IN VITRO PENICILLIN SENSITIVITY OF NEISSERIA GONORRHOEAE STRAINS ISOLATED IN PUNE (INDIA)

P. N. ARORA, A. S. SOHI AND JAGADISH RAI

Summary

Fifty strains of Neisseria gonorrhoeae isolated at random from 55 male patients with gonorrhoea in Pune were subjected to in vitro sensitivity test to penicillin. The range of sensitivity of these strains to penicillin was from 0.0075 to 4.0 intranational units per ml. 27 (54.00%) strains were within the sensitivity range and six (12 %) were sensitive only to 4.0 I.U. of penicillin per ml. The median of sensitivity was 0.062 I.U. (equivalent to 0.038 mcgm) per ml. This is compared to the results obtained by other workers in the field from 1944 to 1975. Strains of gonococci under study did not show absolute resistance to penicillin.

When Neisseria gonorrhoeae (*N. gonorrhoeae*) developed resistance to sulphonamides about thirty years ago, penicillin became the drug of choice[1] in the treatment of gonorrhoea and has remained the mainstay of treatment for several decades. To start with the therapeutic doses were very small[2,3]. In the course of time it became evident that many cases failed to respond to penicillin since the sensitivity of N. gonorrhoeae to this drug had decreased in many of parts of the world as recorded in the literature[4-12].

Chacko and Yogeswari[13] at Madras (South India) isolated 23.4 per cent gonococci resistant to penicillin during the period April 1963 to September 1964; and 60.4 per cent between October 1964 and September 1965. Similar studies in the same centre during 1970[14] revealed 90 per cent resistant strains indicating thereby a rise in the resistance incidence. In Bombay (Western India), the resistant strains isolated during 1968 to 1969 was reported to be 56 per cent[15] as compared to 33.3 per cent isolated in Delhi[16]. The incidence of resistant strains has been shown to have geographical variations[12]. Hence the present study was undertaken.

Material and Methods

The material for this study included 50 strains isolated from 55 male cases of gonorrhoeae picked up at random from outpatient departments of Dermato-Venereology of Armed Forces Medical College, Cantonment General Hospital, Sasoon Hospital and indoor patients of Venereal Disease Treatment Centre, Command Hospital (SC), Poona; from July 1975 to December 1975.

The following procedures were adopted :-

Departments of Dermato-Venereology and Microbiology,
Armed Forces Medical College,
Pune-411001

Present address :
18 Bhagat Road, Jullundur Cantt.

Received for publication on 12—5—1979

Detailed clinical findings were recorded. Staining of smear was done by Gram's method[17] which was followed by cultureon chocolate agar medium[18]. Tetramethylene paraphenylene diamine hydrochloride was used for oxidase reaction[19]. Sugar tests were performed in serum sugar tubes.

Sensitivity of N. gonorrhoeae towards penicillin was performed by disc (6 mm diameter of Whatman No 1 filter) diffusion method. Concentration of penicillin per disc was 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9, 0.95, 0.48, I. U. equivalent to 4, 2, 1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015 and 0 0075 I. U. by tube dilution method respectively as done through a calibration curve. The results of penicillin sensitivity were recorded after 24 hours by measuring the zone of inhibition, if present.

Observations

The values of MIC of penicillin recorded for number of strains of gonococci from 0.0075 to 4.0 I. U. are shown in Table 1.

TABLE 1

Number of Strains with Minimum Inhibitory Concentration (MIC) of Penicillin

MIC of penicillin in I. U. per ml of medium	Strains inhibited by different concentrations of penicillin	
	Number	Percent
0.0075	12	24
0.015	2	4
0.031	6	12
0.062	7	14
0.125	6	12
0.25	2	4
0.5	4	8
1.0	4	8
2.0	1	2
4.0	6	12
Total	50	100

It was observed that 54.00 per cent had decreased sensitivity towards penicillin.

