Penicillin-insensitive gonococci in the Bolton area *Preponderance in young women and immigrants*

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This report relates to 92 patients treated for acute gonorrhoea in the Diagnostic Clinic of the Bolton Health Department between October, 1968, and March, 1969. Gonococci from these patients were cultured and tested for penicillin sensitivity in the Manchester Public Health Laboratory. Retrospectively the laboratory findings and the clinical information were analysed and correlated. The sensitivity study, which was prompted by the fact that penicillin treatment was becoming less and less effective, showed, as expected, that this process was due to a high incidence of insensitive strains. Results of estimations also showed a marked bimodal distribution, consideration of which led to further study of the patient data. Among items of epidemiological interest, it was found that there was a significant difference in sensitivity between the strains from immigrants and those from non-immigrant patients.

Material and methods

Routine examination of males included the taking of material from the urethra on a platinum loop for smear preparation and on a charcoal-treated swab for culture. In females, material was taken from the urethra and from the cervical canal, on plain cotton swabs for smears, and on charcoal swabs for culture. Swabs for culture, as they were taken, were put in Stuart's transport medium and later posted to the laboratory.

After the clinical examination and microscopical examination of smears, patients with positive findings were given either oral tetracycline (500 mg. 8-hrly for 5 days) or penicillin by injection. Male patients were given one dose of 0.9 m.u. procaine penicillin, but female patients received either 0.9 m.u. procaine penicillin on two successive days or a single dose of 1.25 m.u. of a mixture of long-acting penicillins and sodium penicillin (Triplopen*). Patients in whom the infection failed to respond to penicillin were treated with tetracycline and *vice versa*; other drugs were then employed, if necessary.

As the initial treatment was given before the sensitivity results could be known, the choice of therapy was to a large extent arbitrary. However, a preference was shown for tetracycline in the treatment of males, except for those who appeared to be of unreliable character and unlikely to complete the course.

Male patients re-attended usually on the 2nd, 5th, 7th, and 14th days after treatment. On these occasions urethral discharge was looked for and first and second urine specimens were inspected for threads and debris. Females returned on the 4th and 7th days after treatment for testing by smear and culture and then after three successive menstrual periods. All patients had tests for syphilis at the first attendance and before discharge.

When the swabs were received in the laboratory on the next morning, they were plated on two chocolate agar plates. One plate had no antibiotic and the other contained vancomycin 3 µg./ml. and polymyxin B 15 units/ml. (Thayer and Martin, 1964, 1966). Plates were incubated in candle extinction jars. *Neisseria gonorrhoeae* isolates, after confirmation of identity by sugar fermentations, were preserved for sensitivity testing. Growth was scraped with a loop from an 18-hr culture on chocolate agar and emulsified in Bacto-Tryptose broth (20 per cent. glycerol added) to give a turbid suspension. Bijou bottles containing 1.5 ml. broth were used, and the suspensions were immediately placed in the -20°C. cabinet without previous incubation. It has been confirmed that suspensions frozen in this way remain viable for up to one month (Kellogg, Cohen, Norins, Schroeter, and Reising, 1968). The minimum inhibitory concentration (MIC) of sodium penicillin in chocolate agar was determined periodically for collected strains, using freshly prepared plates and a multiple loop device (Tarr, 1958). Suspensions were either the original glycerol broth suspensions (where time of storage was short) or those prepared from subcultures on chocolate agar. Density was such as to give confluent or near-confluent growth. Plates were read after incubation for 40 hrs at 36°C in candle extinction jars. The MIC was taken as the lowest concentration of penicillin giving total or near-total (< 10 colonies) inhibition. The Oxford staphylococcus and representative strains from previous batches were always included. Tetracycline sensitivities were not estimated, though retrospectively we recognize that this would have been desirable for a more complete picture.

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*Triplopen (Benethamine penicillin 0.5 m.u., procaine penicillin 0.25 m.u., benzyl penicillin sodium 0.5 m.u.).

Results

SENSITIVITY DISTRIBUTION

The sensitivity distribution of 95 strains isolated from 92 patients is displayed in Fig. 1. The strains fell into two main groups: *sensitive* (penicillin MIC < 0.03 µg./ml.) and *less sensitive* (MIC ≥ 0.03 µg./ml.). The latter included thirteen strains requiring 0.03 or 0.06 µg./ml. penicillin for inhibition, which are termed *intermediate* in this paper, and 48 strains requiring more than 0.1 µg./ml., which are termed *insensitive*, i.e. insensitive at a level indicating likelihood of treatment failure with dosage current at the time of study (*Brit. med. J.*, 1969). No very resistant strains were encountered, the greatest MIC being 0.24 µg./ml.

