SENSITIVITY OF STRAINS OF GONOCOCCI TO PENICILLIN, SULPHATHIAZOLE, AND STREPTOMYCIN

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Sulphonamides provided the first effective treatment for gonorrhoea. In a short time, however, strains of gonococci appeared which were resistant to sulphonamides, and by 1949 Dunlop was able to report that 86% of patients seen at the London Lock Hospital in 1946-7 were resistant to treatment.

Sulphonamides have now been replaced by penicillin and a careful watch has been kept for the appearance of penicillin-resistant gonococci. Such strains can readily be formed in the laboratory by exposure of the organisms to the drug, and it is perhaps surprising that until very recently there has been no evidence that resistant strains have been produced under natural conditions. In this paper we report the sensitivity of strains of gonococci from 200 patients to penicillin, streptomycin, and sulphathiazole. We have observed the relation between the patient's clinical course and the *in vitro* sensitivity results.

Patients.—Between October, 1957, and February, 1958, cultures were obtained from all male patients with gonorrhoea attending the venereal disease departments at St. Thomas's and St. Bartholomew's Hospital. Cultures were also collected from the female contacts when they attended. Satisfactory cultures for sensitivity-testing were obtained from 200 of 295 patients; in addition, a further 11 cultures were obtained from 10 of these 200 patients in whom the gonorrhoea recurred. Of these 200 patients, 190 were males and 10 were females: four of the female patients' contacts were among the 190 males.

Methods of Isolation and Testing Sensitivity

Material from patients was collected with charcoal-tipped swabs which were stored in Stuart's medium at 4° C. In our hands batches of Stuart's medium varied in their ability to preserve gonococci. Primary cultures were usually made within four days, and for these and for sensitivity tests chocolate agar plates containing 10% horse blood were used. In preparation this medium was heated to 75° C., but not above, for 10 minutes, and then cooled to 50° C., in order to minimize the effects of sulphonamide-inhibitors in the agar.

The cultures were incubated in an airtight jar for two days at 37° C.; carbon dioxide was provided by placing a lighted night-light in the jar immediately before sealing.

Dilution sensitivity tests were done for the three drugs tested. Stock solutions of the antibiotics were kept at 4° C. The penicillin solution was renewed at least every two weeks, and the streptomycin solution every month. For determining sensitivities, serial dilutions of each of the three drugs in chocolate agar were prepared in Petri dishes immediately before use, and a "control" plate containing no drug was prepared at the same time. The plates were

divided into numbered sectors so that batches of up to 12 strains of gonococci could be tested on one set of plates. For some strains the primary cultures could be used to provide the inoculum, but for others subcultures had to be used. In each case one to six colonies were suspended in approximately 0.2 ml. of broth, and one 1-mm. loopful of this suspension was used as the inoculum. The plates were incubated at 37° C. in candle-jars for 48 hours, and the end-points, regarded as the minimum concentration of drug necessary to prevent visible growth, were determined by inspection. As a check of the accuracy of the method the Oxford staphylococcus was inoculated on the penicillin plates on six separate occasions. This organism was inhibited by 0.032 unit/ml. once, and by 0.016 unit/ml. five times.

The nature and purity of the growth were checked for each organism by making films from the first plate which just permitted visible growth. Sugar fermentation was tested for each organism which needed more than 0.064 unit of penicillin per ml. for inhibition. All such organisms were found to have the reaction of *Neisseria gonorrhoeae*, except for one which was discarded from the series. The general sensitivity of the strains to sulphathiazole suggests that sulphonamide inhibitors were not seriously influencing the results.

Laboratory Findings

The main results are shown in Table I. For penicillin, 162 strains tested were inhibited by 0.064 unit per ml., but 16 strains needed 0.256 unit and 5 needed 0.512 unit per ml.

