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CLINICAL ARTICLES

INVITRO DRUG SENSITIVITY OF NEISSERIA GONORRHOEAE STRAINS AND BLOOD PENICILLIN LEVELS AFTER VARIOUS PENICILLIN PREPARATIONS

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Determination of invitro drug sensitivity of pathogenic micro-organisms to various antimicrobial agents plays an important role in evolving successful treatment of infections. This is of paramount importance in case of N. gonorrhoeae, as drug resistant strains are fast emerging during therapy. Failure of treatment with a regime of penicillin is conspicous in many clinics. Hence, this study aims at (i) to determine the range of invitro drug sensitivity of N. gonorrhoeae strains isolated from 100 untreated random cases during the year 1970, (ii) to estimate the blood penicillin levels in ten patients who were given various penicillin preparations, and (iii) to follow-up clinically and bacteriologically, those cases treated with procaine penicillin in oil.

MATERIALS AND METHODS

N. gonorrhoeae strains were isolated from untreated random cases attending the V. D. clinic. Patients on admission were given procaine penicillin G in oil with 2% aluminium monosterate (PAM) 600,000 IU for four days. The cases which were not cured with PAM were given other forms of penicillin. Few cases resistant to PAM were given chloramphenicol and teracycline, orally or intramuscularly. The cases were clinically and bacteriologically followed up after treatment.

Chacko-Nair (CN) medium (Chacko and Nair 1966, 1968) was used for primary isolation of N. gonorrhoeae strains. The strains were tested against sulphadiazine [1] (S), penicillin [2] (P), streptomycin [3] (St), chloramphenicol [4] (C) and tetracycline [5] (T) for minimum inhibitory concentrations (MICs) of drugs

1. Sulphadiazine injection B.P. Tablets Private Ltd., Madras-21.
2. Crystapen (sodium benzyl penicillin injection I.P.) Glaxo Lab., Bombay-18.
3. Streptomycin (sulphate) I P. Hindustan Antibiotics Ltd , Poona-10.
4. Chloromycetin (Chloramphenicol sodium succinate USP) Parke Davis (India) Ltd., Bombay-72.
5. Oxy-cline-125 (Oxy-tetracycline injection) British Pharmaceutical Laboratories, Bombay-12

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using plate dilution technique. Initially, 24 strains isolated in the present series in 1970 were tested under identical conditions in CN medium and ascitic fluid (AF) medium for comparison of MICs in both media. The rest 74 strains were tested in CN medium only. (In an earlier study, 50 strains isolated in 1969 were tested under identical conditions in CN medium AF medium, and Difco GC medium for comparison of MICs – under publication).

Concentrations of S–10, 50, 100 micrograms (ug) /ml; P–0.05, 0.1, 0.2, 0.5, 1.0, 2.0 International units (IU) /ml; St–5, 10, 30 ug/ml; C–5, 20 ug/ml; and T–0. 1, 0.2, 0.5, 1.0 ug/ml were used. Two local strains with known sensitivity range served as controls.

The base medium was prepared according to a method described earlier (Chacko and Nair 1968). A 200 ml of this melted base kept at 55-60 ^{0} C is enriched with one fresh hen's egg content or 20% fresh seitz filtered ascitic fluid. This medium is distributed into appropriate quantities to add the required concentrations of drugs per ml. of the medium, before being poured into plates.

A 2mm diameter loopful (approximately 0.005 ml.) of a suspension of N. gonorrhoeae (18–24 hours old culture in CN medium emulsified in broth) containing 4000 million organisms/ml (Brown's opacity) was spread out in approximately 1.4 x 1.4 cms area (10 ^{6} millions/cm ^{2}), for testing the sensitivity of strains to various drugs. The test was incubated at 37 ^{0} C under 5–10% ^{2} (Candle–jar) for 24 hours and complete inhibition of growth was taken as end point.

