ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1982, p. 897-900
0066-4804/82/110897-04$02.00/0

Vol. 22, No. 5

Susceptibility of Group B Streptococci to 16 β-Lactam Antibiotics, Including New Penicillin and Cephalosporin Derivatives

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Received 7 June 1982/Accepted 27 August 1982

The susceptibility of 100 group B streptococci to 16 β-lactam antibiotics was tested by agar dilution. Penicillin G and *N*-formimidoyl thienamycin were the most active agents tested, both having a 90% minimal inhibitory concentration (MIC_{90}) of 0.06 μg/ml. Ceftriaxone, cefotaxime, cefamandole, and SCH 29482 were almost as active, all having an MIC_{90} of 0.12 μg/ml, and ampicillin, cephalothin, and mezlocillin all had an MIC_{90} of 0.25 μg/ml. The MIC_{90} for piperacillin, cefoperazone, and ceftazidime was 0.5 μg/ml. Least active were carbenicillin, ticarcillin, cefoxitin, and moxalactam, with MIC_{90}s of 1, 2, 4, and 8 μg/ml, respectively. No penicillin-tolerant strains were detected.

Group B streptococci (*Streptococcus agalactiae*) are a major cause of neonatal meningitis, and currently recommended therapy is a penicillin either alone or in combination with an aminoglycoside. The potential for use of new β-lactam antibiotics in the treatment of neonatal meningitis caused by group B streptococci was investigated by determining the minimal inhibitory concentrations (MICs) of selected β-lactams against isolates of group B streptococci.

One hundred clinical isolates of group B streptococci, obtained from blood or cerebrospinal fluid (CSF) of pediatric patients in Buffalo, N.Y., Cleveland, Ohio, and Pittsburgh, Pa. (64 strains) or from adult carriers in Cleveland (36 strains), were used in this study. The identity of all isolates as group B streptococci was confirmed by Lancefield grouping with a coagglutination technique (Phadebact) (3).

The antimicrobial agents used were cefoperazone (Pfizer Inc.); ampicillin (Bristol Laboratories); carbenicillin and ticarcillin (Beecham Laboratories); piperacillin (Lederle Laboratories); mezlocillin (Miles Pharmaceuticals); benzyl penicillin, cephalothin, cefamandole, and moxalactam (Eli Lilly & Co.); cefoxitin and *N*-formimidoyl thienamycin (Merck Institute) ceftriaxone (Hoffmann-La Roche Inc.); cefotaxime (Hoechst-Roussel Pharmaceuticals Inc.); ceftazidime (Glaxo, Inc.); and SCH 29482 (Schering Corp.). These agents were supplied as laboratory standard powders of known potency, and stock solutions were made as recommended by the manufacturers.

MICs were determined by the agar incorporation method in plastic petri dishes (15 by 100 mm) containing 25 ml of Mueller-Hinton agar supplemented with 5% sheep blood and incorporating the above antimicrobial agents in concentrations of 0.008 to 256 μg/ml in doubling dilutions (13). After pouring, the plates were dried, wrapped in plastic, and refrigerated at 4°C before use within 7 days.

For MIC testing, isolates were grown in 2 ml of Mueller-Hinton broth at 35°C for 3 to 5 h, and the turbidity was then adjusted to a turbidity equivalent to that of a 0.5 McFarland barium sulfate standard with a nephelometer (1R, API; Analytab Products). Suspensions were further diluted 1:20 to obtain a final inoculum of 10^4 organisms per 2 μl. Plates were inoculated with a Steers replicator with 3-mm inoculating pins and inoculated overnight at 35°C in room air. The MIC was defined as the lowest concentration of antibiotic completely inhibiting the growth of each isolate. Quality control was ensured if results obtained with recommended controls (13) agreed within 1 doubling dilution with values published or supplied by manufacturers.

