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Surveillance Studies of *Neisseria gonorrhoeae* Sensitivity to Penicillin and Nine Other Antibiotics *

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As increasing numbers of gonococcal strains obtained in routine isolations from patients show reduced sensitivity to penicillin and as more and more patients report that they are allergic to penicillin, other antibiotics are being increasingly frequently resorted to in the treatment of gonorrhoea. It is therefore of importance to determine the susceptibility of both penicillin-sensitive and relatively "resistant" strains to these other chemotherapeutic agents.

The authors report on a study of the in vitro *action of the following antibiotics against routine gonococcal isolates and strains from gonorrhoea cases in which penicillin had failed to effect a cure: kanamycin, leucomycin, chloramphenicol, dextrosulphenidol, oxytetracycline, chlortetracycline, tetracycline, demethylchlortetracycline and synnematin B. It was found that strains of low penicillin susceptibility were as sensitive to these antibiotics —with the exception of synnematin B—as were those of high penicillin sensitivity.*

Emphasis is placed on the need for establishing an international standard procedure for gonococcal sensitivity testing which would make it possible—as it is often not at present—to compare results obtained in different laboratories. The relation between response to treatment and the degree to which patients develop blood and tissue concentrations of penicillin is also discussed.

PENICILLIN SENSITIVITY

In 1955 we reported the penicillin sensitivity of gonococcal strains obtained in routine isolations from female patients in South Carolina to be from 0.005 u/ml to 0.20 u/ml, a 40-fold range. Of these strains 20% required from 0.10 u/ml to 0.20 u/ml to inhibit their *in vitro* growth—concentrations that indicated a marked decrease in susceptibility when compared with the earlier reports by Lankford in 1945 and Romansky in 1946 and the Boston surveillance studies from 1945 to 1954 (Love & Finland, 1955). These authors found only three of 439 strains tested that would have required more than 0.06 u/ml to inhibit *in vitro* growth.

In further studies of gonococcal penicillin sensitivity in North and South Carolina, routine strains isolated in 1957 and 1958 were like those tested in 1955, i.e., about 20% required from 0.10 u/ml to 0.20 u/ml to inhibit growth. In 1959, however, about 30% of 368 strains isolated from patients in Charlotte, North Carolina, required similar concentrations to inhibit growth (Table 1)—a 10% increase over that for strains isolated in the four previous years.

A similar increase in the percentage of strains of lowered penicillin susceptibility has been noted in England (Cradock-Watson et al., 1958 ; Curtis & Wilkinson, 1958), in Germany (Schummer & Hubbes, 1951), and in Denmark (Reyn et al., 1958) ; and in the latter country the range of sensitivity has been expanded to 80-fold, some strains requiring as much as one unit to inhibit *in vitro* growth completely.

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TABLE 1

PENICILLIN SUSCEPTIBILITY OF 368 GONOCOCCAL STRAINS STUDIED IN CHARLOTTE, N.C., USA, 1959

Units per ml	Strains susceptible	
	No.	%
0.20	2	0.6
0.15	14	3.8
0.10	94	25.5
0.05 or less	258	70.1

During 1959 85 cultures of gonococci isolated from treatment failure cases were referred to us for penicillin sensitivity determination from city and State departments of public health in California, Georgia, Florida, Illinois, Michigan, Missouri, North Carolina, Ohio, South Carolina, Texas, and Washington State. This service is being offered by the Venereal Disease Experimental Laboratory to anyone desiring to have gonococci tested or verified for penicillin sensitivity.

The 85 cultures suspected of penicillin "resistance" were isolated from 79 treatment failure cases and ranged in sensitivity from 0.01 u/ml to 0.55 u/ml. Table 2 shows that 85% of these cultures required more than 0.10 u/ml to inhibit *in vitro* growth completely.

Several of the strains of decreased sensitivity were brought into the USA by merchant seamen and migrant workers from other countries.

TABLE 2

PENICILLIN SUSCEPTIBILITY OF 85 CULTURES OF GONOCOCCI SUSPECTED OF PENICILLIN RESISTANCE, ISOLATED FROM 79 TREATMENT FAILURES

Number of cultures	Units per ml inhibiting *in vitro* growth
13	$\leq$ 0.05
16	0.10
12	0.15
19	0.20
10	0.25
13	0.30
1	0.35
1	0.55

The relation of gonococcal strain sensitivity to treatment failure may be shown for some selected cases treated with repository penicillin.