Ten selected volunteer gonorrhoeae patients were tested for their blood–penicillin–levels during therapy. PAM 600,000 IU in 2 ml daily for 4 days, crys–12 (Squibbs – sodium penicillin G one part and procaine penicillin 3 parts) 2.4 or 4.8 million IU single session with or without probenecid, and sodium penicillin 500,000 IU sixth hourly for 5 days. were given as per a clinical schedule followed in this clinic on different patients. Blood samples were drawn at intervals and penicillin levels were estimated using Sarcina lutea' method unpublished document–WHO/ Pharm/250, P^{4}). Simultaneously, the penicillin sensitivity of the N. gonorrhoeae strains isolated from them was also determined.

RESULTS

The MICs of P for all the 24 strains of N. gonorrhoeae in both CN and AF media were the same. Slightly higher MICs were seen in AF medium compared to medium in case of other drugs.

The range of invitro drug sensitivity of N. gonorrhoeae against S, P, St, C and T are shown in Table–1. The blood penicillin levels of 10 patients are given in Table–2. The results of follow–up of patients after treatment (61 out of 100 cases – others could not be studied properly) with PAM, are shown in Tables 3A and 3B.

TABLE 1.

Sensitivity of N. Gonorrhoeae Strains and Correlation Between Penicillin Sensitivity and Sensitivity to Other Drugs (1970).

PENICILLIN. Range IU/ml	No. of strains.	NO. OF STRAINS Tetracycline range ug/ml					Chloramphenicol ug/ml.			Streptomycin ug/ml.				Sulfadiazine ug/ml.			
		0.1 or less	0.2	0.5	1.0	1.0	5.0 or less	20.0	20.0	5.0 or less	10.0	30.0	30.0	10.0 or less.	50.0	100.0	100.0
0.05 or less	9	—	5	4	—	—	5	3	1	6	—	1	2	—	—	3	6
0.1	1	—	—	—	1	—	1	—	—	1	—	—	—	—	—	1	—
0.2	7	—	6	1	—	—	4	3	—	3	1	—	3	1	—	2	4
0.5	20	—	8	10	2	—	3	9	8	2	5	1	12	7	1	7	3
1.0	56	—	13	9	34	—	5	15	36	2	4	—	50	4	—	3	39
2.0	3	—	3	—	—	—	—	1	2	—	—	—	3	2	—	1	—
2.0	4	—	1	—	3	—	—	1	3	—	—	1	3	1	—	2	1
Total	100	—	36	24	40	—	18	32	50	14	10	3	73	15	1	29	55

TABLE-2.

Duration of penicillinaemia after various penicillin preparations given Intramuscularly.

Penicillin preparation	Dose in IU	Patients	Peak concentation IU/ml	Serum levels in IU/ml. (hours after injection) 0	2	4	6	24	48	72
PAM (Procaine penicillin G in oil with	600,000	1	1	0	0.5	1	1	0.5	0.3	—
2% Al. Monostearate)	600,000) x daily)	2	1	0	0.5	1	1	0.5	0.5	0.5
		3	1	0	0.5	1	1	0.5	0.5	0.5
		4	0.8	0	—	0.8	0.8	0.5	—	—
Crys-12	2,400,000	5	10	0	—	10	7	0.4	—	—
		6	10	0	—	10	8	0.5	—	0 4
	4,800,000	7	10	0	8	10	7	1.0	0.5	0.1
	2,400,000*	8	9	0	8	9	7	1 0	0.5	0.5
		9	10	0	9	10	8	1.0	0 5	0.5
	2,400,000**	10	10	0	6	6	10	6	—	—
Sodium penicillin	500,0 0 x 6 hourly	4$	10	—	10	2.0	2.0	—	—	
		4**$	10	—	10	10	8.0	—	—	—

*One gm. probenecid 1 hour before injection. **One gm. probenecid 1 hour before injection followed by 0.5 gm. every 6 hours.

$One patient was given PAM and Sodium penicillin with and without probenecid successively.

TABLE 3 A.

