A SURVEY OF CLINICAL ISOLATES OF NEISSERIA GONORRHOEAE FOR BETA-LACTAMASE PRODUCERS

ARUNA GOHIL * AND S. S. KELKAR †

Abstract

Strains of *Neisseria gonorrhoeae* isolated from smear-positive male patients suffering from acute gonococcal urethritis were studied. Their identity was confirmed on the basis of morphology, oxidase reaction, fermentation of glucose only and failure to grow on nutrient agar. A total of 100 such isolates were tested for beta-lactamase production by use of nitrocefin. None of these isolates were beta-lactamase producers. A WHO reference beta-lactamase positive strain (No. 5731) was the control.

Introduction

The changing antibiotic resistance patterns of clinical isolates of *Neisseria gonorrhoeae* is yet another intriguing drug-parasite effect in the situation of host-parasite-drug interactions. Sulphonamides, introduced in the treatment of gonococcal infections in 1937[1] were a magical cure. However, by 1944 most isolates of *N. gonorrhoeae* were resistant and the drug was ineffective. Penicillin provided the answer. The last 35 years have shown a gradual increase in the minimum inhibitory concentrations of strains of *N. gonorrhoeae* to penicillin. Increasing the treatment dose seemed to provide the solution. An alarming development then was the demonstration of a plasmid-mediated beta-lactamase in *N. gonorrhoeae*[2]. Acquisition of this enzyme led to a sudden increase in resistance and penicillin became ineffective. Many of the isolates of *N. gonorrhoeae* from the Far East showed beta-lactamases and penicillin-resistance[3]. Strains with this enzyme in developed countries were thought to have originated in the Far East and their frequency showed an increasing trend[4]. Monitoring the occurrence of such strains is therefore important. We describe here a study of clinical isolates of *N. gonorrhoeae* in Bombay, screened for beta-lactamase production by use of sensitive Nitrocefin method.

Material and Methods

Strains of Neisseria gonorrhoeae : Male patients suffering from clinically-frank acute gonorrhoea attending the out-patient department of the Sir J.J. Group of Hospitals, Bombay were studied. Only smear-positive cases were processed. Primary isolation was on clarified chocolate agar with the use of a candle-jar[5]. Organisms were deemed to be *N. gonorrhoeae* if they were Gram-negative diplococci, oxidase positive, fermenting glucose

* Technician

† Professor

Department of Microbiology,
The Grant Medical College, Bombay.

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only-glucose, maltose, lactose and sucrose were studied-and unable to grow on nutrient agar[6]. A total of 100 isolates were obtained.

Test for beta-lactamase[7] : A working solution was prepared by dissolving 5 mg nitrocefin in 0.5 ml dimethyl sulfoxide and adding 9.5 ml of 0.1 M phosphate buffer, pH 7. The solution was used within 14 days of its preparation. A thick suspension of the strain was made in sterile saline. Equal volumes of the suspension and nitrocefin solution were mixed in the well of a porcelain tile and allowed to react at room temperature for 30 minutes. A red colour was a positive test. A known beta-lactamase producing strain (WHO standard No. 5731) and a strain derived from a standard *Pseudomonas aeruginosa* (ATCC No. 10662) were the controls. These were tested with every batch of tests.

Results

None of the 100 clinical isolates of *N. gonorrhoeae* showed the presence of beta-lactamases.

Discussion

The demonstration of plasmid-mediated beta-lactamase production in a a strain of *N. gonorrhoeae* by Phillips[4] and Ashford et al[8] led to widespread interest and awareness of the phenomenon. Beta - lactamase producing strains were increasingly isolated in both the U.S. and Britain[2,9]. The rates of isolations in the Far East were as high as 44 per cent of strains from female prostitutes[3]. A single strain was also isolated in Germany[10], and as many as 9 per cent of the isolates in Liverpool in a nine-month period were beta-lactamase producers. The epidemiological features of an outbreak of gonorrhoea caused by beta-lactamase positive *N. gonorrhoeae* in Liverpool were described by Arya and others[11] : cases were initially confined to a localised area with a large immigrant population; there was some spread, but the infections remained circumscribed. In the US, the increased frequency of beta-lactamase producing strains was correlated with army and other personnel returning from postings to bases in the Far East[4,9,12].

The biological and biochemical properties of the beta-lactamases and their plasmids have also been adequately worked out. Robert and Falkow[13] described two separate beta-lactamase coding plasmids with differing masses of 4.4 x 10^6 and 3.2 x 10^6 daltons. Bergstrom and others[14] were of the view that the plasmid was of of TEM 1 type and agreed that it was unrelated to the plasmid of conjugal transfer. Sox and others[15] thought the conjugative (sex) plasmid of *N. gonorrhoeae* preceded the beta-lactamase plasmid in terms of evolution. Enriquo and Amato[16] studied the enzymes by affinity chromatography; it had a molecular weight of 25000 daltons and a pI of 5.4. Its substrate specificity and inhibition pattern was like that of the class III TEM types. Recently Hafix and others[17] found that penicillin-sensitive, non beta-lactamase-containing strains of *N. gonorrhoeae* when "cured" with ethidium bromide, lost sulfafurazole-resistance and showed beta-lactamase activity. The inference was that beta-lactamase production was an inherent property of *N. gonorrhoeae* masked by the gene for sulfafurazole resistance. The current concern is with transfer of TEM type plasmids in Gram-negative organisms. Piott and others[18] have noted such plasmids in nasopharyngeal isolates of *H. influenzae*, *Branhamella cattarrhalis* and *N. flava* and the fear is acquisition of penicillin-resistance by *N. meningitidis*.

The results of the present study indicate that the problem of beta-lacta-

mase producing gonococci has still not reached us. In a study from New Delhi, Bhujwala and others[19] also could not find such strains amongst 95 isolates using an indicator capillary tube method. A report from Ibadan (Africa)[20] also indicates the absence of such strains. It is quite surprising that beta-lactamase producing organisms have still not been encountered in our country considering the increasing ease of international travel. The puritanical habits of the Indian traveller could be one explanation for this. The epidemiological behaviour (cited above) could be another explanation.

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