Minimal bacterial concentrations (MBCs) were determined for selected isolates by broth macrodilution in 1-ml volumes of Mueller-Hinton broth. The MBC was defined as the lowest concentration of antimicrobial agent at which ≥99.9% of the inoculum was killed after overnight incubation (1). The lowest dilution of antimicrobial agent with ≤0.1% of the original inoculum was read as the MBC.

Screening for penicillin tolerance. Screening for penicillin tolerance was performed by tube macrodilution with the same technique as that

TABLE 1. MICs of 16 β-lactam antimicrobial agents for 100 strains of group B streptococci

Antimicrobial agent	MIC (μg/ml)				MBC (μg/ml)[a]
	Range	Mode	50%	90%	
Penicillin G	0.03–0.06	0.03	0.03	0.06	0.03–0.06
N-Formimidoyl thienamycin	0.03–0.06	0.06	0.06	0.06	0.06
Cefamandole	0.06–0.12	0.06	0.06	0.12	0.12–0.25
Ceftriaxone	0.06–0.12	0.12	0.12	0.12	0.12
Cefotaxime	0.02–0.12	0.12	0.12	0.12	0.12
SCH 29482	0.06–0.25	0.12	0.12	0.12	0.12
Ampicillin	0.12–0.25	0.25	0.25	0.25	0.25–0.5
Mezlocillin	0.12–0.25	0.12	0.12	0.25	0.12–0.25
Cephalothin	0.12–0.25	0.25	0.25	0.25	0.25
Piperacillin	0.25–0.5	0.5	0.5	0.5	0.5–1
Cefoperazone	0.12–0.5	0.25	0.25	0.5	0.5–1
Ceftazidime	0.12–0.5	0.25	0.25	0.5	0.5
Carbenicillin	0.5–1	1	1	1	1
Ticarcillin	1–2	2	2	2	2–4
Cefoxitin	2–4	4	4	4	4
Moxalactam	2–8	8	8	8	4–8

[a] The MBC of penicillin G, obtained by macrodilution testing in Mueller-Hinton broth was determined for 20 strains; those of the other antimicrobial agents (obtained in the same way) were determined for 5 strains.

used for MBC determinations, except only a single penicillin G concentration was used (final concentration, 0.2 μg/ml). The penicillin G concentration in broth was assayed by using the large-plate agar diffusion technique, with *Sarcina lutea* as the indicator organism on Mueller-Hinton agar incubated at 35°C overnight (14).

The activities of the 16 agents tested against the 100 strains of group B streptococci are shown in Table 1. The MICs of penicillin G were 0.03 μg/ml for 73 strains and 0.06 μg/ml for 27 strains. Penicillin G and *N*-formimidoyl thienamycin were the most active agents tested, both having a 90% MIC (MIC_{90}) of 0.06 μg/ml. Cefamandole, ceftriaxone, cefotaxime, and SCH 29482 were almost as active as pencillin G, all having an MIC_{90} of 0.12 μg/ml. Ampicillin, mezlocillin, and cephalothin all had an MIC_{90} of 0.25 μg/ml, and piperacillin, ceftazidime, and cefoperazone all had an MIC_{90} of 0.5 μg/ml. Least active were carbenicillin, ticarcillin, cefoxitin, and moxalatam, which had MIC_{90}s of 1, 2, 4, and 8 μg/ml, respectively. The MICs obtained for each antimicrobial agent were all within 1 to 2 doubling dilutions, indicating a homogenous group of strains despite the variations in sources and sites of isolation. There were, therefore, no differences among strains isolated from patients or carriers.

The MBC of penicillin G was determined for 20 strains, and those of other agents were determined for 5 strains. The MBCs of all agents were within 1 dilution of the MICs obtained, and the MICs determined by broth dilution were the same as those determined by agar dilution (Table 1). All 100 strains were screened for penicillin tolerance, but none were found to be tolerant (the penicillin G concentration of the test broth was assayed at 0.21 μg/ml).