Follow-up of 50 Cases Treated with PAM and Sensitivity of N. Gonorrhoeae Strains.

Drug.	Number of patients.		Invitro sensitivity of strains to Penicillin in IU/ml. 0.05	0.1	0 2	0.5	1.0	2.0	2.0
PAM 600,000 IU x 4 days	50	Cured .3 (26%)	1	—	2	8	2	—	—
		Not cured 37 (74%)	—	—	—	8	24	1	4
** Crys-12 2 4 MU Single session.	*7	Cured 7 (100%)	—	—	—	3	3	—	1
		Not cured-nil	—	—	—	—	—	—	—
Sod. Penicillin 500,000 IU 6 hrs x 5 days	* 30	Cured 30 (100%)	—	—	—	5	21	1	3
		Not cured-nil	—	—	—	—	—	—	—

*These are cases resistant to PAM treatment.

**One case was given Crys-12 4.8 MU.

TABLE 3 B.

Follow-up of PAM Resistant Cases Treated with Other Antibiotics

Patient No.	Drug used	Strain sensitivity to that drug	Clinical result	penicillin sensitivity	Cured with
1.	Chloramphenicol-orally 50 mgs x 6 hourly for 5 days	20.0 ug/ml	not cured	1.0 IU/ml	Crys. Penicillin, I. M.
2.	,,	,,	,,	,,	,,
3.	,,	,,	,,	2.0	Crys-12 + probenecid.
4.	,,	5.0	cured	1.0	–
5	Chloramphenicol-injection 1 gm dailyx5 days	20.0	,,	1.0	–
6.	Oxytetracycline-orally 500 mgs 6 hourly x 10 days ...	1.0	not cured	2.0	Crys penicillin I M.
7.	,, ...	,,	,,	1.0	,,
8.	,, ...	0.5	,,	0.5	,,
9.	Oxytetracycline-injection 250 mgs daily x 5 days ...	0.5	cured	0.5	–
10.	,, ...	,,	,,	0.5	–
11.	,, ...	1.0	,,	0.5	–

COMMENTS

A number of laboratory investigations in Far East (Ho and Chang 1967, Holmes et al 1967), United States (Thayer et al 1961), Canada (Snell et al 1963), Germany (Ludwig 1966), France (Durel et al 1967), United Kingdom (Warren 1968), India (Chacko and Yogeswari 1966), Denmark (Reyn et al 1958) and Australia (Smith and Levery 1967) have shown that penicillin is still a highly effective drug against gonorrhoeae, though there is an increasing incidence of treatment failures with it clinically (Holmes et al 1967) even with high doses (Shapiro and Lentz 1967). Laboratory investigations on invitro drug sensitivity of N. gonorrhoeae to penicillin (Guthe 1961) and other antibiotics at various times have shown that the failure of treatment is mainly due to the slow development and circulation of N. gonorrhoeae strains with decreasing drug sensitivity. Hence the importance of drug sensitivity testing of N. gonorrhoeae and the role of a stanard medium for drug sensitivity testing.

In this study, no differences in MICs of P in both CN and AF media were seen. Slightly higher MICs were seen with AF medium compared to CN medium in case of other drugs. But these differnces did not seem significant. All the strains were isolated and maintained in CN medium and it may be a question

of organisms to a new medium. Similar differences in the MICs of drugs were observed in 50 strains isolated in 1969 and tested in CN, AF and Difco GC media (Gopalan and Nair-data to be published). Hence CN medium alone was used for further testing.

In the present study, 9% N. gonorrhoeae strains were sensitive to P 0.05 or less, 1% to P 0.1, 7% to P 0.2, 20% to P 0.5, 56% to P 1.0, 3% to P 2.0 and 4% >2·0 of IU/ml. In 1965, 54.3% strains were sensitive to P 0.05 or less, 8.8% to P 0.125, 17.5% to P 0.5, and 4.4% to P 1.0 of IU/ml (Chacko and Yogeswari 1966). This shows that there is a definite increase in the number of less sensitive strains of N. gonorrhoeae to P in this area. In all other areas also, a similar pattern of increase in the number of less sensitive strains of N. gonorrhoeae to P has been observed (Roiron et al 1961, Willcox 1970).

