

# B-Lactamase Producing Neisseria Gonorrhoea Strains in Karachi

Pages with reference to book, From 70 To 71

Rafiq Khanani, A. Razak Memon, Rasool Bux Shaikh, Ghulam Ali, Mubashir Shaikh, Zahid Hussain ( Department of Pathology, Sindh Medical College, Karachi. )

Tariq Sandila, Nighat Parveen ( Department of Pathology, Skin and Social Hygiene Centre, Karachi. )

## Abstract

Urethral or cervical swab of 255 patients attending Skin and Social Hygiene Centre and found positive for gram negative Intracellular diplococci on direct microscopy were inoculated on Modified New York City (MNYC) medium and chocolate (heated blood) agar for Isolation of neisseria gonorrhoea. Growth of N. gonorrhoea was obtained in 134(52.5%) cases. These strains were tested for penicillin susceptibility by disc diffusion method and for the production of B-lactamase by rapid penicillinase paper strip test and rapid chromogenic cephalosporin method. Penicillin resistance was found in 31(23%) strains, of which twelve (9%) were B- lactamase producers (PPNG), the remaining 19 (14%) strains were penicillin resistant B-lactamase negative (Pen RB Neg). We conclude that PPNG as well as other penicillin resistant strains (Pen RB Neg) of neisseria gonorrhoea are prevalent in our country and appropriate changes in the conventional therapeutic regime are desirable (JPMA 44:70, 1994).

## Introduction

Gonorrhoea has become one of the most common bacterial infections worldwide. Among the factors responsible for this gonorrhoea pandemic are alleged increase in promiscuity, use of oral or parenteral contraceptive methods rather than protective contraceptive methods, a large and gradually increasing reservoir of asymptomatic females and males who unknowingly transmit the disease to their sexual partners and decreasing susceptibility of gonococci to commonly used antibiotics<sup>1</sup>. During the last 30 years, resistance to penicillin G has gradually risen probably by selection of chromosomal mutants<sup>2</sup>. The problem of resistance of gonococci to penicillins has been further aggravated by emergence of B-lactamase producing gonococci since 1976<sup>3</sup>. This resistance is plasmid mediated and gonococci might have acquired it from haemophilus or other gram negative organisms<sup>4</sup>. Focal outbreaks of B-lactamase producing neisseria gonorrhoea have been reported from Phillipines, Thailand, California, New York and elsewhere and endemic foci are being established<sup>5</sup>. This study was conducted to assess the frequency of treatment failures with conventional therapy for gonorrhoea.

## Material and Methods

### Origin of strains:

Skin and Social Hygiene Centre is the only hospital in Karachi specifically designated as a centre for sexually transmitted diseases (STD). This study includes 255 patients who presented to this hospital from March, 1989 to January, 1992 with a history of purulent urethral discharge and showing intracellular gram -ve diplococci on direct microscopy of urethral or cervical smears.

### Isolation and identification:

Specimens were collected by two sterile calcium alginate swabs - one swab was used for marking well on chocolate agar which was subsequently spread thinly by a sterile wire loop<sup>6</sup>. Second swab was seeded by streaking over the surface of the Modified New York City (MNYC) medium while rotating it to ensure that all surfaces of swabs tip come in contact with the medium. Inoculated plates were

incubated immediately in a candle jar with white wax candles, transferred to incubator at 35°C and examined after 24 and 48 hours. Characteristic 0.5-1.0 mm, grey to white, opaque, raised and glistening colonies were subjected to oxidase test. Rapid reacting oxidase positive colonies were picked up for gram staining and biochemical confirmation with rapid carbohydrate degradation tests (oxid).

#### **Susceptibility to penicillin:**

It was determined by picking 5-10 colonies from primary plate, emulsifying it in Muller Hinton broth adjusting inoculum size to  $10^8$  organisms/ml with 0.5 McFarland Standard and inoculating it on 1% IsoVitalax supplemented GC agar base plate and applying a 6 ug penicillin disc. It was incubated in a candle jar at 35°C. A zone size of c 20mm in diameter at 24-48 hours was suggestive of a penicillin resistant strain<sup>7</sup>. Control strains obtained from Centres for Disease Control (CDC), Atlanta, Georgia were also included with each batch. All the strains showing resistance to penicillin, i.e., zone diameter of <20 mm were tested for B-lactamase production.

#### **B-lactamase production:**

It was determined by (i) Rapid Penicillinase paper Strip test<sup>8</sup> and (ii) Rapid Chromogenic Cephalosporin method using nitrocefin impregnated paper discs<sup>9</sup>. Control strains from CDC viz: penicillin resistant  $\beta$ -lactamase negative (Pen RB Neg) and Penicillin resistant B-lactamase positive (Pen RB Pos) were also included with each batch. Penicillin resistant strains which were found B-lactamase negative were once again subjected to penicillin susceptibility test for reconfirmation of its penicillin resistance.

### **Results**

Out of 255 cases, growth of neisseria gonorrhoea was obtained in 134 (52.5%) cases. Of these 103 (77%) were sensitive and 31 (23%) resistant to penicillin. Production of B-lactamase was detected in 12 (9%) strains (PPNG; Pen RB Positive). Remaining 19 (14%) strains were penicillin resistant B-lactamase negative (Pen RB Negative).

