Changing Penicillin Resistance of the Gonococcus in Thailand

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OBJECTIVE: To determine if the penicillin resistance of the gonococcus in Thailand is changing.

BACKGROUND: Although the relative resistance of the gonococcus to penicillin has been shown to be decreasing in two geographical locales (1,2), most reports indicate that the organism is becoming more resistant at a very alarming rate (3, 4).

DESCRIPTION: Test organisms consisted of all clinical isolates of *N. gonorrhoeae* submitted for confirmation to the Department of Microbiology, SEATO Medical Research Laboratory, Bangkok, Thailand between 1 January 1972 and 31 December 1974. Specimens were clinical isolates submitted from clinical facilities in Thailand. These facilities included: Royal Thai Army Hospital, Bangkok; US Army Hospital, Bangkok; and SEATO Medical Research Laboratory venereal disease field unit. The number of isolates evaluated was: 752 in 1972; 622 in 1973; and 867 in 1974.

All organisms were confirmed to be *N. gonorrhoeae* by standard techniques previously reported. Organisms confirmed as *N. gonorrhoeae* were grown on PB plates (Proteose #3 agar + 1% Hemoglobin + 1% IsoVitaleX*) overnight. The colonies were scraped off with a sterilized swab and heavy suspensions were prepared in tryptose phosphate broth. The suspensinos were standardized spectrophotometrically (Coleman Jr. II, Spectrophotometer, Coleman Instruments Maywood, Ill.) with a $BaSO_4$ solution (0.5 ml 1% $BaCl_2$ + 99.5 ml 1% H_2SO_4) to a final bacterial count of 10^7 colony forming units/ml. Inoculum—replicating equipment (Div. of Instrumentation, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.) placed 0.001—0.003 ml of inoculum in a spot on the surface of a plate containing Mueller—Hinton Agar + 5% defibrinated, chocolatized sheep blood and added 1% IsoVitaleX. Serial dilutions of penicillin G were added to the plates for penicillin minimum inhibitory concentration (MIC) determinations. The concentrations tested were: 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 units/ml. One additional plate to which no penicillin was added was also evaluated. A Staphylococcus strain, *S. aureus* ATTCC 6538 P was used as a control for the penicillin content of the plates. The plates were stored at 4°C after preparation and were employed within three days. Before use the plates were dried of excess moisture in the incubator. No moisture droplets were on the surface of the media or petri dish lids when inoculations were made. The inoculated plates were evaluated after overnight incubation (20—24 hrs.). The MIC was determined as the smallest concentration of antibiotic required to inhibit growth completely. In reading the plates, a barely visible haze of possible growth or a single macroscopic colony was disregarded.

Three study populations consisting of all isolates submitted within a given calendar year were compared. In order to apply statistical methods in evaluating the data all isolates with MIC's of less than 0.5 units/ml or more than 2.0 units/ml were excluded. Thus the MIC data for 14 organisms in 1972, 27 in 1973 and 130 in 1974 were not used in calculating means, standard errors and significance of differences. The MIC results of 362 organisms collected between April 1972 and January 1973 have been discussed elsewhere (5). Analysis of variance was utilized to determine if between—group differences existed (6). Student's t test for unpaired variates was employed to determine the significance of differences between the mean MIC's of the organisms tested within a given year (6).

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PROGRESS: The mean penicillin minimum inhibitory concentration ± 2 standard errors for gonococci isolated in 1972, 1973 and 1974 were 0.58 ± 0.02, 0.72 ± 0.04 and 1.05 ± 0.04, respectively. Analysis of variance for between—group differences of these three populations was significant ($F = 215.5$, $df = 2067$, $p < 0.001$).

MIC data obtained in 1972 was significantly different from that of 1973 ($p < 0.001$). An identical result was found when the 1973 data was compared to 1974 ($p < 0.001$).

The data are graphically represented in Figures 1, 2 and 3. Each year the proportion of isolates with a penicillin MIC of more than 2.0 units/ml increased. The mean MIC ± 2 S.E.'s for each of the three years is displayed in Figure 4. Calculations of means and standard errors in Figure 4 were performed excluding the data extremes as explained above.

