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Antibiotic Resistance and Penicillin Tolerance in Clinical Isolates of Group B Streptococci

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The aim of this study was to determine the susceptibility patterns of 100 group B streptococcal strains isolated in our hospital and to ascertain tolerance to penicillin by determining quantitative killing curves. We found two strains with intermediate susceptibility to penicillin and eight strains to ampicillin. Seventeen isolates were tolerant to penicillin, with bacterial counts decreasing 2 to 3 log during the first 8 h but still above 10^2 CFU/ml after 24 h. The kinetic study shows that penicillin tolerance is not rare among group B streptococci isolated in our hospital.

Group B streptococci (GBS) are recognized as a common cause of serious neonatal and maternal infections; they are also isolated in adults from skin infections, wounds, and urinary tract infections, and serious infections in adults due to GBS are being reported with increasing frequency (5, 20, 28, 34). Despite consistent susceptibility of the majority of GBS to penicillin and ampicillin (3, 4, 6, 15, 21, 27), there are several reports of poor clinical responses to appropriate therapy and of relapses in GBS infections in newborn infants and adults (2, 11, 12, 38). These failures have led to changes in the treatment of GBS infections: increase in penicillin dosages or combination with an aminoglycoside has been recommended for treatment of serious GBS infections in adults and neonates (23). Different in vitro studies have shown accelerated killing of GBS when gentamicin is added to penicillin or ampicillin (4, 30, 36). Some investigators have demonstrated penicillin tolerance in these organisms and have suggested that this contributes to treatment failure.

MBCs for tolerant isolates are unusually high relative to MICs, and tolerance has been defined as an MBC/MIC ratio of 1/32 or greater, after 24 h of incubation. However, the significance of the MBC/MIC ratio as a measure of tolerance has been questioned, as the MBC determination is subject to many variables, such as growth phase, antibiotic carryover, medium composition, adherence to test tube walls, and growth in condensate above the meniscus (14, 19, 22, 26, 37). There is controversy in the literature concerning the criteria used to define tolerance, the techniques for determining this phenomenon, and its clinical significance (13, 16, 24, 32, 39). Because of the lack of a standardized method for this determination, the true incidence of tolerance among clinical isolates remains uncertain. Tolerance is better defined as a rate of killing lower than that in nontolerant organisms, and the use of time-kill curves is the most reliable method for detecting tolerance (13, 25). The aim of this study was to determine the susceptibility patterns of GBS isolated in our hospital and to ascertain the incidence of tolerance to penicillin among our GBS strains by time-kill curve analysis.

A total of 100 strains of GBS isolated in 1992 in the Hospital Universitario San Carlos, Madrid, Spain, were studied. The clinical sources were as follows: vagina, 40 strains; urine, 30 strains; skin and soft tissues, 15 strains; urethra, 7 strains; blood, 3 strains; and others, 5 strains. Organisms were identified by standard methods, including coagglutination tests (Phadebact; Pharmacia Diagnostics, Uppsala, Sweden), and only one isolate per patient was studied to avoid duplication.

Antimicrobial susceptibility testing was performed by a broth microdilution procedure (Sensititre; Radiometer, Copenhagen, Denmark) according to the guidelines of the National Committee for Clinical Laboratory Standards (25). The following antimicrobial agents were tested: penicillin, ampicillin, cephalothin, cefotaxime, imipenem, erythromycin, clindamycin, tetracycline, ciprofloxacin, rifampin, sulfamethoxazol-trimethoprim, gentamicin, vancomycin, and chloramphenicol. The breakpoints used and the three-category classification scheme (susceptible, intermediate, and resistant) were those recommended by the National Committee for Clinical Laboratory Standards (25).

Killing kinetics were determined for all 100 GBS strains. Five or more colonies were subcultured from sheep blood agar in 5 ml of Todd-Hewitt broth which was incubated at 37°C until visibly turbid (3 to 4 h); this late-logarithmic-phase culture was diluted in fresh Todd-Hewitt broth to give an inoculum size of approximately 5×10^5 CFU/ml. A quantity of 0.1 ml of the exponentially growing culture was added to acid-washed glass tubes containing 9.9 ml of the same broth with penicillin at a concentration 10 times the MIC; a control tube without antibiotic was always included. At 0, 4, 8, and 24 h, 200-μl aliquots from the culture tubes, with or without antibiotic, were removed and serially diluted 10-fold in sterile saline (0.9% NaCl) containing 1,000 U of penicillinase (Penase; Difco Laboratories, Madrid, Spain) per ml; 100-μl aliquots of each dilution (10^{-1} to 10^{-6}) were inoculated on separate blood agar plates. Duplicate subcultures were performed. CFU were counted after incubation for 24 h and charted on semilogarithmic paper with the survivor colony count on the ordinate (logarithmic scale) and time on the abscissa on an arithmetic scale. We studied the reproducibility of the results, with duplicate tests for each strain. Tolerance was defined as a ≤ 3 $\log_{10}$ decrease in CFU per milliliter after 8 h of incubation and $\geq 10^2$ CFU of viable streptococci per ml after 24 h.

