

Resistance Trends of *Neisseria gonorrhoeae* in the Republic of Korea

MYRON V. PIZIAK,¹ CHARLES WOODBURY,¹ DANIEL BERLINER,² ERNEST TAKAFUJI,¹ JAMES KIRKPATRICK,¹ STEPHEN OPAL,¹ AND EDMUND TRAMONT^{1*}

Department of Bacterial Diseases, Division of Communicable Diseases and Immunology, Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, D.C. 20307,¹ and the Fifth Preventive Medicine Unit, Yongsan Garrison, Seoul, Korea APO 96301²

Received 20 April 1983/Accepted 14 September 1983

Penicillinase-producing *Neisseria gonorrhoeae* has increased in the Far East to the point that penicillin can no longer be recommended as the drug of choice, mandating a change to spectinomycin. As part of an ongoing surveillance of antibiotic susceptibilities, minimal inhibitory concentrations of penicillin, tetracycline, spectinomycin, trimethoprim-sulfamethoxazole, cefoxitin, ceftriaxone, cefotaxime, and moxalactam were determined. A disturbing, steady increase in resistance to spectinomycin was documented.

The advent of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) has necessitated that careful monitoring of antibiotic susceptibility patterns of gonococci be maintained. The incidence of gonococcal infections occurring among U.S. military personnel stationed in the Far East has always been relatively high (4). Beginning in September 1981, an alarming increase in the number of treatment failures associated with penicillin therapy occurred among U.S. military personnel stationed in the Republic of Korea (Fig. 1) and prompted this survey of antibiotic resistance patterns.

MATERIALS AND METHODS

Surveillance for PPNG. Strains of *N. gonorrhoeae* isolated in 1958 (17 strains) and 1974 (67 strains) were randomly collected and isolated as previously described (5). In 1982, 197 consecutive strains were collected and isolated over a 10-day period from four widely dispersed U.S. Army Sexually Transmitted Diseases Clinics serving approximately half of the U.S. military troops stationed in the Republic of Korea. A total of 64 strains (32 PPNG and 32 non-PPNG) were then selected for determination of their minimal inhibitory concentrations (MICs). All strains were confirmed by Gram stain, oxidase reaction, and degradation of dextrose but not sucrose, maltose, or lactose. The presence of penicillinase was determined by beta-lactam reagent disks (Marion Scientific Corp., Kansas City, Mo.). All of the strains collected survived transportation from Korea to Washington, D.C. All isolates were preserved in our laboratory by either lyophilization or freezing at -70°C in skim milk. A strain of *Staphylococcus aureus* of known antibiotic susceptibility was obtained from the Centers for Disease Control, Atlanta, Ga., and used as a control.

Medium. The MICs were determined by agar dilution susceptibility tests carried out on agar prepared from GC medium base (Difco Laboratories, Detroit, Mich.) plus defined supplement (3).

Antibiotics. Reagent solutions of penicillin (Sigma Chemical Co., St. Louis, Mo.), tetracycline (Bristol Laboratories, Syracuse, N.Y.), spectinomycin (The Upjohn Co., Kalamazoo, Mich.), cefoxitin (Merck Sharp & Dohme, West Point, Pa.), trimethoprim-sulfamethoxazole (Hoffmann-La Roche

Inc., Nutley, N.J.), ceftriaxone (Hoffmann-La Roche), cefotaxime (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), and moxalactam (Eli Lilly & Co., Indianapolis, Ind.) were prepared in distilled water, filter sterilized (0.20- μm Nalgene filter, analytical, type A), and adjusted to antibiotic base concentration.

Agar dilution technique. Antibiotic solutions were added to molten agar maintained at 56°C to give the desired concentrations, allowed to cool to 25°C , incubated overnight at 37°C , and then stored at 4°C for no longer than 10 days.

The bacterial inoculum was prepared by scraping the growth from a GC agar plate after 16 to 18 h of incubation at 37°C in a CO_2 candle jar and preparing a suspension in GC broth to a concentration of ca. 10^7 organisms. A 100-fold dilution was then made in GC broth.

With a modified Lidwell phage replicator apparatus, 0.03 ml of each suspension was transferred to each antibiotic-containing plate and incubated at 37°C in CO_2 for 24 h. The MIC was defined as the lowest antibiotic concentration that completely inhibited visible growth (5). *S. aureus* controls (see above) were used on each plate and the results tabulated only if the endpoint was the same \pm one dilution. No test had to be discarded.

