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Non-penicillinase producing *Neisseria Gonorrhoeae*: are they still in existence in Ibadan, Nigeria?

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Summary

In an attempt to evaluate the current prevalence rate of penicillinase producing *Neisseria gonorrhoeae* (PPNG) and whether non-PPNG strains are still in existence in Ibadan, Nigeria, all isolates of *Neisseria gonorrhoeae* from patients that attended our clinic between January and December 1997 were studied. Of the 155 patients that had gonococcal infections, 118 were male (76.1%) and 37 (23.9%) were female with 31 (83.8%) being the partners of infected men. Sixty-four (54.2%) of the male and 19 (51.4%) of the female were aged between 20 and 29 years while 21.2% of the male and 16.2% of the female were in the age of 40 and above. The sex difference is not statistically significant ($\chi^2=1.47, P=0.69$). The present study revealed that non-PPNG strains have reduced considerably to 5.4% from 100% in 1977. This has posed a great threat to the usefulness of penicillin and ampicillin as the drugs of choice in gonococcal therapy in Nigeria.

Keywords: Non-PPMG, Ibadan, Nigeria

Résumé

Dans une tentative d'évaluer le taux de fréquence de pénicilline produisant de *Neisseria gonorrhoeae* (PPNG) et à savoir si des espèces non-PPNG sont toujours existantes à Ibadan (Nigéria), les substances de *Neisseria gonorrhoeae* prélevées des malades qui ont fréquenté notre clinique entre janvier et décembre 1997 avaient été étudiées. Parmi les malades ayant des infections gonococcales, 118 (72,2%) étaient des hommes et 37 (23,7%) étaient des femmes dont 31 entre ces femmes sont des partenaires des hommes infectés. Soixante-quatre (54,2%) de ces hommes et 19 (51,4%) de ces femmes étaient âgés de 20-29 ans tandis que 21,2% des hommes et 16,2% des femmes étaient âgés de 40 et plus. La différence en sexe n'était pas statistiquement significative ($\chi^2=1,47, P=0,69$). Cette étude a révélé que les espèces de non-PPNG ont réduit considérablement de 100% à 5,4% en 1977. Cela a constitué une menace très sérieuse à l'utilité de pénicilline et ampicilline comme des prédilection dans le traitement de gonococcal au Nigéria.

Introduction

As at 1977, uncomplicated gonorrhoea was being treated with 2.4 million units of procaine penicillin or 3 gm of ampicillin plus 1 gm of probenecid. This was the period when Penicillin was the drug of choice in the treatment of gonorrhoea and it was being used freely for the treatment of gonococcal infections. However, there has been a steady increase in prevalence of antibiotic resistant strains of *Neisseria gonorrhoeae* with the attending therapeutic failure with penicillin.

Surveillance screening of *Neisseria gonorrhoeae* isolates in Ibadan for PPNG strains revealed no PPNG in 1977. [1,2]. The prevalence rate of non-PPNG then was 100 percent.

However, it has been reasonably established that strains of PPNG are now circulating freely in Nigeria. [1,2,3,4,5].

In early 1979, Osoba *et al.* [6] found the prevalence of PPNG to be 2.7% while that of Ghana was 36%. There has since been a steady increase in the prevalence rate of PPNG in Ibadan. From the data available to us (Table 1 & Figure), we observed a steady rise from 2.7% of PPNG isolates in early 1979 to 50% by the end of 1981 [3], fluctuating between 70% and 80% by 1989 [5]. The previous work done by the authors showed the prevalence rate of PPNG to be 92.2% in early 1996 [7,8].

At the rate the prevalence of PPNG is increasing, it is doubtful if non-PPNG is still in existence in Ibadan. This has definitely posed a great threat to the usefulness of penicillin (a cheap drug) as the drug of choice in the therapy of gonococcal infections, thereby requiring the use of penicillinase-resistant antimicrobial agents. Most of these drugs such as spectinomycin, Cefuroxime, Augmentin, Ciprofloxacin are expensive and beyond the financial resources of most patients and, furthermore, they have to be given parenterally.

The present study sought to determine the current prevalence rate of PPNG and whether non-PPNG strains are still in existence in Ibadan.

