

SEATO MEDICAL RESEARCH STUDY ON VENEREAL DISEASES

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Study Report

Title: Studies on the causes of Penicillin-failures in the Treatment of Gonorrhoea

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Objective - The objectives of this study were to investigate reported penicillin failures in the treatment of gonorrhoea by studying the sensitivities of the causal organisms and the blood levels of antibiotics of patients.

Description - Studies during this period consisted of monitoring antibiotic sensitivities of strains of Neisseria gonorrhoeae isolated from military personnel and civilians in Thailand and evaluation of therapeutic regimens in gonorrhoea patients. Initial isolations of N. gonorrhoeae were made on Thayer-Martin medium and a medium described by Mandel et al was used for isolation of Mimae-Herellea organisms. Blood agar was used for isolation of other concomitant bacteria. Sensitivity studies of isolates of N. gonorrhoeae to antibiotics were carried out on a solid medium consisting of 4.5 percent proteose~~++~~3, 1.0 percent hemoglobin, 1.0 percent Supplement B, and the desired concentration of the antibiotic to be tested. The tube dilution technique employing trypticase soy broth was used for sensitivity studies of isolates of staphylococci.

Progress - Antibiotic sensitivities of isolates of N. gonorrhoeae isolated from 1 January 1965 to 31 March 1966 are shown in Table 1. None of the more than 300 strains tested were resistant in vitro to readily achievable blood levels of penicillin G or tetracycline. However, there does appear to be a tendency of isolates of N. gonorrhoeae to become gradually more resistant to both antibiotics. Notable was the finding that none of 15 isolates from Udonthani tested in December 1965 were sensitive to 3.0 mcg/ml of tetracycline.

Evaluation of three additional therapeutic regimens were carried out in inmates at the Institution for Socially Handicapped Women at Pakret, Nondhaburi province. It was shown that a single oral dose of 2.0 g of tetracycline was adequate for conversion from positive to negative smears and cultures in the 5 patients studied. The same results were obtained with a regimen consisting of 300 mg oral methacycline q.i.d. for 3 days. At the request of Thai authorities, a regimen consisting of 2.4 megaunits of benzathine penicillin weekly for three weeks was evaluated to see if a penicillin blood level higher than the minimal inhibitory concentration of most strains could be maintained indefinitely in patients at high risk. All but one of the four patients completing the series converted from positive to negative smears and cultures. It is felt that such a regimen is unadvisable because the dosage was very high, patient acceptance was poor, side effects such as fever and nausea were noted, and penicillin blood levels achieved were such as to favour emergence of resistant strains of gonococci.

Thai physicians' reports of the efficacy of combined penicillin and tetracycline in the treatment of gonorrhea implied that these two antibiotics might exert a synergistic rather than the expected antagonistic effect against N. gonorrhoeae. Various mixtures of these antibiotics in culture media indicated a synergistic effect against four strains and an additive effect for six strains. There was no evidence of an antagonistic effect. This study was continued with sera from individuals treated with penicillin, tetracycline, or a combination of these two antibiotics. It was found that sera from all individuals were bactericidal for gonococci and attempts to destroy this effect of the sera resulted in decrease of the amounts of antibiotics therein.

Two therapeutic studies involving American servicemen were carried out during this period. The first was to determine the effectiveness of penicillin therapy in the dosage recommended in TB Med 230 in culture-proven cases of gonorrhea and to examine causes of treatment failures. Twenty-six patients with a history of venereal contact and penile discharge were studied. All but two patients with positive smears were treated with a single intramuscular injection of 2.5 million units of procaine penicillin. Both exceptions had been previously treated with two injections of penicillin followed by a 10 day course of tetracycline without clearing of the discharge, so they were treated with erythromycin. N. gonorrhoeae was isolated from 14 subjects, coexisting with Mimae-herellae in one and coagulase-positive Staphylococcus aureus in another. Stained smears showed intracellular gram-negative diplococci in every patient from whom gonococci were isolated, from 4 of 8 with negative gram cultures and in 3 of 4 patients with two or more organisms other than gonococci.

