime, while 49% were susceptible and 38% were intermediate to ceftriaxone.[5]

Multiresistant *S. pneumoniae* is defined as resistance to three or more antibiotics.[12] Antibiotics with published NCCLS breakpoints (penicillin, erythromycin, co-trimoxazole, rifampicin, cefuroxime, clindamycin, amoxycillin–

Table. Summary of therapeutic indices of and percent resistant and intermediate to commonly prescribed antimicrobials

Antimicrobial	Peak serum concentration (mg/L)	Dose/route	NCCLS breakpoints R (I)	PISP			PRSP		
				MIC/MBC_{90} (mg/L)	therapeutic index	% R (% I)	MIC/MBC_{90} (mg/L)	therapeutic index	% R (% I)
Ceftriaxone	150	1 g iv	≥2 (1)	0.5/0.5	300	0 (5)	1.0/1.0	150	13(38)
Cefotaxime	100	1 g iv	≥2 (1)	0.5/0.5	200	0 (9)	2.0/2.0	50	13(0)
Ceftizoxime	132	1 g iv	NA	4.0/4.0	33	NA	32/32	4	NA
Cefuroxime	100	1.5 g iv	≥2 (1)	4.0/4.0	25	27 (9)	4.0/4.0	25	94 (6)
Cefmetazole	77	1 g iv	NA	16/16	5	NA	32/32	2	NA
Ceftazidime	60	1 g iv	NA	32/32	2	NA	32/32	2	NA
Cefotetan	124	1 g iv	NA	64/64	2	NA	64/64	2	NA
Cefprozil	10.5	500 mg po	NA	16/16	0.65	NA	16/32	0.65	NA
Cefixime	3.0–5.0	400 mg po	NA	32/32	0.09–0.16	NA	32/32	0.09–0.16	NA
Ampicillin/SB	109/150	3 g iv	NA	4.0/4.0	27	NA	16/32	6.8	NA
Amoxycillin/CA	7.6/2.3	500 mg po	≥2/1 (1/0.5)	1.0/1.0	7.6	9 (23)	4.0/4.0	1.9	38 (31)
Imipenem	40	500 mg iv	≥1 (0.25–0.5)	1.0/1.0	10	23 (41)	0.5/1.0	40	13 (44)
Meropenem	26	500 mg iv	NA	4.0/4.0	7	NA	4.0/8.0	3	NA
Clinafloxacin	2.8	200 mg po	NA	0.06/0.06	46.7	NA	0.12/0.12	23.4	NA
Trovafloxacin	2.3	200 mg po	4 (2)	0.5/0.5	4.6	0 (0)	0.25/0.25	9.2	0 (0)
Sparfloxacin	1.0–2.0	400 mg po	2 (1)	1.0/1.0	1.0–2.0	9 (32)	0.5/1.0	1.0–2.0	6 (6)
Ciprofloxacin	4.6	400 mg iv	NA	4.0/4.0	1	NA	4.0/4.0	1	NA
Teicoplanin	112	6 mg/kg	NA	0.06/0.06	1867	NA	<0.03/<0.03	3733	NA
Rifampicin	7.0	600 mg po	≥4 (2.0)	<0.03/<0.03	233	0 (5)	0.12/0.12	58	6.2 (0)
Ramoplanin	NA	NA	NA	0.25/0.25	NA	NA	0.12/0.12	NA	NA
Vancomycin	25	1000 mg iv	– (–)[a]	0.5/0.5	50	0 (–)	0.25/0.25	100	0 (–)
Quinupristin/ dalfopristin	7.5–12.5	7.5 mg/kg iv	NA	0.5/0.5	15–25	NA	0.5/0.5	15–25	NA
Clindamycin	10	600 mg iv	≥1 (0.5)	1.0/1.0	10	0 (18)	64/64	0.156	31 (0)
Linezolid	15.7[b]	625 mg iv bd[b]	NA	1.0/1.0	15.7	NA	1.0/1.0	15.7	NA
Co-trimoxazole	TMP 3–9 SMX 45–100	800 mg SMX iv 160 mg TMP iv	4/76 (1/19–2/38)	8/152 / 8/152	0.38–1.0 0.30–0.65	50 (23)	8/152 / 16/304	0.38–1.0 0.30–0.65	75 (6)
Doxycyline	2.2	100 mg po	NA	16/16	0.13	NA	8.0/8.0	0.275	NA
Tetracycline	2.2	250 mg po	≥8 (4.0)	32/32	0.07	50 (14)	32/32	0.07	71 (0)
Erythromycin	0.9–1.4	500 mg po	≥1 (0.5)	16/16	0.005–0.09	41 (0)	64/64	0.01–0.02	75(0)
Penicillin G	16	12 MU qd iv	≥2 (0.12–1)	1.0/1.0	16	0 (100)	8	2	100 (0)

Abbreviations: CA, clavulanic acid; I, intermediate; R, resistant; SB, sulbactam.