Materials and methods

Study population

The study population comprised of patients that attended the Special Treatment Clinic of the University College Hospital between 15 January 1997 and 15 December 1997. A standard medical history was taken from each patient concerning symptoms and findings, marital status, previous genito-urinary symptoms, date of last coitus and sexual consorts (whether casual or regular). A clinical examination of the lower genito-urinary tract for signs of infection such as urethral discharge, vaginal discharge and endocervical discharge was carried out.

Control group

Thirty male students who had no signs and symptoms suggestive of urethritis served as control subjects.

Laboratory procedures

Specimens obtained from the urethra, cervix and vagina were plated on modified Thayer-Martins media and incubated immediately at 37 °C in a candle extinction jar for 24-48 hours. Smears were made and stained by Gram's method for intracellular gram-negative diplococci. A wet preparation of the genital discharge was examined for *Trichomonas vaginalis*, *Candida albicans*, Clue cells and parasites. All Gram-negative cocci were identified by oxidase reaction and sugar utilization test in serum-free agar medium [9]. All isolates of gonococci were tested on enriched chocolate agar by the disc diffusion method for sensitivities to penicillin, Ampicillin, tetracycline, ofloxacin, ciprofloxacin and spectinomycin. Those that were resistant to 10 units of penicillin and 6 ug Ampicillin disc were taken to be PPNG and these were confirmed by the Iodometric method. [10].

Minimum inhibitory concentration (Mic) determination.

Minimum inhibitory concentrations (MICS) of Penicillin G (Crystapen), Spectinomycin, Tetracycline, Ciprofloxacin, and Ceftriaxone were determined with an agar dilution technique. Gonococcal reference strains were not available as control. An inoculum of 10^4 colony-forming units was delivered on BHIA. This was then incubated at 37°C for 18 hours. The lowest concentration of antibiotics, which completely inhibited the growth of the test organism or caused a decrease of more than 95% in the growth of the organisms, was regarded as the MIC for that strain. A control plate without an antibiotic was included in each test.

Statistical analysis

Data were analyzed by applying the t-test and the chi-squared test.

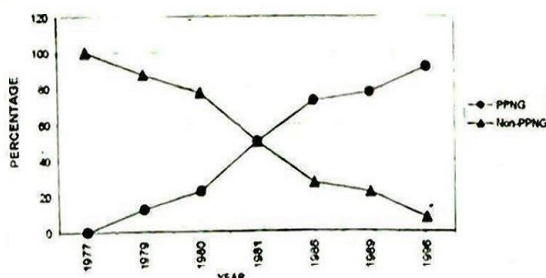
Results

The data available to us from the previous studies done in our center revealed a dramatic and steady rise in prevalence of PPNG (Table 1 & Figure 1).

Table 1: Results of screening of *Neisseria gonorrhoeae* in Ibadan, Nigeria.

Year	% PPNG	% Non-PPNG
1977	0.0	100
1979	12.45	87.55
1980	22.27	77.73
1981	50	50
1986	73	27
1989	78	22
1996	92.2	7.8
1997	?	?

Figure 1 TREND OF OCCURENCE OF PPNG AND Non-PPNG IN IBADAN, 1977-96



During the period of the study, 562 male patients and 196 female patients attended our center and were investigated. Of the 155 patients that had gonococcal infections, 118 were male (76.1%) and 37 (23.9%) were female 31 (83.8%) of who were partners of infected men.

Table 2 shows that a higher percentage of the male patients had NSU (62.1%) than gonorrhoea (21%) while in women gonococci were found in 18.9% of the women investigated.

