7

PENICILLINASE PRODUCING NEISSERIA GONORRHOEAE (PPNG) IN KUMASI, GHANA: THE NEED FOR MORE EFFECTIVE TREATMENT REGIMEN AND CONTROL STRATEGIES

Prof. Pat A-K Addy, Theresa Agbemadzo and P. Aikins-Bekoe

ABSTRACT

During a one-year study period cases referred to the Komfo Anokye Teaching Hospital Clinical Microbiology laboratory with the diagnoses, acute urethritis, pelvic peritonitis, sticky eyes, Ophthalmia neonatorum or vulvo-vaginitis, were examined for penicillinase producing Neisseria gonorrhoeae (PPNG). In all 116 (69.0%) N. gonorrhoeae strains were isolated from a total of 168 cases examined.

Of the 116 culture-positive N. gonorrhoeae isolated, 106 were tested for beta-lactamase activity and 95 (89.6%) were found to be penicillinase producing N. gonorrhoeae (PPNG) strains. Sixty-seven (67) of the 95 PPNGs were from males and twenty-eight (28) were from females.

Among the latter were 2 (two) from children aged 10 and 12 years. Urethral discharge from a 65 year-old female also yielded PPNG.

A number of the PPNG strains were not only resistant to penicillin but to other antimicrobial agents as well including tetracycline, chloramphenicol and co-trimoxazole. The highest PPNG rate was recorded for the age group 16 through 30 years, with the age group 21 - 30 years topping the scale. These findings have grave public health implications not only for the pregnant woman and the neonate but also for the entire community. On the strength of our findings it is suggested that the recommended (Ghana Ministry of Health) treatment regimen for gonococcal infections should be revised and that spectinomycin should be prescribed when absolutely necessary in order to forstall resistance development to the drug.

Keywords: PPND (Penicillinase Producing Neisseria gonorrhoeae), Gonorrhoeae. Kumasi

INTRODUCTION

The first isolations of strains of beta-lactamase producing N.gonorrhoeae capable of degrading penicillin were made in Britain and the United States of America [1, 2]. At the end of 1976, PPNG strains had been identified in Singapore, the Philippines, and Japan, with epidemiological information indicating their presence in Thailand and Hong Kong [3]. Subsequent isolation of PPNG strains were reported from La Cote d'Ivoire [4], Nigeria and Ghana [5, 6] and South Africa [5].

It has been claimed that PPNG strains might have originated in South East Asia and West Africa.

That Africa should be connected with the origin of PPNG, stems, perhaps, from the finding that many Ghanaian and Nigerian patients with gonorrhoeae resident in London had the PPNG variety [3].

Examination of specimens from Ghanaians who had returned from Nigeria (1983) showed that only four of 64 gonococcal strains isolated were sensitive to penicillin. Similarly five out of 11 isolates from Ghanaians who recently (1986) returned from La Cote d'Ivoire were sensitive to penicillin [Addy, unpublished data]

We, therefore, conducted a study to evaluate the incidence of PPNG among patients attending the Komfo Anokye Teaching Hospital in Kumasi, Ghana.

MATERIALS AND METHODS

A total of 168 consecutive patients, aged 2 days through 65 years and made up of 83 males and 85 females, were referred to the Clinical Microbiology Department of the Komfo Anokye Teaching Hospital in Kumasi with diagnoses of acute urethritis, pelvic peritonitis, Ophthalmia neonatorum or acute vulvo-vaginitis, for laboratory investigations.

From the male patients, urethral discharges were

Prof. Pat A-K Addy,
Dept. of Clinical Microbiology
UST, Kumasi

Theresah Agbemadzo,
Department of Clinical Microbiology,
UST, Kumasi

P. Aikins-Bekoe,
Department of Clinical Microbiology,
UST, Kumasi

expressed by gentle squeeze of the penis after thorough cleansing of the penis and the penile orifice with sterile ethyl alcohol-soaked cotton wool swab.

High vaginal smears were obtained from female patients by one of us (T.A.) after placing patients in the lithotomy position, parting both labia and cleaning the vagina with sterile saline-soaked gauze. In certain cases endocervical swabs were collected by the referring physician and transported to the laboratory in Stuart's Medium.

Immediately following specimen collection, smears for Gram's staining were prepared and examined for Gram-negative intracellular diplococci (GNID). Specimens were also inoculated on Thayer-Martin's Medium and incubated at 37°C for 24 - 48 hours in a moist chamber containing 5% carbon dioxide.

Gram-stained smears were made of typical N. gonorrhoeae colonies grown on this medium and when found positive, the colonies were then tested for their reaction to oxidase by applying a drop of one per cent oxidase reagent (tetramethyl-p-phenylene diamine hydrochloride). A change of colony colour, initially to pink and finally to purple is indicative of a positive reaction.

Fermentation reactions of the isolates to 1% solutions of glucose, maltose and suerose in serum-free medium with phenol red as indicator [9] were performed on all oxidase positive colonies. A change of the red colour to yellow in only the glucose-containing medium was diagnostic for N. gonorrhoeae.

Colonies which were oxidase positive and fermented glucose, were tested by the multi-disc (Oxoid) diffusion method for their sensitivities or otherwise to chloramphenicol, co-trimoxazole, tetracycline and penicillin on enriched chocolate agar.

Finally, all isolates resistant to penicillin were tested for beta-lactamase production. In this test a portion of the gonococcal colony was removed with a sterile platinum loop and plated out on to Intralactam Strip (Mast-laboratories, Liver, England). With the production of beta-lactmase (penicillinase) by the test organism, the Intralactam Strip turned pinkish within a few seconds.