[a]The absence of resistant strains precludes defining any result categories other than susceptible.

[b]Unpublished data.

clavulanic acid, imipenem, vancomycin and cefotaxime) were included in the identification of multiple drug resistance.[5] There were 94% of PRSP isolates and 36% of PISP isolates demonstrating multiple drug resistance.

Discussion

Determining the susceptibility of *S. pneumoniae* has become essential throughout most areas of the world because of the widespread occurrence of penicillin resistance. Furthermore, with the high proportion of multiple antibiotic resistance, new alternatives must be sought for the therapy of penicillin-resistant *S. pneumoniae.* The treatment of specific sites of infection by this organism requires not only in-vitro susceptibility data, but also an understanding of the pharmacology of the specific agents.

To eradicate experimentally-induced meningitis in rabbits, the antibiotic concentration in the cerebrospinal fluid must exceed the MBC by ten-fold.[12] Antimicrobial agents which have been used as therapy for PRSP meningitis include penicillin, chloramphenicol, vancomycin, imipenem, cefotaxime, ceftriaxone and erythromycin. There are advantages and disadvantages for each of these antimicrobials when used to treat meningitis.[6] Penicillin is inexpensive and safe, but requires very high doses (600,000 U/kg/day) and may not be active against isolates with high-level resistance. All *S. pneumoniae* isolates to date are susceptible to vancomycin, but this antibiotic is potentially toxic and shows unreliable penetration into the cerebrospinal fluid, and there have been reported treatment failures. Furthermore, as vancomycin resistance has been reported in other Gram-positive cocci, clinicians should anticipate the eventuality of vancomycin-resistant *S. pneumoniae.* If vancomycin *is* used, consideration of combination therapy with an extended-spectrum cephalosporin, and/or rifampicin, has been advocated to achieve an additive or synergic effect. Imipenem is the most active β-lactam but use of this agent has been tempered because of its epileptogenic potential. Cefotaxime and ceftriaxone are safe and generally effective and have been the agents of choice for empirical therapy.[12] These agents may also be combined with vancomycin or rifampicin for isolates with MICs of $\geqslant$2 mg/L.[1] When high-level penicillin resistance is suspected, the advisable agents are vancomycin, imipenem or an extended-spectrum cephalosporin. In our study, the other cephalosporins were not as active as cefotaxime or ceftriaxone, suggesting that substituting within the same class of agents may not be considered unless in-vitro testing confirms the same degree of activity.

When considering treatment for *S. pneumoniae* bacteraemia, several factors have to be considered. It has been shown that the outcome of PRSP bacteraemia is dependent not only on the efficacy of penicillin, but also on the presence of underlying diseases.[6] In adults, mortality may be higher in infections caused by PRSP than in those caused by PSSP.[6] High-dose iv penicillin G (150,000–250,000 U/kg/day) is recommended for PISP. However, there have been reports of resistant strains not responding to therapy with penicillin, or ampicillin and therapy in these cases should also be guided by selecting an agent which is active against PRSP *in vitro.*[6]

The assessment of the incidence of PRSP otitis media may be underestimated because of the infrequency with which cultures are obtained in these cases. Studies have shown that in daycare centres the association of PRSP with otitis media is related to antibiotic prophylaxis.[6] Since meningitis is potentially a life-threatening complication of otitis media in children aged $<$12 months, ongoing surveillance of *S. pneumoniae* susceptibility testing is indicated for isolates from otitis media or its associated complications. Such surveillance data may influence future antimicrobial selections or clinical trial considerations in the treatment of otitis media.

Our in-vitro study provides promising options, such as ramoplanin, quinupristin/dalfopristin, teicoplanin and linezolid for possible clinical trials of PRSP and PISP. Furthermore, marked susceptibility differences within classes such as cephalosporins indicate that antimicrobials within a class are not interchangeable. Similarly, the quinolones also showed substantial intraclass differences in their activities. The avoidance of quinolones such as ciprofloxacin in life-threatening *S. pneumoniae* infections seems appropriate, but newer quinolones—such as trovafloxacin and clinafloxacin—may be useful in treating these infections.

In-vitro antimicrobial activity against PISP and PRSP needs to be monitored to identify changes in susceptibility patterns, and to guide therapeutic decisions. Evaluation of new agents within existing classes or in a novel class of antimicrobial agents may be useful in identifying effective alternative therapies for the grave threat presented by penicillin-resistant *S. pneumoniae.*

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