SUMMARY: Penicillin minimum inhibitory concentrations (MIC) were determined for 2241 gonococcal isolates submitted to the SEATO Medical Research Laboratory between 1 January 1972 and 31 December 1974. Isolates were separated into three groups, determined by the calendar year in which they were submitted. The mean penicillin MIC ± 2 standard errors was calculated for each of the three groups. These values were 0.58 ± 0.02 in 1972, 0.72 ± 0.04 in 1973, and 1.05 ± 0.04 in 1974. These means were found to be significantly different ($p < 0.001$).

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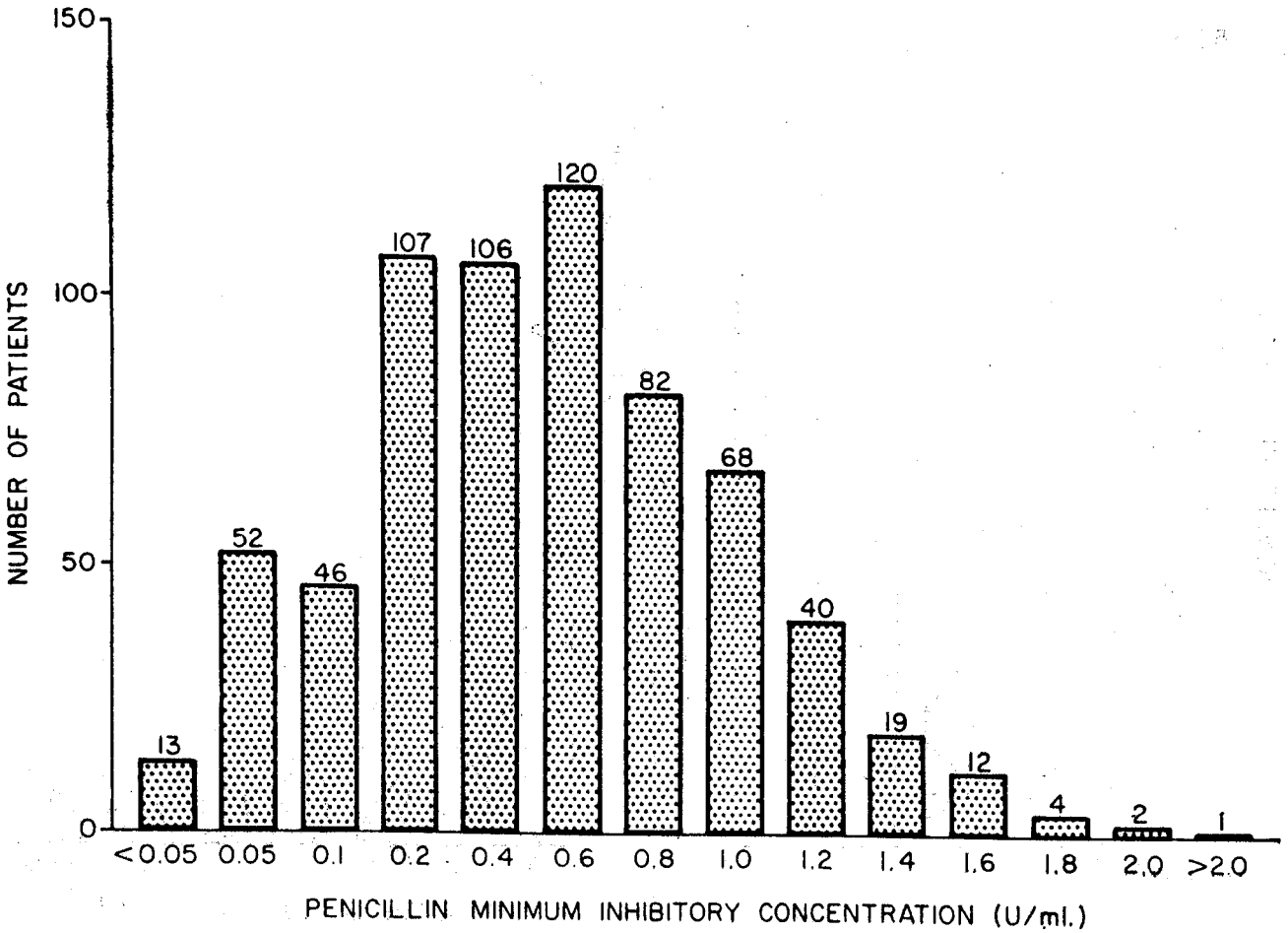