All strains in this series were sensitive to T 1 ug/ml or less. A decreased sensitivity of N. gonorrhoeae to T like that to P, has been observed in United States (Thyer et al 1961), Europe (Reyn 1961) Greenland (Lomholt and Berg 1966) and Philipines (WHO 1963), It is quite probable that in these areas where T is widely used in the treatment of gonorrhoeae, N. gonorrhoeae strains became less sensitive to it. The sensitivity of our strains to T is thought to be due to rare usage of T in the treatment of gonorrhoeae in this area.

Fifty percent strains were sensitive to C 20 ug/ml or less. The rest required more than C 20 ug/ml. This shows that there is a definite increase in the number of C resistant strains compared to 1965 (Chacko and Yogeswary 1966) in this area, probably due to its wide application of this drug in our clinic during 1965-67. There are signs of C resistance in other areas also (Gastrin and Kalling 1964).

Twentyfour percent strains were sensitive to St 10 ug/ml or less. The rest required more than St 30 ug/ml. During 1965, out of 25 strains (Chacko and Yogeswari 1966), 44% required St 10 ug/ml or less The rest required more than St 30 ug/ml. It is observed in many areas that N. gonorrhoeae has acquired a high degree of resistance to St There are instances where complete resistance to St has been easily acquired by certain strains of N. gonorrhoeae (Spitzer and Willcox 1968). Further, it is anticipated (Spitzer and Willcox 1968) that at the present rate of increase of St. resistance, will be of no use in the treatment of gonorrhoeae. Generally, St. resistant strains are found less sensitive to P and vice-versa (56%-strains inhibited at St 30 ug/ml required a concentration of P 1. 0 IU/ml for inhibition). This phenomena of cross resistance between P. and St. in case of N gonorrhoeae is seen in other areas also (Willcox 1970).

Fifteen percent strains were sensitive to S 10 ug/ml or less. Only one strain was inhibited at S 50 ug/ml The rest required S 100 ug/ml or more. Lessened sensitivity to Sulpha (Ho and Chang 1967, Reyn 1961) has been noted in other areas also

From the above findings on the MICs of drugs to N. gonorrhoeae, P still continues to be the drug of choice in this area followed by T, C, St and S, with reference to the percentage of strains inhibited at low concentrations of drugs and the maximum drug levels attained in serum during therapy.

The reports on serum concentrations of P during the administration of different preparations of P or various doses of it are few. Hence relevance of present serum penicillin level estimation (Table–2).

In four patients with 600,000 IU of PAM, the peak concentration of P in serum was 0.8–1. 0 IU/ml at 4–6 hours, which came down to 0.5 IU at 24 hours and 0.3 at 48 hours in one case. With repeated daily injections of PAM. 0.5 IU/ml of P was found at every 24 hours of testing. Other workers have also reported similar results with PAM (Bunn 1959).

In two patients with a single session therapy of 2.4 million IU of Crys–12, the peak concentration of P in serum was 10 IU/ml in 2-4 hours In three other patients who were given probenecid in addition, the peak concentration remained more or less the same. A dose of 4.8 million IU also produced a concentration of 10 IU/ml in one patient in 4–6 hours The peak concentration ot P slowly came down to 7–8 IU/ml at six hours 0.4 – 1.0 IU at 24 hours, 0 5 IU at 48 hours and 0.1 - 0.4 at 72 hours in patients to whom probenecid was given one dose or not given at all. In one case where probenecid was continuously given six hourly, 6 IU/ml was found at 25 hours.