Bicillin. On admittance, 15 male patients positive by smear examination were treated with 1.2 mega-units. All patients returned to the clinic within 2-21 days (median 5.5 days) with positive clinical and cultural findings. The sensitivity of the gonococcal strains from these patients varied between 0.10 u/ml and 0.30 u/ml. For the most part these patients denied re-exposure, but the possibility of reinfection cannot be excluded. All except two patients of this group were subsequently cured by a combined dose of 1.2 mega-units of Bicillin and 0.60 mega-units of PAM.

PAM. Similar findings to those in the Bicillin-treated patients were made for five patients treated with 0.6 mega-units of PAM. The patients returned to the clinic within 4-6 days. Gonococci were sensitive to between 0.10 u/ml and 0.30 u/ml.

Procaine penicillin. Five male patients treated with 1.2 mega-units returned to the clinic within 4-10 days with positive cultures. Strain sensitivity varied between 0.10 u/ml and 0.55 u/ml. Two women patients, positive by culture, failed to respond with a total dose of 3.6 mega-units, given in three injections of 1.2 mega-units each over a four-day period. Both patients were culturally positive seven days after the last treatment. Gonococcal sensitivity of 0.20 u/ml was found for both strains.

The above results of strain sensitivity would be more meaningful if the failure rates were known for the dose and type of repository penicillin used by the contributing clinic.

SENSITIVITY TO THE NINE OTHER ANTIBIOTICS

In a previous paper from this laboratory it was pointed out that therapy for gonorrhoea with antibiotics other than penicillin was resorted to when patients claimed they were allergic (Thayer, Perry, Field & Garson, 1960). Some clinics are attended by increasing numbers of such patients. Further, it was noted that other antibiotic therapy was used when the patient failed to respond to two or more courses of penicillin with clinical or bacteriological cure or both.

As noted above, it has been found that clinical failures to penicillin therapy usually correlate with gonococcal strains of decreased penicillin suscepti-

bility (85% in Table 2). Other laboratories have made similar observations (Curtis & Wilkinson, 1958 ; Reyn et al., 1958 ; Willcox, 1959). Because of the increasing number of these strains of reduced penicillin sensitivity and the untoward allergic reaction of patients to penicillin, it is of importance to determine the susceptibility of routine strains and those of known decreased penicillin sensitivity to the action of other antibiotics and chemotherapeutic agents (Thayer, Field & Garson, 1959 ; Thayer, Perry, Field & Garson, 1960 ; Hirsch et al., 1960).

The *in vitro* action of the following antibiotics was tested against routine gonococcal isolates and strains from penicillin treatment failure cases of gonorrhoea: kanamycin, leucomycin, chloramphenicol, dextrosulphenidol (a methylsulfonyl analogue of chloramphenicol), oxytetracycline, chlortetracycline, tetracycline, demethylchlortetracycline [1] and synnematin B.[2]

The method for performing susceptibility testing of the above antibiotics and penicillin consists of adding varying concentrations of the purified antibiotic to proteose peptone chocolate agar (Difco) and streaking the agar surface with a standardized inoculum of the gonococcal strains to be tested (US Public Health Service, 1956). After 48 hours' incubation, the plates are observed for growth. The concentration of antibiotic completely inhibiting visible growth is taken as the susceptibility of the strain.

The susceptibility range and average minimal inhibitory concentration (MIC) for the strains tested are listed in Table 3.

Gonococci of low penicillin susceptibility were as sensitive to the antibiotics tested, with the exception of synnematin B, as were gonococci of high penicillin sensitivity.

Synnematin B was tested against 54 strains of *Neisseria gonorrhoeae* of known penicillin G sensitivity. The results show that 27 gonococcal strains of reduced penicillin sensitivity (0.10-0.35 u/ml) required high concentrations of synnematin B to prevent growth and that 27 strains of increased penicillin sensitivity (0.005-0.05 u/ml) required low concentrations of synnematin B.