The mean and standard deviation of zone of inhibition of N. gonorrhoeae towards different concentration of penicillin (from 0.0075 to 4 I. U. per ml) are shown in table 2. There was increase in average values of zone of inhibition with increase of penicillin concentration per ml.

TABLE 2

Relation of Penicillin Concentration to Zone of Inhibition

I. U. of penicillin per ml of medium	Mean of zone of inhibition in mm	Standard Deviation
0.0075	8.75	4.73
0.015	10.21	5.80
0.031	11.90	3.71
0.062	13.77	3.21
0.125	15.90	7.20
0.25	16.85	6.57
0.5	17.61	6.27
1.0	17.41	6.54
2.0	18.27	6.57
4.0	18.50	5.94

Discussion

Most of the studies carried out on N. gonorrhoeae strains isolated earlier than 1950 had shown that all strains were sensitive to less than 0.125 I. U. of penicillin per ml, the range of MIC being 0.005 to 0.025[3], 0.002 to 0.033[20] and 0.0038 to 0.024[21]. In 1950, however, the limit of the range of susceptibility was reported to have increased to or beyond 0.125 I. U.[22,23] and 0.515 I.U.[24] along with the emergence of a few resistant strains.

It has been generally agreed that strains requiring 0.125 I. U. per ml or more are relatively resistant[25,26,27]. On the basis of this in our study 23 (46.00 per cent) strains showed relative resistance/decreased sensitivity to penicillin.

In 1958, Reyn and her collegues[21] studied the 90 strains of 1944 by testing them in 1957. The range of sensitivity was 0.0038 to 0.024 I. U. per ml, which

TABLE 3

Median values for the minimum Inhibitory Concentration to Penicillin

S. No.	Authors	Country	Year of study	Number of strains	Mediau mcgm per ml
1.	Reyn et al (1958)[21]	England	1944	90	0.0045
2.	Lankford (1945)[3]	U.S.A.	1945	100	0 0058
3.	Love et al (1955)[20]	U.S.A.	1945	24	0.002
4.	Romansky et al (1947)[32]	U.S.A.	1947	57	0.012
5.	Love et al (1955)[20]	U.S.A.	1954	106	0.003
6.	Gurtis et al (1958)[33]	England	1957	302	0.003
7.	Gradock-Watson et al (1958)[24]	England	1957	200	0.009
8.	Thayer et al (1961)[28]	U. S. A.	1959	368	0.021
9.	Amies (1967)[6]	Canada	1959	320	0.005
10.	Gjessing et al (1964)[5]	Norway	1959–1962	1000	0.026
11.	Snell et al (1963)[34]	Canada	1963	200	0.018
12.	Chacko et al (1966)[13]	Madras (India)	1963–1965	160	0.038
13.	Amies (1967)[6]	Canada	1966	979	0.053
14.	Nicol et al (1968)[9]	England	1966	91	0.022
15.	Smith and Levy (1967)[8]	Australia	1967	104	0.027
16.	Ho and Chang (1967)[7]	Taipei	1967	59	0.059
17.	Moses et al (1971)[15]	Bombay (India)	1968-1969	216	0.0129
18.	Keys et al (1969)[10]	Phillipines	1968–1969	242	0.223
19.	Silverstein (1973)[35]	Taipei	1970	130	0.0256
20.	Panikabutra et al (1973)[11]	Thailand	1967–1970	96	0.133
21.	Duncan (1972)[3]	U.S.A.	1972	81	0.045
22.	Watko et al (1975)[12]	Phillipines	1971	258	0.232
23.	Bhujwala et al (1973)[16]	Delhi (India)	—	93	0.19
24.	Present study (1979)	Pune (India)	1975	50	0.037

was accepted as original range of sensitivity of N.gonorrhoeae to penicillin and considered as a base for comparison results. Out of the strains isolated in 1944, not even one was less sensitive, whereas out of 130 strains of gonococci isolated in 1957, 17 per cent showed decreased penicillin sensitivity. Thayer et al[28] from USA found that during 1955 to 1958, 20 per cent strains were inhibited by 0.10 I. U. of penicillin per ml or more; and in 1959, 25 per cent of 368 strains required the same concentration for inhibition.