The only treatment "failure" was a patient who had a positive smear and culture 24 hours after penicillin treatment. Further treatment was withheld for several days and when a coagulase-positive S. aureus was isolated he was treated with erythromycin in a dosage of 2 g per day for 10 days. The discharge ceased by the third day of erythromycin therapy and cultures were negative. The strains of N. gonorrhoeae isolated were not unusually resistant to penicillin and a single intramuscular injection of 2.5 million units of procaine penicillin appeared to give adequate peak and 24 hour blood levels. This limited study suggests that penicillin therapy as outlined in TB Med 230 was adequate for the strains of N. gonorrhoeae isolated in central Thailand.

In the Bangkok area 120 specimens from 102 U.S. Air Force patients were studied. Thirteen of 47 cultures positive for N. gonorrhoeae were in pure culture and 34 were isolated concomitantly with organisms such as S. aureus, Staphylococcus epidermidis, diphtheroids, streptococci, Mimae-herellae and enterobacteriaceae. Overall there were 21 different species isolated from the urethral exudates of these patients. Penicillin sensitivities of the 47 strains of N. gonorrhoeae ranged from 0.12-0.84 mcg/ml with a median sensitivity of 0.24 mcg/ml while sensitivities to tetracycline ranged from 0.4-3.2 mcg/ml with a median of 2.0 mcg/ml. Penicillin sensitivities of the 71 strains of staphylococci ranged from < 0.78-50 mcg/ml with a median of 6.25 mcg/ml and tetracycline sensitivities ranged from < 0.78 to > 200 mcg/ml. The increasing resistance of staphylococci to tetracycline appears to be part of a pattern evolving in Thailand in which many bacterial species formerly sensitive to that drug are becoming increasingly resistant.

Non-specific urethritis was the initial diagnosis for 9 of the 47 cases of gonorrhea confirmed by culture whereas there were 28 instances in which there was a clinical diagnosis of gonorrhea and the cultures were negative for N. gonorrhoeae. Antibiotics are readily available from commercial sources in Bangkok and it is likely that many of the latter group attempted self-medication prior to cultures being taken.

Thirty-seven gonorrhea patients were treated with a regimen consisting of a single intramuscular injection of benzathine penicillin and 500 mg of tetracycline q.i.d. for 5 days. All but two were considered clinical cures. The urethral exudate of one continued to contain gram negative intracellular diplococci for 4 days but no cultures were taken. He was then successfully treated with a regimen consisting of 2.4 million units of benzathine penicillin plus 500 mg chlortetracycline q.i.d. for five days. The urethral discharge of the second patient contained S. epidermidis sensitive to 12.5 mcg/ml and N. gonorrhoeae sensitive to 0.36 mcg/ml of penicillin. The urethral discharge stopped on the second day of therapy but recurred 4 days later at which time a pure culture of N. gonorrhoeae was isolated. This isolated was resistant to 0.6 mcg/ml of penicillin. The patient was considered a therapeutic failure because the organism apparently persisted and became more resistant to penicillin. He responded to a second course of benzathine penicillin and oral tetracycline.

Summary - These studies indicate that strains of N. gonorrhoeae are gradually becoming more resistant to penicillin and tetracycline. The penicillin therapy recommended in TB Med 230 dated 9 July 1965 or a regimen consisting of 2.4 million units of benzathine penicillin plus 500 mg tetracycline q. i. d. for 5 days is adequate for most strains of gonococci isolated in Thailand at this time.

Table 1

Antibiotic Sensitivities of Isolates of Neisseria gonorrhoeae Isolated in Thailand from 1 January 1965 - 30 April 1966

Month	# of cultures	Penicillin (mcg/ml)		Tetracycline (mcg/ml)	
		Range	Median	Range	Median
January 1965	8	0.06 - 0.48	0.24	0.6 - 2.6	1.4
February	60	0.06 - 0.60	0.36	0.4 - 2.2	1.2
March	55	0.06 - 0.72	0.30	0.6 - 2.2	1.2
April	31	0.06 - 0.18	0.12	1.0 - 2.2	1.8
May-August	17	0.06 - 0.48	0.24	0.6 - 2.4	1.4
September	19	0.12 - 0.72	0.30	1.2 - 2.6	1.8
October	39	0.18 - 0.72	0.30	0.4 - 2.4	2.0
November	8	0.12 - 0.36	0.18	1.4 - 3.2	2.2
December	16	0.12 - 0.72	0.24	3.2 - 3.8	3.2
February 1966	13	0.12 - 0.72	0.24	0.8 - 2.8	1.6
March	29	0.03 - 0.72	0.48	1.2 - 2.6	2.0