Synnematin B differs from penicillin G only by the substitution of the D-4-amino-4-carboxy-n-butyl moiety for the benzyl-methene radical. This difference in chemical structure is responsible for the non-reactivity of synnematin B when injected into the skin of passively or actively penicillin sensitive subjects (Berryman & Sylvester, 1960). The successful treatment of acute gonorrhoea of males by this drug has recently been reported. (Schwimmer & Henderson, 1959 ; Henderson et al. [3])

TABLE 3
IN VITRO SUSCEPTIBILITY OF GONOCOCCI TO NINE ANTIBIOTICS

Antibiotic	No. of strains	Susceptibility range (μg/ml)	Average MIC[a] (u/ml)
Kanamycin	83	4.0-12.0	8.30
Leucomycin	76	0.20-1.0	0.55
Chloramphenicol	117	0.25-0.50	0.44
Dextrosulphenidol	73	0.25-0.50	0.28
Chlortetracycline	56	0.12-0.50	0.26
Oxytetracycline	70	0.06-0.50	0.19
Tetracycline	70	0.06-0.25	0.18
Demethylchlortetracycline	70	0.03-0.12	0.09
Synnematin B	54	0.15-1.83	0.98

[a] Minimal inhibitory concentration.

The time required for kanamycin, leucomycin, dextrosulphenidol, tetracycline, and demethylchlortetracycline to sterilize broth cultures varied with the strain of gonococci. Using concentrations of antibiotics which were twice the minimal inhibitory dose for the test strains, killing began within 12 hours but sometimes as long as 48 hours were required to sterilize the culture. Clinical failures have occurred in some patients treated for less than two days with most of the drugs reported above. Blood concentrations in excess of the minimal inhibitory concentration for the most resistant gonococcal strains tested may be readily established and maintained by adequate dosage of the antibiotics reported here.

DISCUSSION

At present, it is impossible to compare gonococcal sensitivity determined by one author with that found by others. This is due to the lack of a common

[1] Supplied by Dr J. A. McMillen, Lederle Laboratories, New York.

[2] Supplied by Dr W. D. Henderson, Division of Laboratories, Michigan Department of Health.

[3] Henderson, N. D., Schwimmer, B. & Olson, B. H. (1960) *Treatment of acute gonorrhea in males with synnematin B* (paper read before the 11th Annual Symposium on Recent Advances in the Study of Venereal Diseases, Chicago, Ill., April 1960).

method for performing sensitivity testing ; present methods are as diverse as the numbers of authors performing the test. There is a paramount need to establish a standard international procedure for sensitivity testing of gonococci that will allow for interlaboratory comparisons and surveillance of gonococcal resistance in the world-wide study of gonorrhoea.

A few of the factors that cause variability of minimal inhibitory concentration for a given gonococcal strain within a given testing method may be mentioned : the medium may be unsuitable for the development of small numbers of gonococcal strains difficult to grow ; different lots of commercial peptones contain toxic substances that add their effect to penicillin in inhibiting growth ; some media contain substances that excessively bind or destroy penicillin.

The sensitivity of the test procedure is influenced by the number of gonococci in the inoculum. All unknown and control strains should be standardized to the same density.

The increments of penicillin concentrations over the range tested should not be too great ; a false impression of gonococcal sensitivity may be obtained, especially in the upper range, when twofold increments are used. Of course, only standardized penicillin G of known potency should be used since commercial injectable drugs may vary widely.

The factors in the medium itself which cause variability may be readily detected by including in the day-to-day assay two control cu'tures, i.e., a gonococcus strain of decreased penicillin sensitivity and *Sarcina lutea*, which is highly sensitive to penicillin. Variation in end-point of these cultures in excess of two standard deviations would indicate failure of the method for that day's test.

It will be noted from Table 2 that 15% of the strains isolated from treatment failure cases were presumed to be " resistant " but were actually highly sensitive, requiring 0.05 u/ml or less to inhibit growth. While some of these strains might have been from patients reinfected by sexual exposure during the follow-up period, careful interviewing elicited denial of any such re-exposure. The return of these patients to the clinic within five days or less lends support to a diagnosis of relapse rather than reinfection.

To understand the failure of presumed adequate dosage to cure gonococcal infections due to strains highly sensitive to penicillin, or, conversely, to understand the successful cure of patients infected with relatively " resistant " strains, one must look elsewhere than to the susceptibility of the gonococcal strain. A consideration of the relation of certain host factors to the drug and parasite may shed light on these anomalous observations.

Perhaps the most important host factor operating to explain the above findings is the manner in which and the degree to which treated patients develop blood and tissue concentrations of penicillin.