The penicillin MICs obtained in this study agree with those reported by Baker et al. (2) for a group of 179 group B streptococci from clinical isolates throughout the United States (the MIC_{90} of penicillin obtained in this report was also 0.06 μg/ml). Strains with penicillin MICs of up to 0.8 μg/ml, as reported by Jokipii and Jokipii (6), were not found and are presumably rarely detected.

Penicillin tolerance has been described in 4% of strains of group B streptococci (15). Tolerant strains had MICs of penicillin that were similar to those of nontolerant strains (mean MIC, 0.04 μg/ml), but MBCs of penicillin G were 0.3 to 1.25 μg/ml (mean, 0.6 μg/ml) for tolerant strains as compared with 0.04 to 0.3 μg/ml (mean, 0.08 μg/ml) for nontolerant strains (8). None of the strains used in this study were found to be tolerant, nor were any in a previous study of 179 strains (2). If strains are tested in tryptose-phosphate broth, 87% appear to be tolerant, but the clinical significance of tolerance and of differences among results obtained with different media are unknown (8, 9).

Despite the availability of new and effective antimicrobial agents for the treatment of life-threatening bacterial infections, neonatal meningitis continues to be associated with an unacceptable mortality and morbidity rate (11). Initial treatment is often empirical and has traditionally relied upon combination chemotherapy active against the wide range of microorganisms capable of infecting the neonatal central nervous system (12). These regimens have serious limitations, including the increasing prevalence of

ampicillin-resistant microorganisms, the narrow therapeutic index and poor cerebrospinal fluid pharmacokinetics of parenterally administered aminoglycosides, and the toxicity and lack of bactericidal activity of chloramphenicol. Accordingly, recent efforts have been directed towards identifying new agents which might prove efficacious in the treatment of neonatal meningitis.

The new β-lactam antimicrobial agents, particularly the third-generation cephalosporins and the 1-oxa-β-lactam antibiotic moxalactam, are potentially useful in the treatment of neonatal meningitis. These agents offer a number of theoretical advantages over the traditional combination regimens used for the initial empirical treatment of neonatal central nervous system infections. These advantages include a broad spectrum of antimicrobial activity, a wide therapeutic index, and the ability to achieve bactericidal concentrations in CSF. One potential shortcoming of many of these new agents is their limited activity against gram-positive microorganisms, including group B streptococci, as compared with those of the penicillins.

Of the penicillins tested, ampicillin has good CSF penetration (which is independent of meningeal inflammation in neonates) (7) and good activity against group B streptococci (MIC_{90}, 0.25 μg/ml) and is second only to penicillin G (MIC_{90}, 0.06 μg/ml). Ticarcillin can also achieve CSF levels significantly higher than its MIC_{90} against group B streptococci and may also be useful in treating group B streptococcal meningitis. Penicillin was the most active agent studied, but achievable CSF levels are not very high, and current practice is to continue its administration for 10 to 14 days after cultures become sterile to prevent recurrence of disease in group B streptococcal infections.

Application of in vitro results to patients is complicated by various factors. CSF concentrations of group B streptococci are often as high as 10^7 to 10^8/ml, and MICs of penicillins and chloramphenicol are much higher when an inoculum of 10^7/ml rather than 10^4/ml is used (4). Ampicillin and chloramphenicol show in vitro antagonism against streptococci, including group B streptococci, and their use together may be contraindicated (15). Penicillin-treated group B streptococci, however, show increased susceptibility to the bactericidal activity of human polymorphonuclear leukocytes (5). The results of the combination of inoculum, pH, drug antagonism, and leukocyte effects on the outcome of disease are difficult to assess.

The range of susceptibility of group B streptococci to the drugs used in this study was extremely narrow, and all drugs showed a variation of only 1 or 2 dilutions in MIC range. Prediction of susceptibility to these agents can be made by determination of the MIC of penicillin G, and this determination should be performed on all CSF isolates of group B streptococci. In addition, MBC determinations should also provide useful information about tolerance.