TABLE I.—Sensitivity of Strains of *N. gonorrhoeae* from 200 Patients to Penicillin, Streptomycin, and Sulphathiazole

Penicillin		Streptomycin		Sulphathiazole	
Minimum Inhibitory Concentration (unit/ml.)	No. of Strains Inhibited	Minimum Inhibitory Concentration (μg./ml.)	No. of Strains Inhibited	Minimum Inhibitory Concentration (mg./100 ml.)	No. of Strains Inhibited
0.008	10	0.25	6	0.03	6
0.016	94	1	13	0.125	30
0.032	46	4	139	0.5	82
0.064	12	16	42	2	69
0.128	17			8	12
0.256	16			>8	1
0.512	5				

For streptomycin, 158 of the strains were inhibited by 4 μg./ml. and all by 16 μg./ml. To our surprise 199 of the 200 strains were inhibited by 8 mg. or less of sulphathiazole per 100 ml.

Clinical Findings

At both hospitals the routine treatment for gonorrhoea was a single intramuscular dose of 300,000 units of procaine penicillin, and this was given to 190 of the patients. Following treatment the gonorrhoea recurred in 25 patients; all had received the standard dose except one who had been given 600,000 units. In attempting to correlate therapeutic results with the laboratory sensitivity tests it was decided on arbitrary grounds to consider strains needing 0.128 unit or more of penicillin per ml. for inhibition as possibly resistant. There were 38 patients with such strains, and analysis showed that gonorrhoea had recurred in 13. Three of these admitted further intercourse and may have been reinfected. Of the remainder, 2 defaulted immediately after treatment, and the remaining 23 had no observed recurrence in a follow-up ranging from one day to three months.

Among the 162 patients infected with sensitive strains there were 12 in whom a recurrence was observed: five of these admitted further intercourse and may have been reinfected. Ten patients defaulted after treatment, while there was no recurrence of gonorrhoea in 140 patients with a follow-up again ranging from one day to three months.

Further sensitivity tests were obtained in 10 of the 25 patients in whom the gonorrhoea recurred. In two patients, thought to be reinfected from the original source, both strains of gonococci were "sensitive." In seven patients, at

TABLE II.—Clinical Course of 10 Patients with Repeated Sensitivity Tests

Patient:	1	2	3	4	5	6	7	8	9	10
1st Attendance:										
Gonococci	+	+	+	+	+	+	+	+	+	+
Penicillin sensitivity (unit/ml.)	0.128	0.256	0.256	0.032	0.016	0.256	+	0.256	0.256	0.256
Procaine penicillin (100,000 units)	3	3	3	3	3	3	3	3	3	3
1st Failure:										
Gonococci	+	+	+	+	+	+	+	+	+	+
Penicillin sensitivity (unit/ml.)	0.512	0.512	0.512	0.016	0.016	+	+	0.128	0.128	0.256
Procaine penicillin (100,000 units)	6	6	6	3	3	6	6	6	6	6
2nd Failure:										
Gonococci	+	—	—	—	—	+	+	—	—	+
Penicillin sensitivity (unit/ml.)	0.512	—	—	—	—	0.016	0.256	—	—	+
Streptomycin (g.)	1	—	—	—	—	1	1	—	—	1
3rd Failure:										
Gonococci	—	—	—	—	—	—	+	—	—	+
Penicillin sensitivity (unit/ml.)	—	—	—	—	—	—	0.512	—	—	+
Streptomycin (g.)	—	—	—	—	—	—	1	—	—	2
Gonococci	—	—	—	—	—	—	—	—	—	—

¹ = 1,200,000 units procaine penicillin. ² = 2,400,000 units procaine penicillin. ³ = Admitted further intercourse with original contact. ⁴ = Possible intercourse with fresh contact.

least six of whom were thought to be suffering from a relapse rather than reinfection, the original "resistant" strain was recovered from the second specimen. In one patient the original resistant strain was replaced by a sensitive strain, but this patient was known to have at least two homosexual contacts. The results of treatment in these patients are shown in Table II.

