ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1989, p. 1622–1623
0066-4804/89/091622-02$02.00/0

Vol. 33, No. 9

Comparative Activity In Vitro of 16 Antimicrobial Agents against Penicillin-Susceptible Meningococci and Meningococci with Diminished Susceptibility to Penicillin

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Broad-spectrum cephalosporins were very active against strains of *Neisseria meningitidis* with both penicillin susceptibility and diminished penicillin susceptibility. Ceftriaxone was the most active antibiotic. Increases in MIC for 90% of meningococci with diminished susceptibility to penicillin of ≥16-fold were observed for amdinocillin, cefuroxime, aztreonam, and imipenem; 2-fold increases were observed for ceftazidime, mezlocillin, and piperacillin. No differences were observed for non-β-lactam antibiotics.

Penicillin has been the treatment of choice for meningococcal infections for more than 40 years. In contrast to *Neisseria gonorrhoeae*, until very recently *Neisseria meningitidis* has conserved its susceptibility to penicillin. Both β-lactamase-producing and non-β-lactamase-producing penicillin-resistant *N. meningitidis* strains have been isolated (1, 3, 5, 7, 9). Although isolation of strains with enzymatic resistance is still exceptional, non-β-lactamase-producing strains of meningococci for which benzylpenicillin MICs are ≥0.25 μg/ml (meningococci with diminished susceptibility to penicillin [MDSP]) are becoming increasingly common in some geographic zones (5). To study possible therapeutic alternatives and to better comprehend the mechanisms of this resistance, we thought it would be interesting to compare the susceptibilities in vitro of a group of penicillin-susceptible meningococci (MS) (n = 40; 1 serogroup A strain; 33 serogroup B strains; 3 serogroup C strains; 2 serogroup Z strains; and 1 serogroup Y strain) and a group of MDSP (n = 30; 9 serogroup B strains and 21 serogroup C strains).

Strains for which penicillin MICs were ≤0.12 μg/ml were considered susceptible (MS). Nonduplicated organisms from recently obtained clinical isolates (cerebrospinal fluid or blood cultures and pharyngeal swabs from carriers) were maintained as stock cultures at −70°C until just before testing. Stock cultures were thawed, and samples were inoculated onto plates containing 5% chocolate horse blood agar. After incubation for 20 to 24 h, isolated colonies were subcultured to a second chocolate agar plate, which was incubated for another 20 to 24 h. Growth from this plate was used to prepare inocula. MICs were determined by an agar dilution method on GC agar base (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% chocolate horse blood; sulfonamides were tested on Iso-Sensitest agar (Oxoid Ltd., Basingstoke, England) supplemented with 5% laked horse blood. The antimicrobial agents used in this study were supplied by their manufacturers. Fresh dilutions of all compounds were made daily. For the inoculum, a McFarland 0.5 turbidity standard was prepared by using a nephelometer. A further 1:20 dilution in broth was placed in a Steers replicator and spotted onto the surface of each antimicrobial agent-containing plate (final concentration, $5 \cdot 10^3$ to $1 \cdot 10^4$ CFU). Antimicrobial agent-containing plates were examined after 20 to 24 h of incubation. Incubation conditions were always 35°C in a humid 5% CO_2 atmosphere. *Staphylococcus aureus* ATCC 29213, *N. meningitidis* ATCC 13090, and *N. gonorrhoeae* CDC 98 were included as controls. No strain produced β-lactamase when tested by a chromogenic method (Cefinase; BBL).