At present, clinical information on the new β-lactam agents is limited (10). Selected new penicillins and cephalosporins show considerable potential for the initial therapy of neonatal meningitis of undetermined etiology and may allow the current dependence on aminoglycosides to be greatly reduced (16). However, ampicillin and penicillin G are highly active against group B streptococci, and therefore, if the etiology is known to be group B streptococci, these drugs should not be abandoned in favor of the as yet unproven third-generation cephalosporins.

LITERATURE CITED

1. **Anhalt, J. P., L. D. Sabath, and A. L. Barry.** 1980. Special tests: bactericidal activity, activity of antimicrobics in combination, and detection of β-lactamase production, p. 478–484. *In* E. H. Lennette, A. Balows, J. R. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
2. **Baker, C. N., C. Thornsberry, and R. R. Facklam.** 1981. Synergism, killing kinetics, and antimicrobial susceptibility of group A and B streptococci. Antimicrob. Agents Chemother. **19**:716–725.
3. **Christensen, P., G. Kahlmeter, S. Jonsson, and G. Kronvall.** 1973. New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. Infect. Immun. **7**:881–885.
4. **Feldman, W. E.** 1976. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. J. Pediatr. **88**:549–552.
5. **Horne, D., and A. Tomasz.** 1981. Hypersusceptibility of penicillin-treated group B streptococci to bactericidal activity of human polymorphonuclear leukocytes. Antimicrob. Agents Chemother. **19**:745–753.
6. **Jokipii, A. M. M., and L. Jokipii.** 1976. Presumptive identification and antibiotic susceptibility of group B streptococci. J. Clin. Pathol. **29**:736–739.
7. **Kaplan, J. M., G. H. McCracken, L. S. Horton, M. L. Thomas, and N. Davis.** 1974. Pharmacologic studies in neonates given large doses of ampicillin. J. Pediatr. **84**:571–577.
8. **Kim, S. K., and B. F. Anthony.** 1981. Penicillin tolerance in group B streptococci isolated from infected neonates. J. Infect. Dis. **144**:411–419.
9. **Kim, S. K., R. N. Yoshimori, D. T. Imagawa, and B. F. Anthony.** 1979. Importance of medium in demonstrating penicillin tolerance of group B streptococci. Antimicrob. Agents Chemother. **16**:214–216.
10. **Landesman, S. H., M. L. Corrado, P. M. Shah, M. Armengaurd, M. Barza, and C. E. Cherubin.** 1981. Past and current roles for cephalosporin antibiotics in treatment of meningitis. Am. J. Med. **71**:693–703.
11. **McCracken, G. H.** 1976. Neonatal septicemia and meningitis. Hosp. Pract. **11**:89–97.
12. **McCracken, G. H., and Nelson, J. D.** 1977. Antimicrobial therapy for newborns: practical application of pharmacology to clinical usage. Grune and Stratton, New York.
13. **National Committee for Clinical Laboratory Standards.** 1980. Standard methods for dilution antimicrobial susceptibility tests for bacteria which grow aerobically. Proposed standard PSM-7. National Committee for Clinical

Laboratory Standards, Villanova, Pa.
14. **Sabath, L. D., and J. P. Anhalt.** 1980. Assay of antimicrobics, p. 485–490. *In* E. H. Lennette, A. Balows, J. R. Hausler, Jr., and J. P. Truant, (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
15. **Weeks, J. L., E. O. Mason, and C. J. Baker.** 1981. Antagonism of ampicillin and chloramphenicol for meningeal isolates of group B streptococci. Antimicrob. Agents Chemother. **20**:281–285.
16. **Wilkinson, P. J.** 1982. β-Lactam antibiotics in the newborn. J. Antimicrob. Chemother. **9**(Suppl. B):21–29.