### **Discussion**

Sexually transmitted diseases (STD) are hyperendemic in many developing countries and their incidence is increasing<sup>10</sup>. Treatment failures with conventional regimes of penicillin or ampicillin led us to conduct this study to find out susceptibility of neisseria gonorrhoea to penicillin, the most widely used drug for treatment of gonorrhoea. Present study showed resistance to penicillin in 23% strains, which is similar to 20 to 31% reported in other series<sup>11,13</sup>. However, it is much lower than that of 41% reported by Gedebo and Tassew<sup>14</sup> and 62% reported by Ng et al<sup>15</sup>. In the present study 12(9%) PPNG strains were isolated. This is comparable to 16% PPNG strains isolated by Chowdhary et al<sup>16</sup> 14.6% reported by Urabe et al<sup>17</sup>. . However, the figure is much lower compared to 40-50% PPNG strains reported from USA and Singapore<sup>5</sup>. The case histories of patients included in the present study indicated that eight (Pen RB Positive) strains isolated were acquired within the country and four strains were imported by sailors. However, due to stigma attached to STDs, concealment of facts by patients, lack of education, limited availability of STD clinics and almost non-existence of contact tracing and epidemiological studies, it is difficult to ascertain whether there are established endemic foci of PPNG and other penicillin resistant strains or these represent importation and only occasional secondary cases. Systematic multicentre studies for isolation, identification and susceptibility of neisseria gonorrhoea are needed to formulate recommendations for treatment of gonorrhoea, as conventional measures in vogue are bound to result in treatment failures leading to spread of disease and increased incidence of

complications of gonorrhoea.

## Acknowledgements

We sincerely thank Dr. Tahira Naqvi, Director, Skin and Social Hygiene Centre for help and guidance in patient selection. Our thanks are also due to Miss Amna and Mr. Rustam for technical assistance and to Mr. Muhammad Zamiruddin Kauser for typing the manuscript.

## References

1. WHO Technical Report Series. Beta-Lactamase-Producing neisseria gonorrhoea, 1978;616:123-42.
2. Biswaa, GD., Blackman, E.Y. and Sparling, PP. High frequency conjugal transfer of a gonococcal penicillinase plasmid. *J.Bacteriol.*, 1980; 143:1318-24.
3. Phillips, I.B. lactamase producing penicillin resistant gonococcus. *Lancet*, 1976;ii:556-57.
4. Sykes, RB. and Matthew, MJ. *Antimicrob. Chemother.*, 1976;2:115-57.
5. Centers for Disease control. Penicillinase producing neisseria gonorrhoea-United States, Worldwide. *Morb. Mort.Wkly. Rep.*, 1979;28:85-87.
6. Reyn, A. Antibiotic sensitivity of gonococcal strains isolated in the South-East Asia and Western Pacific Regions in 1961-68. *Bull. WHO.*, 1969;0:257-62.
7. Thornsberry, C., Gavan, T.L. and Garlach, EN. New developments in antimicrobial agent susceptibility testing. *Cumitech 6*, American Society for Microbiology, Washington, D.C., pp. 1-2.
8. Jorgensen, J.H., Lee, J.C. and Alexander, GA. Rapid penicillinase paper strip test for detection of beta-lactamase producing haemophilus influenzae and neisseria gonorrhoea. *Antimicrob. Agents, Chemother.*, 1977; 11:1087-88.
9. BBL Microbiology Systems. Cefinase Package insert BBL Microbiology systems. Cockeysville Md., 1986.
10. DeSchryver, A. and Meheua, A. Epidemiology of sexually transmitted disease: the global picture. *Bull. WHO.*, 1990;68:639-54.
11. Renkonen, O.V., Sivonen, A., Lassus, A. and Salo, O.P. Current status of the in vitro sensitivity of gonococcus to penicillin in Finland. *Acta. Derm. Venereol.*, 1970;50:151-53.
12. Moatter, T. A study of neisseria gonorrhoea in Karachi (thesis). University of Karachi, 1983, pp. 33-35.
13. Seth, A.D., Kolator, B. and Wilkinson, A.E. Sensitivity of neisseria gonorrhoea to antibiotics in London 1976-78. *Br.J. Vener.Dis.*, 1979;55:325-28.
14. Gedebo, M. and Tassew, A. Neisseria gonorrhoea isolates from Ethiopia: in vitro susceptibility pattern to live antibiotics. *Bull. WHO.*, 1980;58:67-71.
15. Ng. W.S., Anton, P. and Arnold, K. Neisseria gonorrhoea strains isolated in Hong Kong: in vitro susceptibility to 13 antibiotics. *Antimicrob Agents. Chemother.*, 1981;79:12-17.
16. Chowdhury, M.N.H., Pareek, S.S. and Mshgoub, El-S. Penicillinase producing neisseria gonorrhoea in Riyadh, Saudi Arabia. *Br.J.Vener.Dis.*, 1981;57:256-58.
17. Urabe, S., Yoshida, S. and Mizuguchi, Y. Epidemiology and treatment of gonorrhoea caused by penicillinase producing strains of neisseria gonorrhoea in Fukuoka, Japan. *Br.J.Vener.Dis.*, 1983; 59:37-40.