FIGURE I. PENICILLIN MINIMUM INHIBITORY CONCENTRATION FOR NEISSERIA GONORRHOEAE ISOLATED IN 1972. DATA EXTREMES (0.05U/ml. >MIC >2.0U/ml.) WERE NOT INCLUDED IN CALCULATIONS OF MEAN AND STANDARD ERROR

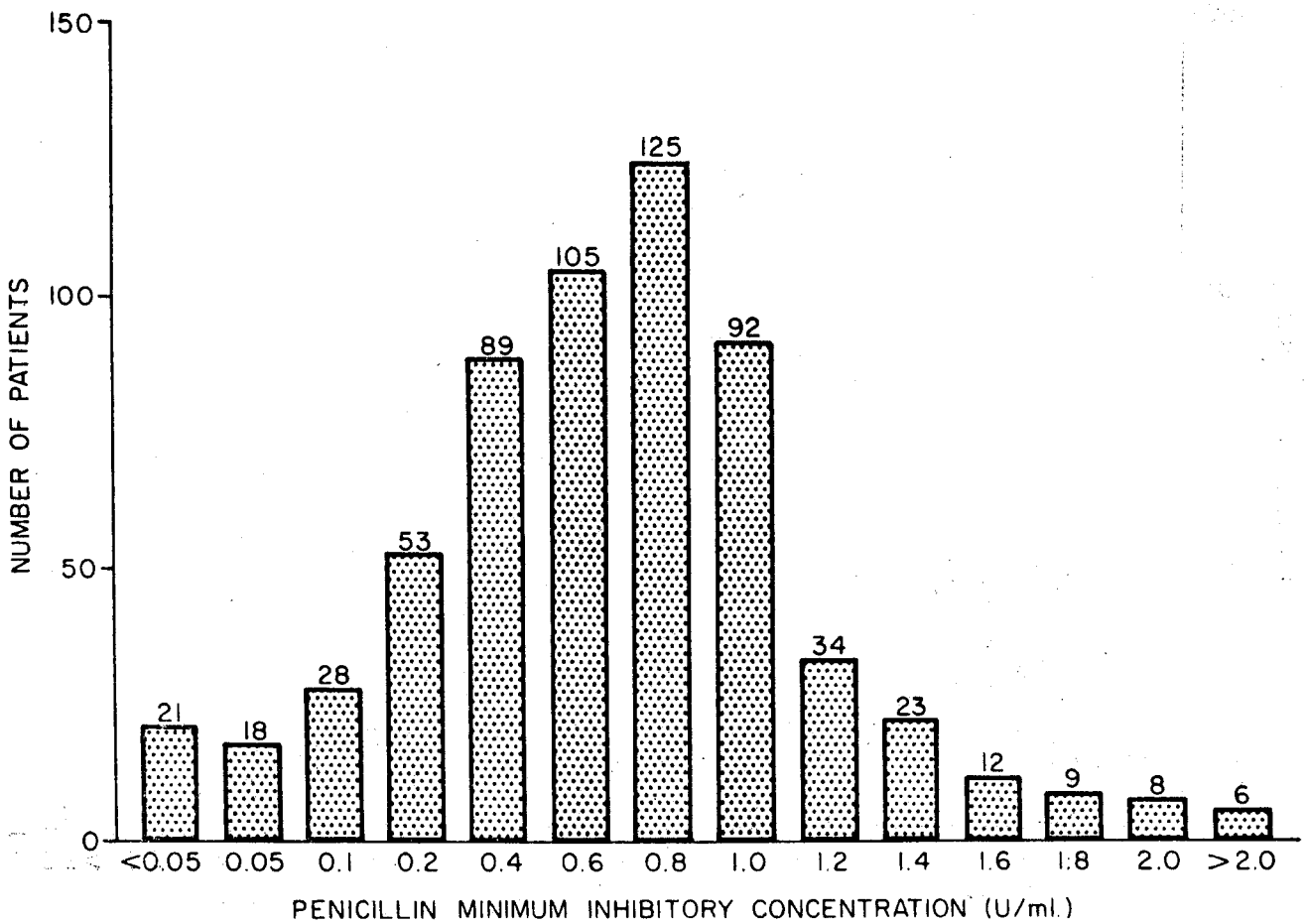

FIGURE 2. PENICILLIN MINIMUM INHIBITORY CONCENTRATION FOR NEISSERIA GONORRHOEAE ISOLATED IN 1973. DATA EXTREMES (0.05 U/ml. >MIC >2.0 U/ml.) WERE NOT INCLUDED IN CALCULATIONS OF MEAN AND STANDARD ERROR