Table 3 shows the age distribution of those that had gonorrhoea, 64 (54.2%) of the male and 19 (51.4%) of the female were aged between 20 and 29 years while 21.2% of the

Table 2: STDs diagnosed by sex

Diagnosis	Male (n=562)		Females (n=196)	
	No.	%	No.	%
Gonorrhoea	118	21.0	37	18.9
Genital warts	29	5.1	14	7.1
Candidosis	24	4.3	83	42.4
<i>Trichomonas vaginalis</i>	42	7.5	55	28.1
Non Specific Urethritis	349	62.1	0	0

Table 3: Age distribution of patients with gonorrhoea by sex

Age (yrs)	Males (n=118)		Females (n=37)	
	No.	%	No.	%
10-19	7	5.9	4	10.8
20-29	64	54.3	19	51.4
30-39	22	18.6	8	21.6
40 and above	25	21.2	6	16.2
Total	118	100	37	100

$$\chi^2 = 1.47 \quad df = 3 \quad P = 0.69$$

male and 16.2% of the female were in the age of 40 and above. The sex difference is not statistically significant ($\chi^2 = 1.47, P = 0.69$). Of the 155 cases analyzed, 147 (94.6%) were PPNG strains while 5.4% were non-PPNG strains.

All the isolates were susceptible to Spectinomycin (MIC<64ug/ml), Ceftriaxone (MIC<0.25ug/ml) and Ciprofloxacin (MIC<0.06ug/ml). The PPNGs were resistant to both penicillin and ampicillin with MIC over 0.25 ug/ml of each antibiotics (Table 4).

Table 4: Cumulative percentage of mics (ug/ml)

Antimicrobial agent	0.0625	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Penicillin G											
Ampicillin											
Spectinomycin											
Ciprofloxacin											
Ceftriaxone											

Amongst the 30 control male subjects, *Trichomonas vaginalis* were recovered from three of them. None of them had gonorrhoea. The difference in the occurrence of *T. vaginalis* between the patients and the male control group was highly statistically significant ($P < 0.001$).

Discussion

Extensive surveillance screening of *Neisseria gonorrhoeae* in Ibadan showed no strain of PPNG as at 1977, hence almost 100 percent of the circulating strains were non-PPNG [11]. However, various studies have shown that the PPNG strains that were introduced into Nigeria early 1979 have now become well established and are circulating freely in the country [3]. The upsurge in the incidence of PPNG was attributed to the large number of nationals from ECOWAS countries particularly Ghana that migrated to Nigeria in 1978 in search of employment, while most of the men were gainfully employed many of the women engaged in prostitution [2]. Table 1 shows a steady and progressive decrease in the incidence of non-PPNG in Ibadan, Nigeria as at 1996. This has posed a great threat to the usefulness of penicillin and ampicillin as the drugs of choice in gonococcal therapy due to the production of β -lactamase by the PPNG [3]. Unfortunately these are still the drugs of choice of general practitioners and pharmacists outside this hospital.

The present study revealed that non-PPNG strains in Ibadan have reduced considerably to 5.1%. It has now been reasonably confirmed that, though non-PPNG strains still exist in Ibadan, their number is insignificant. The last two decades have witnessed a dramatic decrease in the incidence of the non-PPNG strains in this environment and in many parts of Africa as a result of which PPNG strains have become endemic [3]. There is the possibility that the incidence of PPNG being highlighted here may not be representative of the reservoir in the community as many patients often decide to treat themselves with the range of antibiotics, both oral and injectable that are available in open market. In order to establish a true picture of the incidence of PPNG strains, a multi-centre research programme would be necessary at every level at which the patients present rather than the teaching hospital based method employed in this study.

However, with the present incidence rate (94.5%) of PPNG strains in our environment, though not representative, it is clear that they have become well established and within the next decade, non-PPNG strains are not likely to exist.

Unfortunately, the drugs with proven efficacy against these PPNG strains such as spectinomycin, cefuroxime, cefotaxime and 4-quinolones are relatively expensive and beyond the resources of most patients.

In Nigeria at present, there are organized S.T.D. clinics only in the teaching hospitals where patients pay for consultation, laboratory investigations and treatment. In some of these clinics, laboratory facilities are inadequate to distinguish between PPNG and non-PPNG, and hence the drugs of choice should be effective on both strains and these drugs are usually expensive. The help of the government and international agencies are required in the control of STDS/AIDS including gonorrhea in Nigeria.

The introduction of syndromic management of STDS

is a significant and welcome advance in the management of STDS in Nigeria, but the drugs especially for gonococcal infections should be effective against both PPNG and non-PPNG, and affordable.

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