

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 in terms of antibiotic sensitivity of N. gonorrhoeae and concomitantly occurring organisms. Laboratory support was provided the U.S.A.F. dispensary, Takhi, Thailand, in an attempt to better evaluate the extent of therapeutic problems and to perhaps determine an effective therapeutic regimen.

A limited number of antibiotic sensitivity tests were conducted on isolates of N. gonorrhoeae during this reporting period. All of these isolates were from caucasian males stationed at Takhli RTAFB (central plains area). The range of penicillin was 0.06 to .72 mcg/ml, with a median of .18 mcg/ml. The range of tetracycline sensitivity was 1.0 to 2.8 mcg/ml, with a median of 1.8 mcg/ml. (Table I). These data are too limited to make comparison with the antibiotic sensitivities of N. gonorrhoeae determined in 1965. A concerted effort will be made during the next two months to obtain from cases of gonorrhoea occurring in both males and females at Korat (central plateau), Bangkok, Udorn (northeast), Ubol (northeast), Songkhla (south), and Sattahip (southeast).

A study was initiated by LTC Kenneth Gould at the USAF dispensary, Takhli RTAFB, in order to evaluate the efficacy of 7 therapeutic regimens for urethral disease. The need for such a study was obvious since there is limited coordination and uniformity of policy on the treatment of gonorrhoea among the military stationed in Thailand. The Department of Bacteriology & Mycology furnished the laboratory support for this study, and SSG John Bell was placed on temporary duty at Takhli to provide this support. N. gonorrhoeae cultures were returned to SMRL for confirmatory studies. The following regimens were studied: A-procaine penicillin, 2.4 M units IM STAT; B-procaine penicillin, 2.4 M units IM STAT X 2 days; C-procaine penicillin, 2.4 M units IM STAT plus tetracycline, 250 mgm p.o. QID X 5 days; D-tetracycline, 250 mgm p.o. QID X 5 days; E-tetracycline, 2.0 gms p.o. STAT (single dose); F-procaine penicillin, 1.2 M units IM STAT plus tetracycline, 250 mgm p.o. QID X 5 days; and G-tetracycline, 500 mgm p.o. QID X 5 days. A brief summary of this study will be included since this laboratory acted only in a collaborative capacity. A total of 730 patients complaining of urethritis were seen in the dispensary, however, only 288 of these met the requirements of the protocol and were placed on one of the above therapy regimens. The most effective regimens were C, G, B, and F, with essentially identical cure rates (83.3% — 88.7%). Regimens A and E were discontinued after preliminary results showed them to be inadequate. These data will be collated and submitted for publication by LTC Kenneth Gould.

Table II lists the organisms isolated from 208 moles with urethritis. N. gonorrhoeae was isolated from 73.6% which is an unusually high recovery. Perhaps the reason for this success, was the great care exercised in the processing of these cultures. Specimens were inoculated and incubated immediately after collection. The organism most frequently associated with N. gonorrhoeae, or in combination with other

organisms, was Staphylococcus epidermidis (144/208, 69.2%). Mimae-herella, which have been implicated as etiologic agents in penicillin resistant urethritis, were isolated from only 9 patients. Isolation of N. gonorrhoeae was also made from two of these 9 patients. The significance of staphylococci in urethritis requires further investigation in terms of its ability to perpetuate a urethritis after the initiating N. gonorrhoeae have been eliminated by penicillin therapy. These data suggest that gonorrhea in South East Asia may frequently be complicated by several concomitantly occurring organisms which could influence not only the course of the disease but also the therapeutic efforts.

Early in this reporting period, an investigation was initiated to evaluate a rapid (10-15 minute) presumptive diagnostic test for gonorrhea. This test, as reported (Pub. Health Rep. 81:318, 1966) uses commercially available paper-test-strips for the detection of cytochrome oxidase in urethral exudates as a presumptive test for N. gonorrhoeae. A simple, reliable, inexpensive and rapid diagnostic aid for gonorrhea has practical application in many military dispensaries where adequate laboratory support is not available to the clinician. For these reasons, evaluation of this technique was undertaken. A collaborative study was begun with the USAF dispensary, Don Muang in August 1966. After several months, it became obvious that the difficulties involved in maintaining a fresh supply of media and transportation for the return of inoculated media resulted in an unsatisfactorily low percentage of isolations of N. gonorrhoeae. A similar study was then undertaken at the Takhli USAF dispensary. To standardize the techniques and to handle the extra workload, SSG John Bell from the Department of Bacteriology & Mycology was assigned temporary duty at Takhli.

A history was obtained from each patient, the paper-strip oxidase test performed, a gram-stained smear prepared, and cultures inoculated and incubated immediately. The results of these three tests (oxidase smear & culture) were compared to determine the reliability of the oxidase test as a rapid diagnostic aid. Urethral exudate was inoculated onto the following media; chocolate agar (GC medium plus hemoglobin, supplement B, ristocetin and polymyxin B sulfate); blood agar; and Mimae-herella agar. Approximately 100 specimens were also inoculated onto PPLO agar. Cultures were incubated at 37°C, with 5% added CO₂, for 48 hours. Plates were examined at 24 hours for sufficient growth, and suspicious colonies of N. gonorrhoeae subcultured for confirmation at SMRL. Organisms other than N. gonorrhoeae were also identified. Occasionally, difficulties with transportation of the fastidious N. gonorrhoeae subcultures from Takhli to Bangkok resulted in the loss of viability. Therefore, more significance had to be placed on the identification procedures used at Takhli. The capability for detection of N. gonorrhoeae by fluorescent antibody microscopy was developed at SMRL. Subsequently, duplicate smears were sent to Bangkok for examination by this procedure.