In one patient, the peak concentration of P with 500,000 IU of sodium penicillin was 10 IU/ml at 2 hours which came down to IU/ml at 4-6 hours. Wormer et al (1955) reported a peak concentration of 8-9 IU/ml of P which came down to 5 IU at 4–5 hours in a similar experiment. When probenecid produced a concentration of 8–10 IU/ml of P throughout.

In this estimation, continued administration of probenecid was found helpful to retain a high concentration of P in serum. Earlier workers (Gibaldi and Schwarts 1968, Evans 1966) have also reported such retention of high concentration of P in serum when probenecid was given with 2.4 million IU of procaine penicillin. White et al (1956) reported a peak concentration of 8 IU/ml of P with procaine penicillin which was raised to 10 IU/ml with probenecid. This effect of probenecid in raising the peak concentration of P in serum could not be assessed in our experiment, as the sodium penicillin in Crys–12 was helpful to give the maximum peak concentration of P (10IU/ml) attainable in blood.

Often, there is little or no correlation between invitro sensitivity of microorganisms to various drugs and invivo response of microorganisms during treatment. Further the terms 'Sensitive' and 'Resistant' strains of microorganisms create confusion. There is no accepted criteria for sensitive and resistant lcassification of strains, as the range of sensitivity invitro is no measure of

sensitivity or resistance. This is because, in vitro, the organisms are in direct contact with the drug. Invivo, certain drugs need to metabolise to become effective, e. g. Sulphanilamide (Waterson 1967). Sometimes, the potency of drug may be reduced by protein binding invivo and/or invitro (Rolison 1967, Quinn-1964, Bond 1964), or non-specific phenomena such as production of drug inactivating enzymes by associated microorganisms invivo (Findland 1955 a, b). The wide differences in drug concentration in various of tissues (Bunn 1959), blood and other body fluids (Winnighan et al 1968) may affect the outcome of a particular treatment of infections with a particular regime Here, it seems worthwhile to adopt creteria such as the maximum drug concentration attainable in serum as a base (wide infra) to classify the strains into 'Sensitive' or 'Resisant' to a particular drug, as it is possible to maintain that drug level by repeated doses at frequent intervals.

The reports show that Sulphadizine even when given in high doses (4 gms + 1 gm four hourly - oral) produces only a maximum serum concentration of 100ug/ml (Waterson 1967, Goodman and Gillman 1967) Hence, 84% strains of gonorrhoeae in our series which required S 100ug/ml or more may have failed to respond to S-treatment. The rest 16% strains or a majority of them would have responded to S treatment. Similarly, streptomycin 1gm i. m. was found to produce a serum concentration of St 25-45 ug/ml (Goodman and Gillman 1965). In this series, 75% strains which required St 30 ug/ml or more for complete inhibition, may not have responded to St treatment. The rest 24% strains or a majority of them would have responded to St treatment. The sensivity of certain percentage of currently infecting strains of N. gonorrhoeae to St and S may be due to the discontinuance of St and S for the treatment of gonorrhoeae in this area, for the last 15-18 years. In other areas also sulphonamide (Willcox 1970) have been much less used than other drugs. The increase in the percentage of St resistant strains in 1970 compared to the figures in 1965 in this series has to be attributed to this cross resistance acquired against St by P less sensitive strains (Table-1).

Sodium penicillin 500,000 IU given I. M. produces a peak serum concentration of 10 IU/ml of P in our patients at two hours Thus all strains were sensitive to P. despite the increase in the number of less sensitive strains of N. gonorrhoeae to P in this area.

Table-4 shows that 3 strains of N. gonorrhoeae sensitive to P 0 5 IU/ml or less responded clinically to PAM treatment. Further, in majority of cases the minimum serum penicillin levels maintained during therapy were equal to or more than the invitro MICs of strains. Hence, it seems to us that for successful treatment of gonorrhoeae, it is necessary to choose penicillin preparations which will maintain a minimum serum drug concentration above the invitro MIC of strains at least for 24 hours, regardless of the peak concentration. A high peak drug concentration seems helpful to achieve a high tissue concentration in short

TABLE-4

Correlation Between invitro penicillin sensitivity of N. Gonorrhoeae Strains and Maximum Blood Penicillin Levels.