Susceptibility data are summarized in Table 1. A total of 11 strains exhibited resistance or an intermediate susceptibility to two or more antibiotics other than gentamicin and tetracycline. In the study of time-kill curves, tolerance occurred in 17 of the

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TABLE 1. Antimicrobial susceptibility of GBS strains

Antimicrobial agent	MIC (μg/ml)[a]			% of strains[b]	
	Range	50%	90%	Intermediate	Resistant
Penicillin	≤0.03–0.5	≤0.03	0.06	2	
Ampicillin	≤0.06–1	≤0.06	0.12	8	
Cephalothin	≤0.12–16	0.5	0.5	4	
Cefotaxime	≤0.12–16	≤0.12	≤0.12	2	
Imipenem	≤0.12–2	≤0.12	≤0.12		
Erythromycin	≤0.25–>4	≤0.25	1	6	8
Clindamycin	≤0.12–>2	≤0.12	1	4	8
Tetracycline	≤1–>8	>8	>8	3	89
Ciprofloxacin	≤0.5–>2	1	2	19	1
Rifampin	≤0.5–>2	≤0.5	≤0.5		1
Sulfamethoxazole-trimethoprim	≤0.5–>2	≤0.5	≤0.5		1
Gentamicin	≤4–>8	>8	>8	25	70
Vancomycin	0.5–4	1	2		
Chloramphenicol	≤0.25–>16	4	4		3

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

[b] MICs from the National Committee for Clinical Laboratory Standards (25) for intermediate and resistant isolates, respectively, are as follows: penicillin and ampicillin, 0.25 to 2 and ≥4 μg/ml; cephalothin and chloramphenicol, 16 and ≥32 μg/ml; cefotaxime, 16 to 32 and ≥64 μg/ml; erythromycin, 1 to 4 and ≥8 μg/ml; clindamycin, 1 to 2 and ≥4 μg/ml; tetracycline and gentamicin, 8 and ≥16 μg/ml; and ciprofloxacin and rifampin, 2 and ≥4 μg/ml.

100 GBS strains examined. Penicillin caused a rapid killing of nontolerant GBS isolates, with a >3 log decrease in bacterial counts during the first 8 h, and no viable counts could be demonstrated after 24 h, while the tolerant strains did not decrease more than 2 to 3 log within 8 h, and after 24 h $\geq 10^2$ CFU/ml could still be demonstrated. The results of kinetic studies of a nontolerant and a tolerant GBS strain are shown in Fig. 1 and 2. We ascertained that our results were reproducible by means of the kill kinetic method.

The results of the susceptibility studies showed that the β-lactam agents tested displayed good activity in vitro, although this was less than that noted by the majority of investigators (3, 4, 6, 15, 21). We found two strains with intermediate susceptibility to penicillin and eight with intermediate susceptibility to ampicillin. The presence of such strains is rare but has been described in the literature (7, 17, 29, 31). The resistance rate of 89% for tetracycline was comparable to the rates reported by Baker et al. (3) and Jokipii and Jokipii (17). Rates of resistance to erythromycin and clindamycin obtained in this study (8% for both antibiotics) agree with those reported by Berkowitz et al. (6).

The incidence and clinical significance of penicillin tolerance in GBS infections have not been established. The different methods used by researchers may explain the discrepant results observed in published studies; the occurrence of penicillin tolerance in clinical isolates of GBS varies widely and ranges from 0% (15) to 4% (18, 33), 13% (8), 30% (1), and 87% (19). Tolerance is best detected by quantitative killing curve procedures. The results of our antibiotic kill kinetics showed that 17% of GBS studied were penicillin tolerant.

Reports of poor therapeutic responses to penicillin observed in infected neonates or in serious infections in adults have been described with increasing frequency (2, 11, 12, 38), but only scattered reports have confirmed the presence of clinically significant tolerance among GBS infections. Broughton et al. (9) and Siegel et al. (33) reported recurrent infections among neonates associated with penicillin-tolerant GBS. Steinbrecher (35) published the first report of a case of serious GBS infection caused by a penicillin-tolerant organism in an adult, and after an initial failure to respond to penicillin therapy alone, synergistic therapy with gentamicin and penicillin was commenced with clinical improvement. In 1992 Cunningham et al. (10) reported a case of prosthetic hip joint GBS infection that recurred after 3 months of treatment with amoxicillin; the isolate was found to be amoxicillin tolerant, and with the use of a combination of amoxicillin and gentamicin the patient has remained asymptomatic.