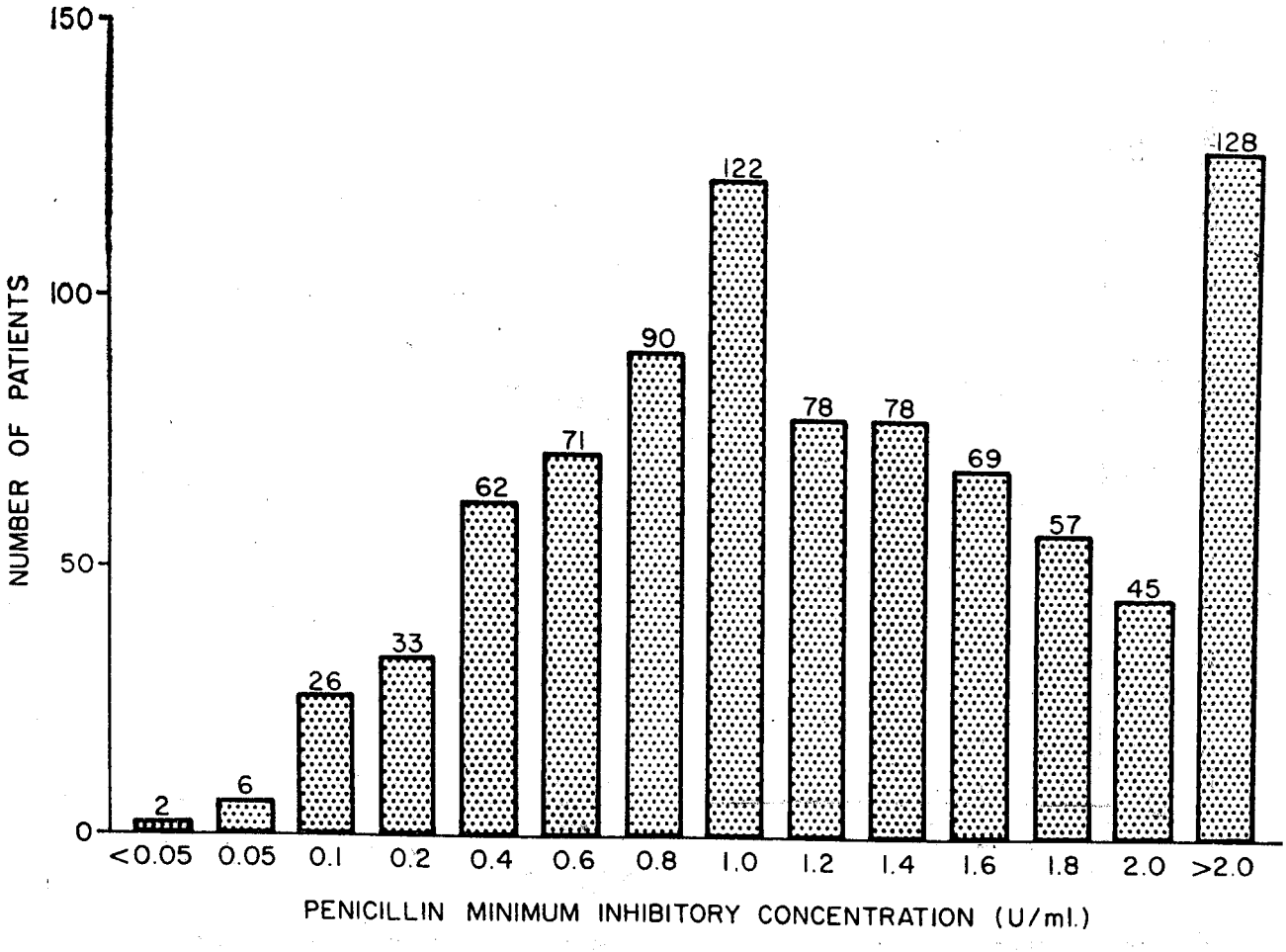

FIGURE 3. PENICILLIN MINIMUM INHIBITORY CONCENTRATION FOR NEISSERIA GONORRHOEAE ISOLATED IN 1974. DATA EXTREMES (0.05 U/ml. >MIC >2.0 U/ml.) WERE NOT INCLUDED IN CALCULATIONS OF MEAN AND STANDARD ERROR

