ORIGINAL CONTRIBUTIONS

PENICILLIN RESISTANT GONOCOCCI

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Minimum inhibitory concentration (MIC) of penicillin was estimated for 25 strains of *N. gonorrhoeae*, isolated over a period of 10 months (September 1983 - June 1984). In 16 strains, the MIC of penicillin ranged between 0.01 μg/ml and 0.63 μg/ml and all were negative for penicillinase production. In 9 strains, the MIC was >1.25 μg/ml, and 5 of these strains produced penicillinase. Of these 5 penicillinase producing strains, 4 had MIC >10μg/ml and in one it was 2.5 μg/ml.

Key Words : Penicillin, Resistance, Gonococci, Penicillinase.

Strains of *N. gonorrhoeae* resistant to penicillin have been reported from all over the world including our country.[1-6] This increasing resistance to penicillin has been further complicated by the appearance of penicillinase producing strains of *N. gonorrhoeae* (PPNG).[7] These PPNG strains have been reported to have very high minimum inhibitory concentrations (MIC's) of penicillin.[8-11] Available data shows that PPNG constitute 10-30% of all the isolates in various parts of Asia and Africa. Such strains have also been reported from our country in the recent past.[8,13-16] The present communication describes the penicillin sensitivity pattern and penicillinase production in *N. gonorrhoeae* in this part of our country.

Material and Methods

Over a period of 10 months (September 1983 to June 1984), 25 strains of *N. gonorrhoeae* were isolated from patients suffering from acute gonococcal urethritis attending the Nehru hospital attached to the PGIMER, Chandigarh, India. The organisms were isolated and identified by the conventional techniques.[17]

All the isolates were screened for resistance to penicillin by the disc diffusion technique using 6 μg (10 i u) disc of penicillin.[18] The strains showing an inhibition zone diameter of <20 mm were recorded resistant to penicillin and suggestive of penicillinase production.

The minimum inhibitory concentration of penicillin was tested in all the strains by the plate dilution technique.[18] Doubling dilutions of penicillin G (1640 units/mg) obtained from Sarabhai Chemicals (India) were incorporated in GC agar (Difco) with 1% haemoglobin (Difco) and 1% isovitalex (BBL) to give concentrations from 0.01 to 10 μg/ml. The inoculum was prepared by making a suspension of *N. gonorrhoeae* taken from 18-20 hour growth on chocolate agar. The suspension was made in proteose peptone No 3 broth (Difco) and was adjusted to contain 10^8 colony forming units/ml by matching the suspension to a 0.5 McFarland standard. The suspension was then diluted in proteose peptone No 3 broth to contain 10^6 colony forming units/ml. A drop (2-5 μl) of this working dilution was then delivered on the surface of antibiotic containing agar plate. inoculation was also made on a chocolate agar

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plate without the antibiotic and Oxford strain of *Staphylococcus aureus* (MIC of penicillin 0.03 μg/ml) was used as control. The plates were incubated at 36°C in 10% CO_2 for 18 hours and the MICs were read the next day. An organism having an MIC of ≤0.08 μg/ml was considered to be highly sensitive to penicillin; MIC between 0.16-0.63 μg/ml moderately resistant and MIC of >1.25 μg/ml as highly resistant.

The penicillinase production in all the isolates was checked by the rapid iodometric technique.[18] The following strains were used as controls : (1) Penicillinase producing *N. gonorrhoeae* strains WHO 5731/77 and 6788/71 obtained from Dr Inga Lind, Neisseria department Staten Serum Institute, Copenhagen, Denmark. (2) A local penicillinase producing strain of *Staphylococcus aureus*, and (3) A local penicillin sensitive strain of *N. gonorrhoeae*.

Results

Five of the 25 strains showed an inhibition zone diameter of <20 mm in disc diffusion technique using 6 μg disc of penicillin and produced penicillinase, whereas the other 20 strains had an inhibition zone diameter of <20 mm and were negative for this enzyme. The results of MIC values of 25 strains of *N. gonorrhoeae* are shown in table I. Nine (36%) strains showed MIC of <0.08 μg/ml of penicillin (sensitive), 7 (28%) had MIC of 0.016-0.63 μg/ml (moderately resistant), and 9 (36%) showed MIC of >1.25 μg/ml (highly resistant).

Out of the 9 resistant strains (MIC range 1.25 μg/ml - 10 μg/ml), 5 produced penicillinase (PPNG), while 4 were negative for this enzyme (Non-PPNG). Four of the five PPNG had MIC >10 μg/ml, whereas in one it was 2.5 μg/ml.

Patients from whom resistant strains were isolated did not respond to 4.8 mega units of procain penicillin with 1 gm probenecid and were treated with 2 gm kanamycin sulphate intramuscularly. Of the 5 patients infected with PPNG strains, 2 were Nigerians and 3 Indians.

Comments

Penicillin resistance in *N. gonorrhoeae* was first reported in 1955.[19] Since then, resistance has been reported with greater frequency from different parts of the world ranging between 38.4 per cent to 86 per cent.[3-6,20-24] Different reports from India show wide variations in the degree of resistance to penicillin. In Madras, out of 197 gonococci isolates tested, 65.5% had MIC of 0.44 μg/ml or more.[6] Fifty six per cent of 216 isolates from Bombay were reported to have MIC values of 0.15 μg/ml.[5] In Delhi, over a period of 14 years (1969 to 1982) the increase in resistance has ranged from 9.1 per cent to (51.68%) per cent.[4] In the present study, 16 (64%) of 25 strains showed moderate to high resistance to penicillin (MIC >0.08) μg/ml).

Out of 9 strains with MIC >1.25 μg/ml, 5 with MIC range of 2.5 to <10 μg/ml produced penicillinase (PPNG), while 4 with MIC of 1.25 μg/ml showed intrinsic resistance (Non-PPNG). Such observations have also been made earlier by Chowdhury et al,[9] where 15 per cent of the non-PPNG strains showed MIC of penicillin ranging between 0.5 to 2 μg/ml.

Table I. Minimum inhibitory concentration of penicillin in *N. gonorrhoeae*.

	Number of strains with the MIC value in μg/ml										
	>10	5	2.5	1.25	0.63	0.32	0.16	0.08	0.04	0.02	0.01	Total
Penicillinase negative *N. gonorrhoeae*	—	—	—	4	—	4	3	1	1	—	7	20
Penicillinase positive *N. gonorrhoeae*	4	—	1	—	—	—	—	—	—	—	—	5

The isolation of 5 PPNG strains from our population suggests that there is a reservoir of such strains in this part of our country. Isolation of such strains is not surprising in a cosmopolitan city like Chandigarh where students from other parts of Asia and Africa come for higher studies. Since two of the strains were isolated from Nigerian students, it is quite possible that these strains came from the African continent. Four of the 7 patients infected with PPNG strains were reported to be foreign students (3 Nigerian, 1 Iranian) in our earlier study.[16] Epidemiology of the disease could not be investigated due to non-cooperation of the patients and as such the exact source of infection could not be ascertained.

We conclude that the increasing resistance to penicillin and the discovery of PPNG strains in our environments should alert us to a periodical reappraisal of penicillin sensitivity patterns. There should be routine screening and confirmation of all the strains for penicillinase production.

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