Of the female patients who had male contacts in the series, three of the pairs each had sensitive strains: the remaining pair were each infected by resistant gonococci.

Of the 200 patients, 127 were of European or Asiatic racial origin, the remaining 73 being of the negro race. Twenty-three of the "resistant" strains were isolated from the first group and 15 from the second.

Discussion

During the period in which gonorrhoea was treated with sulphonamides the number of clinically resistant cases progressively increased. This increase was associated with a rise in the numbers of *in vitro* sulphonamide-resistant gonococci. Lankford, Scott, and Cooke (1943) reported that all of 100 strains were inhibited by 5 mg. of sulphathiazole per 100 ml., whereas by 1949 Love and Finland (1955) found that the majority of 63 strains needed more than 40 mg. of sulphadiazine per 100 ml. When penicillin replaced sulphonamides in the treatment of gonorrhoea the proportion of sensitive strains began to increase, and by 1954 Love and Finland found that 73% of 43 strains could be inhibited by 2.5 mg. of sulphadiazine per 100 ml., although there were still some strains which required more than 40 mg./100 ml. Our findings presumably reflect the abandonment of sulphonamides for the treatment of gonorrhoea in this country in recent years.

Penicillin produced a welcome improvement in treatment, but the fear remained that gonococci resistant to penicillin might emerge. Until very recently there has been no confirmed evidence that this has happened. Penicillin is effective so rapidly that it might be difficult to distinguish relapse from reinfection, and it is probable that some apparently clinically resistant cases can be accounted for in this way. This was the view of Cohn and Seijo (1944), who investigated 35 supposedly penicillin-resistant cases and successfully treated 34 with penicillin alone. They were supported by Hughes and Carpenter (1948), who examined 216 cases of alleged penicillin-resistant gonorrhoea. Gonococci could be found in only 19 of these cases, and these were all cured with one or two doses of penicillin in oil and beeswax.

There has also so far been no evidence from sensitivity tests that penicillin-resistant strains have been emerging. Six papers have reported the sensitivity of 1,012 strains of gonococci between 1944 and 1954 (Cohn and Seijo, 1944; Lankford, 1945; Kenedy and Alföldy, 1947; Hawks and

Greay, 1948; Cohn *et al.*, 1949; Love and Finland, 1955). Of all these strains, only five have been reported as requiring more than 0.128 unit of penicillin per ml. for inhibition, and three of these five may have in fact have needed less, as the immediate lower inhibitory levels were not tested.

In contrast to these findings, we found that 38 of our strains from 200 patients required 0.128 unit or more per ml. of penicillin for inhibition and five needed 0.512 unit. The clinical course of patients infected with these strains suggested that the increased *in vitro* resistance to penicillin might be reflected in the results of treatment with conventional doses of penicillin. Ignoring the possibility of reinfection, 13 of the 38 patients (34%) infected with resistant strains relapsed, as opposed to only 12 of the 162 patients (7%) who were infected with sensitive strains. That resistance was constant for at any rate a short period was indicated by the few results in which relapses occurred with the original infecting strain, and by strains of similar resistance which were isolated from infected partners.

The overall cure rate, using 300,000 units of procaine penicillin, is not unsatisfactory (87.5%), but in view of the experience described in this paper a dosage of 600,000 units will be used in future, and clinical relapse will be treated with streptomycin and sulphonamides.

Summary

The sensitivity of strains of gonococci from 200 patients has been tested to penicillin, streptomycin, and sulphathiazole. 158 of the strains were inhibited by 4 µg. of streptomycin per ml. and all by 16 µg./ml. Only one strain needed more than 8 mg. of sulphathiazole per 100 ml. for inhibition. Thirty-eight strains needed 0.128 unit or more of penicillin per ml. for inhibition, and patients infected with these strains relapsed after treatment more often than those infected with more sensitive strains (34% to 7%).

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