In 1963, a study was undertaken by World Health Organisation on 452 strains isolated from Africa, Phillipines, Ceylon, Poland and Greenland. Out of these 1.0 per cent strains from West Africa during 1960—62; 27 per cent of strains from Ceylon in 1961; 10 per cent obtained from Poland in 1962; 10 per cent during 1960-61 and 83 per cent in 1962 from Greenland were less sensitive to penicillin; and all the strains from Phillipines isolated in 1961 revealed decreased sensitivity. Not a single strain was found absolutely resistant to penicillin[29]. Watko et al[12] reported only 9.3 per cent strains sensitive to 0.05 mcgm (equivalent to 0.1 I.U.) of penicillin and all the rest of the

strains sensitive at a higher concentration. The strains least sensitive to penicillin reported in the literature had MIC of 4.0 I. U. per ml[30]. Six strains in the present study were sensitive only to 4.0 I. U. of penicillin.

Though MIC values are most frequently used for reporting the susceptibility of gonococci to penicillin in vitro, Watko and Bownlow[12] found the median to be a useful single statistic for the comparison of ranges of MIC values. In order to compare out data with earlier reports in the literature, the median MIC values of data from other studies have been calculated, converted into mcgm and shown in Table 3. The MIC of N. gonorrhoeae to penicillin in this study (0.062 I. U. per ml equivalent to 0.037 mcgm per ml) was almost similar to those reported by Chacko and Yogeswari in 1966[13]; and Duncan in the year 1972[31].

Acknowledgement

It is a privilege to thank Vice Chancellor, University of Poona, to grant permission to publish this paper; as it formed a part of the dissertation submitted for MD (Derm, Ven & Lep) by the first author.

References :

1. Reyn A : Sensitivity of N. gonorrhoeae to antibiotics, Brit J Vener Dis, 37 : 145, 1961.
2. Cohn A and Seijo IH : In vitro effect of penicillin on sulphonamide resistant and sulphonamide susceptible strains of gonococci, J Amer Med Assn, 124 : 1125, 1944.
3. Lankford CE : The in vitro tolerance of gonococcus for penicillin, Amer J Syph, 29 : 56, 1945.
4. Gjessing H : Increasing failure rate after using the same preparation and dosage of penicillin in treatment of gonorrhoea, Brit J Vener Dis, 35 : 256, 1959.
5. Gjessing HC and Odegard K : Sensitivity of the gonococcus to antibiotics and treatment of gonorrhoea (1000 cases) Acta Derm Venereol, 44 : 132, 1964.
6. Amies CR : Development of resistance of gonococci to penicillin, Canad Med Assn J, 96 : 33, 1967.
7. Ho TJ and Chang SS Quoted by Watko and Brownlow, Far East Med J, 3 : 320, 1967.
8. Smith DD and Levy JM : Quoted by Watko and Brownlow, Med J Aust, 1 : 849, 1967.
9. Nicol CS, Ridley M, Symonds MAE : The problem of penicillin resistant gonococci, Brit J Vener Dis, 44 : 315, 1968.
10. Keys TF, Halverson CW and Clark FJ : Single dose treatment of gonorrhoea with selected antibiotic agents, J Amer Med Assn, 210 : 857, 1969.
11. Panikabutra K and Suvanmalik S : Sensitivity to penicillin of Neisseria gonorrhoeae in Bangkok - Relation to the results of treatment, Brit J Vener Dis, 49 : 209, 1973.
12. Watko LP and Brownlow WJ : Antibiotic susceptibility of Neisseria gonorrhoeae isolated in Western Pacific, Brit J Vener Dis, 51 : 34, 1975.
13. Chacko CW and Yogeshwari L : A survey of the range of sensitivity in vitro of N. gonorrhoeae to penicillin, Indian J Med Res, 54 : 823-828, 1966.
14. Sowmini CN and Nair CM : In vitro drug sensitivity of Neisseria gonorrhoeae strains and blood penicillin levels after various penicillin preparations, Indian J Derm Vener, 37 : 149, 1971.
15. Moses JM, Desai MS, Bhosle CB and Trasi MS : Present pattern of antibiotic sensitivity of gonococcal strains isolated in Bombay, Brit J Vener Dis, 47 : 273, 1971.
16. Bhujwala RA, Sood P, Pasricha JS, Singh OP, Omprakash and Kandhari KC : Susceptibility of N. gonorrhoeae in vitro to antibiotics (penicillin, oxytetracycline, chloramphenicol and Rifampicin), Indian J Med Res, 61 : 156 , 1973.
17. Burrows W : Text book of microbiology, 19th Ed, Saunders, Philadelphia, 1968.
18. McLeod JW, Coats JC, Happold FC, Priestley DP and Wheatley BB : Cultivation of gonococcus as a method in the