FIG. 1 Distribution of strains according to penicillin MIC—proportion from immigrants also shown

The actual number of cultures tested was 115, but 23 of these were repeat isolations from returning patients; and unless a strain isolated by repeat culture differed significantly in sensitivity (i.e. by more than one step in the penicillin dilution series) from that previously obtained, it was not regarded as a new

strain. Three returning patients gave new strains by this criterion and these have been included in the total. The other isolates have been excluded even though they may have represented new infections.

AGE AND SEX OF PATIENTS

The distribution of patients by age and sex is shown in Table I. The great majority were young adults. The ratio of males to females was 2 : 1.

SENSITIVITY OF STRAINS FROM IMMIGRANTS

Fifteen male and two female patients were of overseas origin (six Pakistani, four Indian, three West African, three Jamaican, and one Portuguese). Of eighteen strains cultured from this group, thirteen (72.4 per cent.) were *insensitive*. This compares with 35 of 77 strains (45.4 per cent.) from patients native to the British Isles. A comparison of MIC levels in the immigrant and the non-immigrant groups by the Kolmogorov Smirnov test (Siegel, 1956) showed a significant difference between them (0.005 > P > 0.001). Not only were *insensitive* strains more frequent in immigrants but the degree of their insensitivity was greater (Fig. 1). Only twelve of 35 *insensitive* strains from non-immigrants required 0.24 µg. penicillin/ml. for inhibition, but eleven of thirteen *insensitive* strains from immigrants required this concentration. No strains of *intermediate* sensitivity were cultured from immigrants.

SENSITIVITY OF STRAINS FROM YOUNGER AND OLDER NON-IMMIGRANTS

The distributions are shown in Table II. There was a preponderance of *insensitive* strains in young females. Statistical analysis showed significant differences in ages between the female patients of the three sensitivity groups (0.05 > P > 0.025), but no significant differences in the case of the corresponding groups of male patients.

RESPONSE TO INITIAL TREATMENT

Results of initial treatment with penicillin and with tetracycline in patients with *sensitive* and *insensitive* strains are compared in Table III. The total of 93 cases includes three of re-infection and excludes two treated from the start with streptomycin for reasons

TABLE 1 Age-sex distribution of patients

Age (yrs)		15-19	20-24	25-29	30-34	35-39	40-44	50-60
Sex	Male	12	23	13	6	3	1	4
	Female	9	11	7	1	1	1	0
Total		21	34	20	7	4	2	4

TABLE II Sensitivity distribution of strains from non-immigrants by age and sex

Sex	Age group (yrs)	No. of strains	Sensitive	Intermediate	Insensitive
Male	15-21	16	7	2	7
	22 and over	33	15	5	13
Female	15-21	14	2	1	11
	22 and over	14	5	5	4

TABLE III Results of initial treatment with penicillin and with tetracycline

Penicillin MIC of strain	Treatment	No. treated	Result		
			Cured	Failed	Failure rate (per cent.)
< 0.1 µg./ml. (46 cases)	Penicillin	15	14	1	6.7
	Tetracycline	31	30	1	3.2
> 0.1 µg./ml. (47 cases)	Penicillin	24	9	15	62.5
	Tetracycline	23	16	7	30.4

of allergy. Patients with *intermediate* strains responded in the same way as those with *sensitive* strains and are included with them in the Table.

In patients with *sensitive* strains, treatment, whether with penicillin or with tetracycline, was effective. In patients with *insensitive* strains, penicillin, in the dosage used, often failed to cure, and tetracycline, though more effective, also showed an increased failure rate, which would suggest a correlation between penicillin and tetracycline insensitivity such as has been shown *in vitro* by Amies (1969) in Canada and by Phillips, Rimmer, Ridley, Lynn, and Warren (1970) in London.

TREATMENT FAILURE/RE-INFECTION

27 patients, almost all of whom were infected with insensitive strains, failed to respond to initial treatment or had later episodes of active infection. They could be grouped as follows:

Initial failure

Five men and four women did not respond to initial treatment and were immediately given the alternative drug, whether tetracycline or penicillin, and recovered without having a repeat positive culture.