A summary of data obtained from 318 caucasian males, reporting to the dispensary with urethral disease, shows an 89.30% (284/318) correlation of the oxidase test with smear or culture. There were, however, 9.43% (30/318) of the patients who had a positive oxidase test and a negative smear and culture. The oxidase test results were, therefore, considered to be "false positives" among these individuals. Negative oxidase tests were obtained in only 4 persons (1.26%) with either positive smears or from whom N. gonorrhoeae was recovered. Our data show a closer agreement of the cytochrome oxidase test with smear and culture than found in the original report (89.3% vs 78%). The closer agreement in our study is undoubtedly due to the population studied, since most of the urethral discharges were classical gonorrhea. The majority of the 9.4% "false positives" were noted among patients with scanty or non-purulent discharges. The cytochrome oxidase test is certainly not the best diagnostic technique available, but might have a place in small dispensaries where adequate laboratory facilities are not available. The oxidase test in this study approaches the reliability of the gram-stained smear (89.3% vs 94.7%) even when performed by an experienced technician. The low (1.26%) "false negatives" might make this a useful screening test for ruling out N. gonorrhoeae.

A preliminary investigation was begun to determine the reasons for the "false positive" oxidase reactions. Urethral infections with organisms, other than N. gonorrhoeae, which produce cytochrome oxidase (i.e. pseudomonas, Aeromonas, vibrios, Alcaligenes and Flavobacteria) were ruled out on the basis

of the culture results. Empirical testing showed WBC's, from the buffy coat of peripheral blood, capable of producing a typical blue color on the oxidase strip within the 10 to 15 minute period of the test. Additional testing is required to determine whether intact WBC's are capable of releasing cytochrome oxidase, or whether they must be ruptured before the enzyme can be released. Pus from a variety of sources was cultured and tested for cytochrome oxidase activity. Most of the specimens, even when not containing the organisms listed above, produced a blue color, but usually the time required was from 30 minutes to 2 hours. It, therefore, becomes extremely important to standardize the time for reading the test strips, and to restrict all readings to less than 30 minutes. An interesting observation which requires confirmation and further investigation, is the finding that fluid from blisters will produce a positive cytochrome oxidase reaction within 10 to 15 minutes after application to the test paper.

Summary — Antibiotic sensitivities of N. gonorrhoeae were determined on a limited number of strains and none were found to be exceptionally resistant to either penicillin or tetracycline. Seven therapeutic regimens for urethritis were evaluated among 288 caucasian males, and five regimens were found to be of equal efficacy (83.3% — 88.7%). Two regimens were inadequate and dropped from the study. N. gonorrhoeae was the predominating organism (73.6%) recovered from 208 males with urethritis. The organism most frequently associated with N. gonorrhoeae, or in combination with other organisms was S. epidermidis (69.2%). Mimae-herella were isolated from only 9 patients. Isolation of N. gonorrhoeae was also made from 2 of these 9 patients. A simple, inexpensive, rapid diagnostic aid, cytochrome oxidase paper test strips, were found to have good correlation (89.3%) with gram-stained smears and cultures. Only 1.26% of the oxidase tests could be considered as "false negatives", whereas 9.4% were "false positives". The good correlation can be attributed in part to the population studied, since the percentage of classical gonorrhea was high and a minimum number of patients had scanty discharges or "NSU". It was among the latter patients that the majority of the false positives were noted. This test may have application in remote areas where laboratory support is minimal or non-existent.

Table I

Antibiotic Sensitivity of isolates of Neisseria gonorrhoeae Isolated in Thailand

	Conc. of Drug (mcg/ml)									
	.72	.6	.48	.36	.30	.24	.18	.12	.06	.03
Penicillin	1	—	—	—	1	3	14	7	1	—

Median .18 mcg/ml

	Conc. of Drug (mcg/ml).									
	2.8	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.0
Tetracycline	1	7	2	3	1	3	2	3	3	2

Median 1.8 mcg/ml.

Table II

Organisms recovered from 208 Males with Urethritis

Organisms	No. isolates
<u>N. gonorrhoeae</u>	46
<u>N. gonorrhoeae</u> + Staph. epidermidis	41
" " + alpha streptococci	16
" " + micrococci	12
" " + staphylococci + alpha streptococci	11
" " + diphtheroids	10
" " + staphylococci + enterococci	4
" " + diphtheroids + alpha streptococci	3
" " + staphylococci + klebsiella	2
" " + alpha streptococci + E. coli	1
" " + pseudomonas	1
" " + mimaë-herella group	2

The following organisms were isolated single and in various combinations

<u>Staphylococcus epiaermidis</u>	103
<u>Staphylococcus aureus</u>	6
<u>Alpha streptococci</u>	49
<u>Enterococci</u>	41
<u>Micrococci</u>	37
<u>Diphtheroids</u>	37
<u>Pseudomonas</u>	6
<u>Candida</u>	6
<u>Klebsiella-Aerobacter</u>	2
<u>Hemophilus</u>	4
<u>E. coli</u>	3
<u>Mimæ-Herella group</u>	7
<u>No growth</u>	26