The following twofold dilutions were used: penicillin, 0.12 to 32.0 $\mu\text{g}/\text{ml}$; tetracycline, 0.0125 to 32 $\mu\text{g}/\text{ml}$; cefoxitin, 0.188 to 6.0 $\mu\text{g}/\text{ml}$; ceftriaxone, 0.0008 to 0.025 $\mu\text{g}/\text{ml}$; cefotaxime, 0.0008 to 0.4 $\mu\text{g}/\text{ml}$; moxalactam, 0.0156 to 128 $\mu\text{g}/\text{ml}$; trimethoprim-sulfamethoxazole, 0.03125 to 16 $\mu\text{g}/\text{ml}$ and 0.594 to 30 $\mu\text{g}/\text{ml}$, respectively. Spectinomycin dilutions were 32, 16, 14, 12, 10, 8, 4, 2, and 1 $\mu\text{g}/\text{ml}$, respectively.

Isolation and characterization of plasmids. The isolation and characterization of plasmids were performed by agarose gel electrophoresis as previously described (2).

RESULTS

Of 197 consecutive strains collected over a 10-day period in October 1981, 91 (46%) were found to be beta-lactamase positive. The antibiotic susceptibilities of 32 randomly selected non-PPNG strains, in contrast with those of isolates collected in 1958 and 1974, demonstrated a trend towards a steadily increasing resistance to penicillin (Table 1). On the other hand, resistance to newer and lesser-used antibiotics had not yet occurred. Resistance to tetracycline appears not to have increased over the 8-year period of 1974 to 1982.

* Corresponding author.

TABLE 1. Antibiotic susceptibilities of *N. gonorrhoea* isolated from U.S. military personnel in the Republic of Korea^a

Antibiotic	MIC ₉₅ ^b (μg/ml) in indicated yr:				MIC range (μg/ml) in indicated yr:			
	1958	1974	1982		1958	1974	1982	
			Non-PPNG	PPNG			Non-PPNG	PPNG
Penicillin	0.25	4.0	8.0	>32.0	0.001-0.5	0.02-2.0	0.05-8.0	0.12->32.0
Tetracycline	0.4	>3.2	>3.2	>3.2	0.1-0.4	0.1-3.2	0.2-3.2	0.2-3.2
Spectinomycin	10.0	14.0	≥32.0	≥32.0	1.0-10.0	1.0-14.0	8.0-≥32.0	1.0-32.0
Cefoxitin	ND ^c	6.0	6.0	6.0	ND	0.19-6.0	0.19-3.0	0.19-6.0
Cefotaxime	ND	0.2	0.4	0.2	ND	0.003-0.4	0.003-0.2	0.0125-0.1
Ceftriaxone	ND	0.0015	0.006	0.003	ND	≤0.0008->0.025	≤0.0008->0.025	≤0.0008-0.006
TMP/SMZ ^d	ND	4/76	4/76	2/38	ND	0.125/2.3-2/38	0.06/1.18-4/76	0.125/2.3-2/38
Moxalactam	ND	2.0	2.0	0.5	ND	≤0.01-2.0	0.125-2.0	0.125-0.5

^a *N. gonorrhoeae* is generally considered susceptible at the following MICs (micrograms per milliliter): penicillin, <2; tetracycline, 1; cefoxitin, <1; cefotaxime, 0.062; trimethoprim-sulfamethoxazole, 0.95 and 15, respectively; and moxalactam, ≤2. The MIC of spectinomycin has not yet been determined.

^b MIC₉₅, MIC at which 95% of strains tested were inhibited; 32 strains isolated in 1974 and 64 strains isolated in 1982 were tested. Only 17 strains from 1958 were available.

^c ND, Not done.

^d TMP/SMZ, Trimethoprim-sulfamethoxazole.

Resistance to spectinomycin showed a gradual increase. Spectinomycin usage increased coincident with treatment failures with penicillin. None of the strains required the

massive amounts of spectinomycin (>1,000 μg/ml) that previously reported treatment failures required (1).

The plasmid profile of 15 randomly selected PPNG strains revealed the 24-, 4.4-, and 2.4-megadalton plasmids previously described for strains isolated in the Far East. (2). All PPNG strains tested had the plasmid.