All strains were susceptible to chloramphenicol (Table 1). The most active agent of those tested was ceftriaxone, with an MIC for all strains of ≤0.008 μg/ml. In general, the broad-spectrum cephalosporins showed very good activity, which should be emphasized since broad-spectrum cephalosporins, cefotaxime and ceftriaxone, are the firmest candidates for drugs of choice in the empirical treatment of bacterial meningitis in the near future. The in vitro activities of the antibiotics used in the chemoprophylaxis of meningococcal infection—sulfadiazine, rifampin, ciprofloxacin, and tetracycline (minocycline)—were similar for MS and MDSP; the strains exhibited the typical patterns of susceptibility of strains isolated in Spain (resistance to sulfadiazine and susceptibility to the other three agents) (8). There was a slightly greater frequency of strains resistant to sulfadiazine and rifampin among MDSP. The differences in the susceptibilities of MS and MDSP were more marked for the β-lactam antibiotics, especially amdinocillin (mecillinam), cefuroxime, aztreonam, and imipenem; as with penicillin, MDSP were 5 to 50 times more resistant in vitro to these agents than were MS.

Amdinocillin and other β-lactam antibiotics of dubious indication in the therapy of meningococcal infections were included in the study to examine the effect of the mechanisms of resistance of MDSP on these agents. The MICs of amdinocillin, cefuroxime, aztreonam, and imipenem for 90% of MDSP were ≥16-fold greater than those for MS; the ceftazidime, mezlocillin, and piperacillin MICs for 90% of strains were barely 2-fold greater. Once the possibility of antibiotic destruction by β-lactamase is excluded, the mechanism involved in MDSP resistance could be a penicillin-binding protein (PBP) alteration that impairs antibiotic affinity and/or a reduced permeability of the outer membrane. Recently, a decreased affinity for benzylpenicillin was described in an ~59-kilodalton PBP in six MDSP (4). This

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TABLE 1. Antimicrobial susceptibilities of 40 MS and 30 MDSP

Antibiotic	MIC (μg/ml)[a]					
	50%		90%		Range	
	MS	MDSP	MS	MDSP	MS	MDSP
Penicillin	0.03	0.5	0.06	1	0.01–0.12	0.25–2
Amdinocillin	1	8	1	32	0.5–1	1–32
Mezlocillin	≤0.008	0.01	0.01	0.03	≤0.008–0.01	≤0.008–0.03
Piperacillin	0.01	0.03	0.03	0.06	≤0.008–0.03	0.01–0.06
Aztreonam	0.03	0.12	0.03	0.5	0.03–0.06	0.06–1
Cefuroxime	0.12	1	0.12	4	0.06–0.25	0.25–8
Moxalactam	≤0.008	0.01	≤0.008	0.06	≤0.008	≤0.008–0.06
Cefotaxime	≤0.008	0.01	≤0.008	0.03	≤0.008	≤0.008–0.03
Ceftizoxime	≤0.008	0.01	≤0.008	0.03	≤0.008	≤0.008–0.06
Ceftazidime	0.01	0.03	0.03	0.06	≤0.008–0.03	≤0.008–0.06
Ceftriaxone	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008
Imipenem	0.01	0.12	0.01	0.25	0.01–0.03	0.06–0.25
Sulfadiazine	64	64	≥128	≥128	2–≥128	16–≥128
Rifampin	≤0.03	≤0.03	≤0.03	0.12	≤0.03–0.12	≤0.03–≥0.5
Ciprofloxacin	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
Tetracycline	1	1	1	1	0.5–1	0.5–2
Chloramphenicol	1	1	1	1	0.5–1	1–2

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

protein was designated PBP 3 (4), although there is doubt as to whether it might be more correctly designated PBP 2. The pathogenic *Neisseria* species *N. gonorrhoeae* and *N. meningitidis* are very closely related genetically (6). Dougherty et al. (2) included a strain of *N. meningitidis* in a study of the PBPs of *N. gonorrhoeae* and, after finding no appreciable differences between the masses of PBPs 2 and 3 in the two species, designated the ~60-kilodalton PBP as PBP 2. The different activities of amdinocillin against MS and MDSP observed in our study provide another reason for thinking that the altered protein could be PBP 2.

This work was supported in part by Fondo de Investigaciones Sanitarias grant 89/0923 from the Ministerio de Sanidad y Consumo, Spain.

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