Patient	Treatment	Maximum blood penicillin levels in IU/ml.	Minimum 24 hrs.	Strains MIC in IU/ml.	Clinical and bacteriological result
1.	PAM 600,000 units x 4 days	1 0	0.5	0 2	Cured
2.	do	1 0	0.5	0.2	,,
3.	do	1.0	0.5	0.5	,,
4.	Crys-12. 2 4 MU- Single injection	10 0	0.4	0 5	,,
5.	do	10 0	0.5	1.0	,,
6.	Crys-12. 4 8 MU-Single injection	10.0	1 0	0.5	,,
7.	Crys-12. 2 4 MU-Single injection with 1 gm probenecid 1 hr befor injection	9.0	1.0	0.5	,,
8.	do	10.0	1.0	1.0	,,
9.	Crys-12. 2.4 MU-Single injection with 1 gm probenecid before injection followed by 0.5 gm every 6 hrs	10.0	6.0	1.0	,,
10.	Penicillin Sodium G 500,000 IU 6 hourly for 5 days	10.0	2 0	2.0	,,

time, thus shortening the duration of therapy required for cure. Considering this criteria, in 1965, 96.5% strains sensitive to P 0.5 IU/ml or less (of which 78.1% were sensitive to P 0 25 IU/ml) might have been cured with PAM. Similarly, in 1970,37% of strains which were sensitive to P 0 5 IU/ml or less (of which 17% were sensitive to P 0.25 IU/ml) should have been cured with PAM. In actual practice, controlled clinical trials in this series showed 26% cure rate (which includes all strains of P 0.2 IU of less, 50% of P 0.5 IU and 8% of 1.0 IU) and uncontrolled trials in 1965 showed a 80% cure rate. These differences in the calculated and actual cure rates with PAM may be due to discrepancies in penicillin levels in different patients. In fact on many occasions, different drug serum level values have been produced by different form of preparation of the same drug (penicillin–Bunn 1959, tetracycline–Olon & Holvey 1961); by different workers on the same preparation (penicillin–Griffith & Peck 1958, White et al 1956, Cohen 1950); and sometimes by the same worker at different time of experiment (Irons 1950). Variations even on the same patient have also been reported (Lucas 1968 – as quoted by Willcox 1970). Hence, a good correlation is seen between invitro penicillin sensitivity of N. gonorrhoeae and invivo response of

N. gonorrhoeae to penicillin, during treatment with different penicillin preparations.

Good clinical results have been reported in some area by single injections of 1.2 – 1.4 mega units (MU) (Klaska et al 1963, Marshall and Curtis 1967) or 2.4 MU (Staheli 1964, Ashmalla et al 1966) of aqueous procaine penicillin. In our clinic, though single session therapy with 2 4 MU of Crys 12 have been found to give good clinical response in all six cases tested, this regime of penicillin is not followed routinely. High doses (4.8 MU) of Crys–12 are not favoured, as these higher doses are found not helfpul in retaining the drug over long periods or producing higher penicillin concentration, compared to lower doses (2.4 MU). It seems to us that 2.4 MU of crys–12 with 1 gram probenecid before injection followed by 0.5 grams six hourly may produce high and prolonged penicillin levels in blood. Sodium penicillin 500.000 IU six hourly with or without probenecid may be given only in exceptionally penicillin–less sensitive strains of N. gonorrhoeae. Thus, Penicillin continues to be the best drug for the treatment of gonorrhoeae.

Tetracycline 250 mgs given orally every six hours produces a drug concentration of 2–4 ug/ml in serum, while one injection 250 mgs I M., produces a concentration of 10ug/ml (Goodman and Gillman 1965). In our study, all strains were sensitive to T 1.0 ug/ml or less. Hence, all of them would have responded well to T given either orally or intrampuscularly. Chloramphenicol 2 gms given orally produces a drug level of 15–25 ug/ml (Goodman and Gillman 1965). In present series, 82% strains required C 20 ug/ml or more. The rest 18% strains would have responded to C given orally.

