Change in Susceptibility of Group B Streptococci to Penicillin G from 1968 Through 1975

M. J. SEVERIN* AND J. L. WILEY

Creighton University, School of Medicine, Omaha, Nebraska 68178, and Omaha-Douglas County Health Department, Omaha, Nebraska 68105*

Received for publication 5 March 1976

This report describes a study of 212 isolates of group B streptococci from sore throats over an 8-year period. A small but increasing percentage showed increased resistance to penicillin G when tested in an in vitro system.

In 1938, Fry reported that group B, beta-hemolytic streptococci were causative agents of human disease (1). Subsequently, the syndromes of neonatal sepsis (5) and neonatal meningitis (4) due to group B streptococci were characterized.

During the evaluation of a throat culture program serving physicians and dentists in our community, it was apparent that non-group A streptococci were being isolated from throat swabs of clinically ill individuals on a constant basis. Dacron swabs received from clinicians were routinely processed by culturing them on blood agar plates containing 5% sheep erythrocytes. After overnight incubation at 35°C in an atmosphere of 5% CO₂, these plates were examined for streptococcal colonies demonstrating beta-hemolysis. All such isolates were screened by the fluorescent antibody technique (3) to identify group A streptococci. Non-group A isolates were examined by precipitin testing methods (2) to determine the isolates of groups B, C, D, and G. Isolates identified as group B streptococci, which by colony count appeared to represent at least 25% of the throat flora, were suspended in 0.5 ml of sterile sheep erythrocytes. These suspensions were stored at -20°C until antibiotic susceptibility tests could be performed. Before freezing, one of every 10 isolates was tested against penicillin G to determine a preliminary minimal inhibitory concentration (MIC). Representative isolates of group A streptococci (one per month) and group D streptococci (one per month) were similarly isolated, identified, tested, and stored. Later comparison with these preliminary MICs enabled us to determine the effects of time and freezing on the stored streptococci.

Streptococcal isolates were tested against penicillin G in a twofold dilution series using Todd-Hewitt broth as the antibiotic diluent. The concentrations of penicillin G prepared ranged from 2.50 to 0.009 units/ml. The MIC for

each isolate was measured in duplicate with the same lot number of culture medium and antibiotic. Each series was inoculated with a suspension of 10⁴ colony-forming units of test isolate per ml. All cultures were incubated at 35°C in an atmosphere of 5% CO₂. Results were read after 24 h of incubation. When a duplicate series differed from the original, the isolate was restudied to establish the true MIC. Streptococci isolated during the years 1968 through 1973 were evaluated in an initial study. A second study evaluated the isolates of 1974 and 1975.

A total of 212 group B streptococci isolates were studied during an 8-year period. The range of susceptibility to penicillin G was 0.62 to 0.009 unit/ml or below (Table 1). All the isolates obtained in years 1968 through 1971 were susceptible to 0.009 unit or less per ml. For some of the isolates obtained in years 1972 through 1975, the MIC was at least fourfold above the 0.009-unit/ml level (Table 2). In 1972, the MIC for 10% of the group B streptococcal isolates were 0.075 unit or greater per ml. During 1973 this figure rose to 21%, and in 1975 it was 24%. During this same 4-year period, 6% of group B streptococci were not susceptible to 0.15 unit/ml, and 3% were not susceptible to 0.3 unit/ml.

Each of the 96 group A control isolates was inhibited by MICs at or below 0.009 unit/ml.

TABLE 1. Range of susceptibility of group B streptococci to penicillin G-212 isolates (1968-1975)

MIC (U/ml)	No. of isolates
0.009	108
0.018	37
0.037	31
0.075	10
0.150	8
0.310	12
0.620	6

TABLE 2. *Change in susceptibility of group B streptococcal isolates to penicillin G (1968-1975)*

Year	No. of isolates	% Requiring for inhibition MIC > 0.075 U/ml
1968	15	0
1969	15	0
1970	17	0
1971	18	0
1972	30	10
1973	37	21.6
1974	39	17.6
1975	41	24

The MIC levels for the group D controls varied widely and are not included. There were no detectable differences in the susceptibility before and after freezing and storage.

This preliminary report of group B strepto-

cocci isolated from human sore throats indicates that a small but significant percentage of streptococci showed increased resistance to penicillin G. We cannot be sure that this same pattern will apply to group B streptococci from vaginal cultures.

LITERATURE CITED

1. Fry, R. M. 1938. Fatal infections by hemolytic streptococcus Group B. *Lancet* 1:199-201.
2. Lancefield, R. C. 1933. A serological differentiation of human and other groups of hemolytic streptococci. *J. Exp. Med.* 57:571-595.
3. Moody, M. D., A. C. Siegel, B. Pittman, and C. C. Winter. 1963. Fluorescent antibody identification of Group A streptococci from throat swabs. *Am. J. Public Health* 53:1083-1092.
4. Rantz, L. A. 1942. Streptococcus meningitis. *Ann. Intern. Med.* 16:716-726.
5. Rantz, L. A., and W. M. Kirby. 1942. Hemolytic streptococcus bacteremia. *N. Engl. J. Med.* 227:730-733.