diagnosis of gonorrhoea with special reference to the oxidase reaction and the value of air reinforced in its carbon dioxide content, J Path Bact, 39 : 221, 1934.

19. Price INO : Oxidase reaction in the laboratory diagnosis of gonorrhoea, Brit Med J, 1 : 199, 1929.

20. Love BD and Finland M : Susceptibility of Neisseria gonorrhoeae to eleven antibiotics and sulphadiazine, Arch Int Med, 95 : 66, 1955.

21. Reyn A, Korner B and Bentzon MW : Effects of penicillin, streptomycin and tetracycline on N. gonorrhoeae isolated in 1944 and in 957, Brit J Vener Dis 34:227, 1958.

22. Schummer H and Hubbeg A : Die penicillin and streptomycin resistender gonokoken in vitro (penicillin and streptomycin resistance in gonorrhoea in vitro), Hautartz, 2 : 500, 1951.

23. Marcuse K and Hussels H : Quoted by Guthe, Derm Wschr, 130 : 1031, 1954.

24. Gradock-Watson JE, Shoolre RA and Nicol CS : Sensitivity of strains of gonococci to penicillin, sulphathiazole and streptomycin, Brit Med J, 1:1091, 1958.

25. Guthe T : Failure to control gonorrhoea, Bull Wld Hlth Org, 24 : 297, 1961.

26. Reyn A and Bentzon MW : A study of relationships between the sensitivities of N. gonorrhoeae to sodium penicillin G, four semisynthetic penicillins, spiramycin and fusidic acid, Brit J Vener Dis, 44:140, 1968.

27. Morton RS : Recent advances in sexually transmitted diseases, Churchil Livingstone, 1 : 32, 1975.

28. Thayer JD, Field FW, Ferry MI, Martin JE (Jr) and Garson W : Surveillance studies of Neisseria gonorrhoeae sensitivity to penicillin and nine other antibiotics, Bull Wld Hlth Org, 24:327, 1961.

29. Hossain ASMT : Gonococcal resistance to penicillin, Indian J Derm Ven, 33:25, 1967.

30. Reyn A : Antibiotic of gonococcal strains isolated in South East Asia and Western Pacific regions in 1961-68, Bull Wld Hlth Org, 40 : 245, 1969.

31. Duncan WC : Epidemic venereal diseases, Proc, 2nd Int Vener Dis Symposium, St. Louis, 1972.

32. Romansky MJ and Robin EVD : A comparison of the in vitro susceptibility of the gonococcus to penicillin G and X., Amer J Syph, 31 : 271, 1947.

33. Curtis FR and Wilkinson AE : A comparison of the in vitro sensitivity of gonococci to penicillin with the results of treatment, Brit J Vener Dis, 34 : 70, 1958.

34. Snell E, Norries DA and Strong J : Susceptibility of Neisseria gonorrhoeae to antibiotics — A study of 200 consecutive strains isolated in Winnipeg in 1962, Canad Med Assn, 89 : 601, 1963.

35. Silverstein DM : Milit Med, 138 : 18, 1973. (Quoted by 12)