Early recurrence

Five men and two women responded to initial treatment but soon, within 14 days, showed further evidence of active infection and were then treated successfully with the alternative drug. They all had repeat positive cultures.

Late recurrence

Three men and five women responded either to the drug first given or, if that failed, to the alternative drug, but later, after intervals of 14 to 29 days in the case of the men, and 19 to 119 days in the case of the women, showed further evidence of active disease confirmed by culture. These late recurrences did not readily respond to treatment and a variety of agents, including penicillin in higher dosage, was used in order to achieve cure.

Re-infection

Three men, after clinical cure, returned many weeks later with clinical and bacteriological evidence of re-infection. In one, an *insensitive* strain succeeded a *sensitive* one, in another the reverse occurred, and in the third a less *sensitive* succeeded a more *sensitive*.

Except in the case of two patients in the *initial failure* group and the re-infection cases noted, all the above patients had *insensitive* strains. Some of the recurrences may have been cases of re-infection, but the repeat cultures showed no significant differences in penicillin sensitivity as compared with the original cultures.

DATA RELATING TO POSSIBLE CHANGES IN STRAIN SENSITIVITY

Sensitivity differences, not significant on an individual basis, were surveyed for possible group significance:

In cases of recurrence

These provided twenty instances of repeat positive culture after intervals ranging from 4 to 119 days. In fifteen the penicillin MIC value was identical with that first obtained and in five there was a one-step difference in the direction of greater resistance. The patients had all been treated with penicillin between cultures except for two in the 'no-difference' category.

In contact pairs

Thirteen pairs of contacts, marital and extra-marital, were identified. In ten pairs the MICs of the strains were identical and in three there were one-step differences.

Discussion

The gonococci in this series were equally divided into those having a penicillin MIC less than and greater than 0.1 µg./ml. The clinical value of this division was confirmed by a high rate of treatment failure in patients with strains insensitive at this level. But clinical considerations apart, the natural bacteriological division between strains occurred at a lower MIC value, *i.e.* 0.03 µg./ml. There was virtually a discontinuity of the spectrum of sensitivities at this value (Fig. 1). This was also seen in a recent series reported from London by Phillips and others (1970); their figures show a higher proportion of sensitive strains (56 of 96) than ours (34 of 95), but, as in our series, only two strains fell in the 0.03 µg./ml. column. The trend may be seen if earlier reports from London are compared (Fig. 2). Taking it as axiomatic that the emergence of strains with

increased penicillin resistance is related to the use of the drug, then this effect must be related to the use of penicillin in previous years. In this clinic, until the end of 1965, the routine dose was 0.6 m.u. for males and the same on two consecutive days for females. The level at which discontinuity in the spectrum of MIC values is tending to occur may have been determined by this or by an earlier regime of dosage or by dosage elsewhere than in the clinics. If not determined by the magnitude of therapeutic levels in the past, then the effect must reflect an innate characteristic of gonococci when interacting with sub-bactericidal concentrations of penicillin. In any case, the effect is one which must be explained by any satisfactory theory of the emergence of penicillin insensitivity in gonococci. The routine dosage of penicillin has been much increased since this study took place, and we must ask ourselves whether the process which has caused this effect will continue to operate? If so, we may expect the emergence of a group of strains of greatly increased resistance which will form a serious therapeutic problem.

Not only initial treatment failure but recurrence of active infection occurred more often in patients with *insensitive* strains than in those of other groups. Among 46 patients with *sensitive* and *intermediate* strains there were only two cases of initial treatment failure and two of re-infection, but among 47 patients with *insensitive* strains there were seven cases of initial treatment failure, one of definite re-infection, and fifteen of recurrence. It was not clear if these recurrences were due to relapse or to re-infection, though if many were due to re-infection, it is hard to

FIG. 2 Changes in distribution by penicillin MIC of gonococci from London clinics patients, 1958-1970 (Based on reports by various authors)

understand why these patients should be so much more prone to this than the others and why their re-infecting strains should be so uniformly *insensitive*. But whether relapse or re-infection be incriminated, we have a situation in which *insensitive* strains are persisting in a partially 'penicillinized' community. In the laboratory, the best way to produce *insensitive* strains from *sensitive* gonococci is to subculture alternately on penicillin-containing and penicillin-free media (Miller and Bohnhoff, 1945). Is a similar process occurring in these patients? Our numbers are insufficient for significance, but more studies of cultures from cases of recurrence and their contacts might show changes which, though insignificant individually, together would indicate a slow upward 'creep' in resistance.