Repository penicillins were designed to allow slow continuous absorption that would maintain blood concentrations well above the excretion rate. However, the extent to which individual patients develop blood levels is quite variable. Two examples of such individual patient variation to the injection of procaine penicillin G in aluminium monostereate and oil (PAM) and benzathine penicillin G (Bicillin) may be mentioned.

Thayer, Field, Magnuson & Garson (1957) reported the average concentration for 98 patients treated with 0.60 mega-unit of PAM to be 0.20 u/ml. However, examination of individual patient blood levels at 24 hours revealed 1% to have developed levels below 0.03 u/ml and 9% below 0.10 u/ml. At the other extreme, 7% had concentrations in excess of 0.40 u/ml. The second example concerns the work of Wright et al. (1959), who injected subjects with 1.2 mega-units of benzathine penicillin. Here, the average concentration for 21 patients was 0.10 u/ml. However, study of individual patient response at 24 hours revealed great variability; 2.6% of the subjects showed no detectable levels, 11% developed concentrations below 0.05 u/ml and 2.6% showed concentrations in excess of 0.40 u/ml. Further, the injection of 2.5 mega-units of benzathine penicillin into 9 patients shows similar variability of blood levels (Elias et al., 1951).

Since gonococci will survive and multiply in levels of penicillin below their minimal inhibitory concentration, it can be seen that such an opportunity for survival may occur in patients whose blood levels are inordinately low. Therapeutic failure would thus occur if other host factors of resistance do not enter the picture. Similarly, when the gonococcal strain is relatively " resistant " to penicillin, it may be readily killed by the excessively high concentrations developed by some patients, thus resulting in successful therapy.

Another host factor that may sometimes militate against the adequacy of treatment, particularly in the female, is the inability of penicillin to kill gonococci that have been phagocytized by fixed

tissue cells (Thayer, Perry, Magnuson & Garson, 1957 ; Thayer, Perry, Field & Garson, 1957).

While penicillin remains the drug of choice for treatment of gonorrhoea, the problems of increasing numbers of allergic patients and the ever-decreasing susceptibility of the gonococcus must alert the clinician to the possible use of other antibiotics for the control of this disease.

RÉSUMÉ

La résistance à la pénicilline, signalée dans un nombre croissant de souches de gonocoques, et l'allergie à la pénicilline de plus en plus fréquente chez les malades, motivent désormais l'emploi d'autres antibiotiques dans le traitement de la blennorragie. Il est donc important de savoir comment se comportent les souches sensibles ou résistantes à la pénicilline vis-à-vis de ces nouvelles substances thérapeutiques.

Les auteurs ont étudié à cet égard l'action in vitro de diverses substances inhibitrices: kanamycine, leucomycine, chloramphénicol, dextrosulphenidol, oxytétracycline, chlortétracycline, tétracycline, demethylchlortétracycline, et synnematine B. Cette dernière est un dérivé substitué de la pénicilline G, qui a donné de bons résultats dans le traitement de la blennorragie aiguë chez des malades allergiques à la pénicilline.

L'expérience a montré que les souches résistantes à la pénicilline étaient aussi sensibles à ces antibiotiques que les souches non résistantes, exception faite pour la synnematine B, dont il fallut élever la dose pour inhiber les souches résistantes à la pénicilline G. Le temps nécessaire à la stérilisation de cultures de gonocoques en bouillon par le double de la dose inhibitrice minimum de kanamycyne, leucomycine, dextrosulphenidol, tétracycline et demethyltétracycline a varié selon les souches, entre 12 et 48 heures. Des échecs du traitement clinique par ces antibiotiques, poursuivi pendant moins de deux jours, ont été signalés. Une antibiothémie supérieure à la concentration inhibitrice minimum peut aisément être atteinte et maintenue par l'administration appropriée des antibiotiques susmentionnés.

Il est actuellement impossible de comparer entre eux les degrés de sensibilité du gonocoque obtenus par différents chercheurs, faute de méthodes d'épreuve uniformes (milieu de culture fixant ou détruisant la pénicilline, peptone contenant des traces de substances toxiques pour le gonocoque, nombre inégal de germes inoculés, etc.). Il devient urgent d'établir une technique standard internationale d'épreuve de sensibilité du gonocoque, qui permettrait des comparaisons d'un laboratoire à l'autre et faciliterait l'étude, à l'échelle mondiale, de la résistance du gonocoque.

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