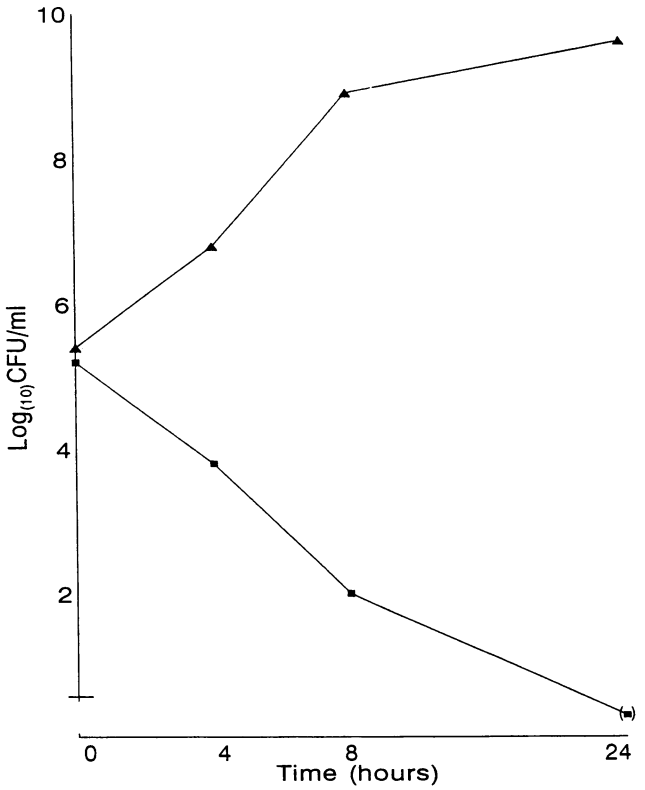

FIG. 1. Killing curve for a nontolerant GBS strain. The penicillin MIC was 0.06 μg/ml. Symbols: ▲, in the absence of antibiotic; ■, in the presence of 0.6 μg of penicillin per ml. Point at 24 h is in parentheses to indicate that lowest number of detectable colonies was 10^2/ml. For nontolerant strains, colony counts at 24 h were less than 10^2 CFU/ml.

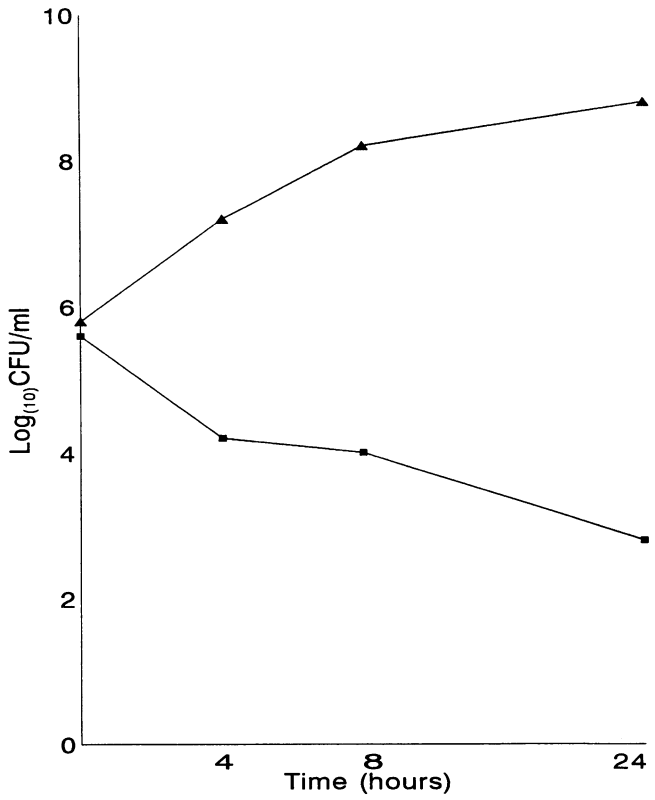

FIG. 2. Killing curve for a tolerant GBS strain. The penicillin MIC was 0.06 μg/ml. Symbols used are the same as in Fig. 1.

The intermediate resistance to penicillin and ampicillin and multiple-antibiotic resistance patterns of GBS observed in this study indicate the need for continued surveillance of susceptibility in these organisms. In addition, the kinetic study data show that penicillin tolerance is not rare among GBS isolated in our hospital, and we suggest that this phenomenon contributes to the poor therapeutic responses observed in some patients with serious GBS infections.

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