Of all the recurrences, those in certain female patients occurred after the longest intervals. In view of the known capacity of the gonococcus to survive in females over long periods and in spite of treatment, this finding is not unexpected, but it should be considered in context with our more unexpected finding that the young female patients attending this clinic are mostly those infected with *insensitive* strains. Those infected with *sensitive* strains do not appear to be finding their way to the clinic. This suggests that many girls infected with gonorrhoea first get treatment elsewhere and come to the clinic only if this fails. Some may be having treatment under unqualified advice, but probably most are getting treatment from general practitioners who may or may not be aware of the fact that these patients are venereally infected. Even if treatment is given knowingly, the bacteriological control, follow-up and contact tracing, which are difficult enough to obtain under clinic conditions, must be almost impossible to establish. The inference is that the population of female carriers is being augmented in a way boding ill for the future.

Conditions favouring the development of resistance in endemic strains also favour the dissemination of more highly resistant strains if introduced from abroad. Our data suggest that immigrants more often become infected with *insensitive strains*, and these of a higher degree of insensitivity, than the indigenous population. It would be unwise to assume that these persons were harbouring their strains on arrival in Great Britain—it is more likely that they acquired them in the post-entry period—but it may well be that some of the strains represent importations over the years. Nearly all the immigrant patients were men, who, though difficult to cure, rarely returned with fresh infections. They did not reveal their history and we are left to wonder about the source of their infection.

In conclusion, our results show that, though upward 'creep' of the level of sensitivity may be occurring, the penicillin MIC is a sufficiently stable character of gonococcal strains to make its estimation of more use than just to guide treatment in the individual patient or to monitor changes in the incidence of insensitive strains in the populace. In fact, it provides a crude substitute for typing if the results are correlated case by case with information gained by clinician and social worker. In this way an understanding may be reached of current epidemiological patterns and of the mechanisms of emerging resistance which will serve to guide future efforts in the field.

Summary

The minimum inhibitory concentrations of penicillin were estimated for gonococci isolated from 92 patients attending a Bolton clinic between October, 1968, and March, 1969. Half of the strains were found to be insensitive to 0.1 µg./ml. The sensitivity distribution was bimodal with separation at the value of 0.03 µg./ml. A significant difference in sensitivity was found between strains from immigrants and those from non-immigrant patients. The majority of immigrants had strains of MIC 0.24 µg./ml. Non-immigrant female patients showed significant age differences when grouped according to whether their strains were *sensitive* (MIC ≤ 0.015 µg./ml.), *intermediate* (MIC 0.03–0.06 µg./ml.), or *insensitive* (MIC ≥ 0.12 µg./ml.). The majority of females aged 21 years or under had insensitive strains. High rates of initial treatment failure and of later recurrence were found in patients infected with strains insensitive to 0.1 µg. penicillin/ml. These observations are discussed and attention is drawn to the value of correlating clinical, sociological, and laboratory findings in this field of study.

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Gonocoques non sensibles à la pénicilline dans la région de Bolton. Prépondérance chez les jeunes femmes et les immigrants

SOMMAIRE

Les concentrations inhibitrices minima (CMI) de pénicilline furent établies pour les gonocoques isolés chez 92 malades s'étant présentés à une clinique de Bolton entre Octobre et Mars, 1969. La moitié des souches se

montrèrent insensibles à 0,1 µg./ml. La distribution de la sensibilité se manifesta en deux groupes, de part et d'autre de la valeur de 0,03 µg./ml. Une différence significative dans la sensibilité fut trouvée entre les souches provenant d'immigrants et celles provenant de malades non immigrants. La plupart des souches des immigrants avaient une CMI de 0,24 µg./ml. Les malades femmes non immigrantes montrèrent des différences d'âge significatives entre les groupes formés selon que les souches étaient *sensibles* (CMI < 0,015 µg./ml.), *moyennes* (CMI 0,03–0,06 µg./ml.) ou *insensibles* (CMI ≥ 0,12 µg./ml.). La majorité des femmes âgées de 21 ans ou moins avaient des souches insensibles. De hauts taux d'échecs du traitement initial ou de rechutes ultérieures furent retrouvés chez les malades infectés avec des souches insensibles à 0,1 µg./ml. Ces observations sont discutées et l'on attire l'attention sur les corrélations constatées au cours de cette étude entre la clinique, la sociologie, et les constatations de laboratoire.