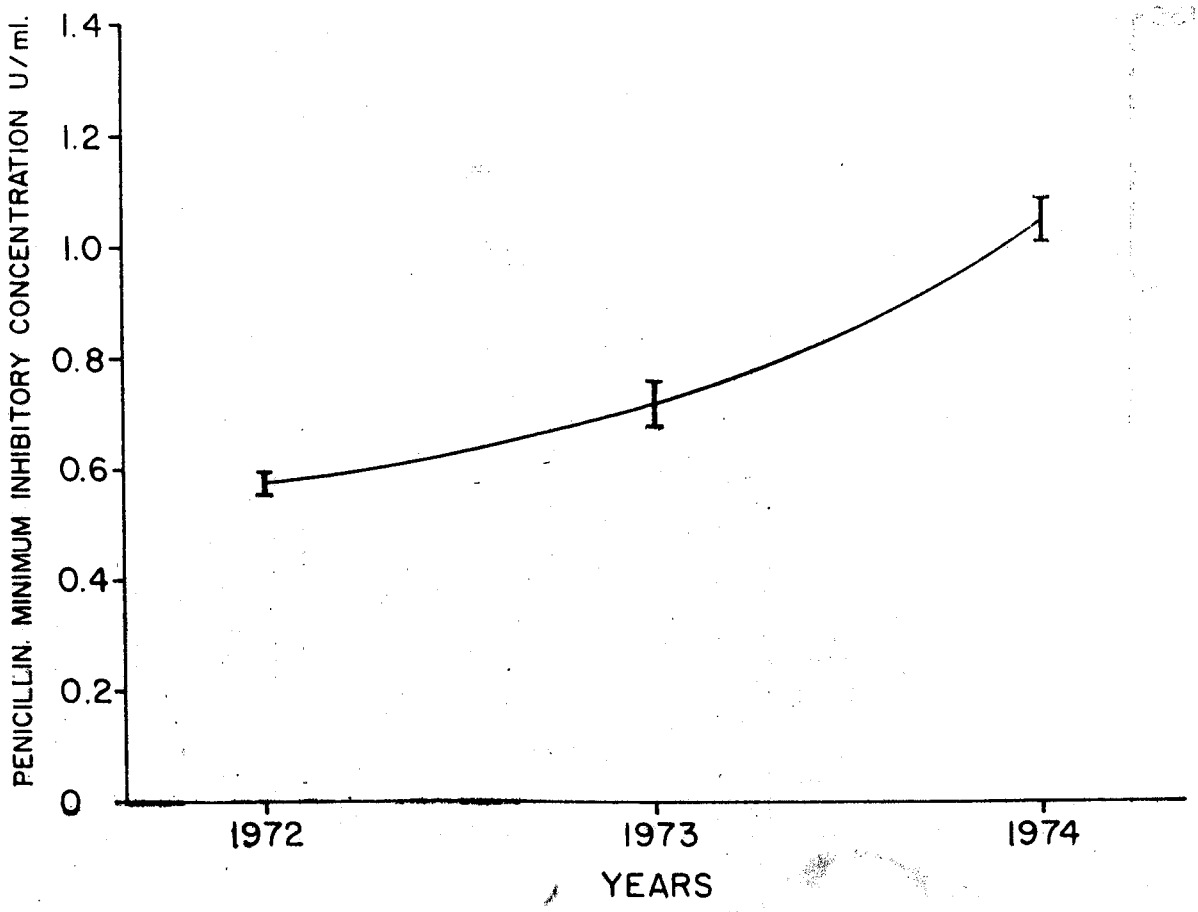

FIGURE 4. MEAN PENICILLIN MINIMUM INHIBITORY CONCENTRATION ± 2 S.E. FOR NEISSERIA GONORRHOEAE ISOLATED IN 1972, 1973 AND 1974. DATA EXTREMES (0.05U/ml. >MIC >2.0U/ml.) WERE DELETED IN MEAN AND STANDARD ERROR CALCULATIONS