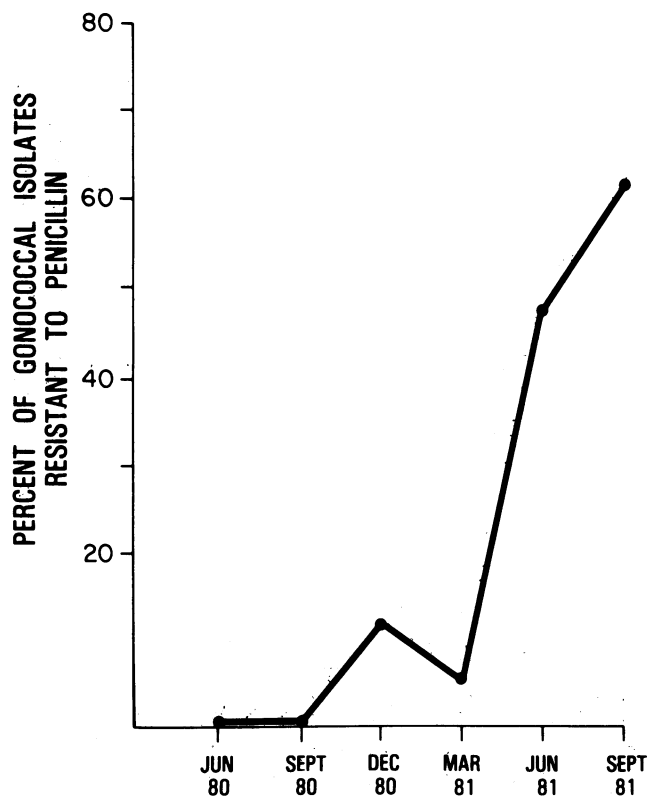


FIG. 1. Prevalence of PPNG isolated from patients cultured for gonorrhea. Curve represents cultures done at the 121 Evacuation Hospital, Seoul, Korea, and principally represents cultures taken from female patients and treatment failures. Until September 1981, penicillin resistance was determined by the use of a 6-μg penicillin disk (6). Most treatment failures were sent to the hospital for evaluation. Thus, simple, routine testing with a penicillin disk was used to monitor the incidence of penicillin resistance. Actual testing for PPNG during 1976, 1977, and 1978 revealed less than five isolates per year (<0.005%), and all were traced to transients at a U.S. military air base. The increase in PPNG isolates was dramatic. Of 197 consecutive cultures, 46% were shown to be PPNG (Epicon Report, Walter Reed Army Institute of Research, 1982).

DISCUSSION

Since the emergence of PPNG in the Far East, an active surveillance system has been maintained by the U.S. military to mark the spread of these strains. However, the emergence of PPNG and the rapidity at which it attained such high levels was unexpected. The resistance plasmids were the same as those described for PPNG strains previously isolated in the Far East. Thus, this epidemiological phenomenon cannot be explained on the basis of new or markedly different plasmids or the incorporation of beta-lactamase genes into the chromosomal DNA.

The degree of resistance of non-PPNG strains to penicillin has also shown a steady increase, whereas the degree of resistance to tetracycline appears to have leveled off (Table 1). Previous studies have demonstrated that resistance to these two antibiotics tend to parallel each other (5).

One of the more disturbing findings in this survey was the apparent steady increase in resistance to spectinomycin from the base-line levels of 1958. The clinical utility of spectinomycin as the drug of choice in areas where PPNG occurs in significant numbers (i.e., >5% of cases) appears to be threatened and probably will be less useful in the near future. Resistant strains have already been isolated (1; unpublished data).

As expected, the resistance patterns to the newer and heretofore little-used cephalosporin antibiotics have remained constant, and the organisms have remained susceptible. Resistance to trimethoprim-sulfamethoxazole has also remained constant. It will be of interest to follow resistance patterns as the use of this antibiotic increases for other purposes (i.e., urinary tract infections and diarrhea prophylaxis).

On the basis of these findings, the recommended treatment for gonorrhea in U.S. military troops in Korea has been changed to 2 g of spectinomycin administered intramuscularly, but continued surveillance is being maintained to monitor trends in antibiotic resistance.

ACKNOWLEDGMENTS

We thank Janet Morales and Bruce Innis for technical support, Gerald Seitter for command support, and Jean Guidas for secretarial support.

LITERATURE CITED

1. Ashford, W. A., H. J. Adams, S. R. Johnson, C. Thornsberry, D. W. Potts, J. C. English, J. W. Biddle, and H. W. Jaffe. 1981. *Lancet* ii:1035-1037.
2. Elwell, L. P., M. Roberts, L. W. Mayer, and S. Falkow. 1977. Plasmid-mediated beta-lactamase production in *Neisseria gonorrhoeae*. *Antimicrob. Agents Chemother.* 11:528-533.
3. Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *J. Bacteriol.* 85:1274-1279.
4. Patient Administration Office. 1981. RCS-MED 302 Reports. Office of the Surgeon General, U.S. Army, Washington, D.C.
5. Wilson, C., D. L. Rose, and E. C. Tramont. 1976. Increased antibiotic resistance of *Neisseria gonorrhoeae* in Korea. *Antimicrob. Agents Chemother.* 9:716-718.
6. World Health Organization. 1978. *Neisseria gonorrhoeae* and gonococcal infections. W.H.O. Tech. Rep. Ser. 616:138.