In our experience, there seem to be wide disparities between the invitro sensitivity range of N. gonorrhoeae to C and T and invivo response of strains to them during treatment. Three strains sensitive to T 1 0 ug/ml invitro did not respond to T given orally, while one strain of T 0.5 ug/ml range responded to T orally.

Two other strains of T 0.5 and T I.0 ug/ml responded to T given intramuscularly. Similarly, three strains sensitive to C 20 ug/ml did not respond to C given orally, while one strain with a sensitivity range of C 5 ug/ml responded. But C when given intramuscularly seems to produce good clinical results in gonorrhoeae, as one strain in our trials sensitive to C 20 ug/ml responded to C 1 gm daily for five days. Thus the route of administration of C and T seems to be very important in the treatment of gonorrhoeae. Only when the strains are sensitive to C and T in very low concentrations, oral administration of both these drugs seems effective against gonorrhoeae. In other areas, tetracycline 250 mgm. I M. plus 1 gm orally out single session, or one injection of T and multiple oral doses of it; as well as C 1 gm I.M, or single oral doses of C (Willcox 1970) are found to have given good clinical response against gonorrhoeae.

SUMMARY

One hundred N. gonorrhoeae strains isolated in 1970 were tested against sodium penicillin (P), streptomycin sulphate (St), Chloramphenicol (C), Oxytetracycline (T) and sulphadiazine (S) for their minimum inhibitory concentrations (MICs) by plate dilution method. In 10 patients, serum penicillin levels were estimated by 'Sacrina lutea' method after intramuscular injections of procaine penicillin G in oil with 2% aluminium monostereate (PAM), crys-12 (Squibb-procaine penicillin + sodium penicillin) and sodium penicillin. The PAM treated cases were clinically and bacteriologically followed up for invivo and invitro correlation of drug sensitivity test results.

The percentage of strains sensitive to various concentrations of drugs were : P 0.05 or less = 9, P 0.1 = 1, P 0.2 = 7, P 0.5 = 20, P 1.0 = 56, P 2.0 = 3, P > 2.0 = 4 of IU/ml; St 5 or less = 14, St 10 = 10, St 30 = 3, St > 30 = 73 of ug/ml; C 5 or less = 18, C 20 = 32, C > 20 = 50 of ug/ml; T 0.2 or less = 36, T 0.5 = 24, T 1.0 = 40 of ug/ml and S 10 or less = 15, S 50 = 1, S 100 = 29, S 100 = 55 of ug/ml. The maximum and minimum penicillin levels in IU/ml of serum with various penicillin preparations of therapeutic doses were : PAM 60 ,000 IU = 1.0 and 0.5; crys-12 2.4 MU = 10 and 0.4-0 5; crys-12 4.8 MU = 10 ahd 1.0; crys-12/2. 4 MU + 1 gm probenecid one hour before injection = 10 and 1.0; crys-12/2.4 MU = 1 gm probenecid one hour before injection followed by 0 5 gms 6 hourly = 10 and 6 0; Sodium penicillin 500,000 IU 6 hourly = 10 and 2.0; and Sodium penicillin 500,000 6 hourly + probenecid = 10 and 8.0. The cure rates with various treatment regimes in percentage figures were : PAM = 26, crys-12 = 100, and sodium penicillin = 100. Intramuscular injections of oxytetracycline and chloramphenical gave good clinical results.

There is a definite increase in the percentage of P-less sensitive strains in this area. Penicillin resistant strains showed cross resistance against St. Penicillin continues to be the best drug followed by T and C against gonorrhoeae in this area. Good invivo and invitro correlation of drug sensitivity test results were seen with penicillin. Less correlation was found witih T and C. Details are discussed.

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