RESULTS

Of the 168 referred cases examined which consisted of 83 males and 85 females, 116 gonococcal strains were isolated, giving a gonococcal isolation rate of 69.0%

Of the 116 gonococcal strains isolated, 106 were tested for beta-lactamase activity and ninety-five (89.6%) were found to be PPNG strains. Since ten (10) gonococcal isolates could not be tested for beta-lactamase activity, the calculated PPNG prevalence rate among the isolates tested was 60.1%.

The monthly detection rate of gonococci isolated and the number of PPNG strains identified, is depicted in Figure 1, from which distinct peak gonorrhoea incidence periods may be recognized. In Kumasi, in other words, most cases of gonorrhoea were detected/reported between December and February, followed by a case reporting decline in March.

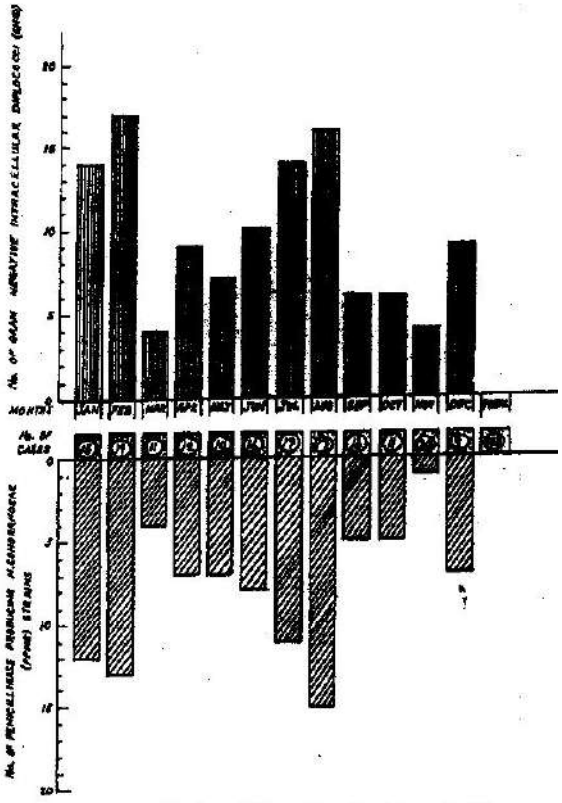

Fig. 1 Distribution of Gram Negative Intracellular Diplococci (GNIB) and PPNG strains isolated, January - December

Gradual increases were noticed from April and August, with peak incidence in August. From September through November, one noticed a gradual decline in incidence only to be confronted with high case reporting in December which peaked in February.

Age and sex distribution patterns of gonorrhoeal cases are shown in Table 1. There were 82 (71.0%) males and 34 (29.3%) females amongst whom were 12 male and 15 female children in the age-range 0 - 5 years. Vaginal discharge taken from 16 (59.3%) of 27 mothers of babies with Ophthalmia neonatorum, were also found to harbour gonococci [6].

Table 1 also shows that both males and females aged between 16 years and 30 years were the most affected; gonococcal isolation rates were 74.4% for males and 38.2% for females. Two girls aged 10 and

12 years had gocococcal infection. The gonococcal strain isolated from the 10 year-old girl was of the PPNG variety. PPNG was also isolated from a 65 years old woman.

It is interesting to note that of the 27 gonococcal strains isolated from eye discharges of 27 neonates (Table 1), 22 (81.5%) were PPNG strains. Of the 61 PPNG strains isolated from adolescents and young adults (16 to 30 years old), 52 (85.2%) were from males whilst only 9 (14.8%) were from females.

In vitro assays to test for bacteria sensitive to penicillin showed that only 10.4% (11 of 106) of the isolates were inhibited (Table 1). In addition 16 PPNG strains were resistant to one other microbial agent (Table 2).

Table 1
Age and sex specific distribution of gonococcal isolates in Kumasi

AGE GROUPS (in years)	MALES			FEMALE		
	Number of Gonococcal Isolates	Beta-lactamase reaction		Number of Gonococcal Isolates	Beta-lactamase reaction	
		No. Positive	No. Negative *		No. Positive	No. Negative *
0 - 5**	12	8	4	15	14	1
6 - 10	0	0	0	1	1	0
11 - 15	0	0	0	1	0	1
16 - 20	26	22	4	5	5	0
21 - 30	33	30	5	8	4	4
31 - 40	7	5	2	3	3	0
Over 40	2	2	0	1	1	0
Total	82	67 (81.7%)	15 * (18.3%)	34	28 (82.4%)	6* (17.6%)

* Exclude 10 gonococcal strains not tested for beta-lactamase activity: 9 from males and 1 from a female
** All were neonates aged below 22 days

Table 2
Age and sex distribution pattern of PPNG Isolates resistant to one antimicrobial agent (inside of Penicillin) tested.

Age groups (in years)	Isolated PPNG Strains		Actual ages of patients	
	Resistant to	Number	Males	Females
0 - 5	Cotrimoxazole	2	-	3/52; 3/52
6 - 10	-	-	-	-
11 - 15	-	-	-	-
16 - 20	Cotrimoxazole	2	18; 20	-
	Tetracycline	1	17	-
21 - 30	Chloramphenicol	3	22; 25; 28	-
	Tetracycline	4	21; 27	25; 30
	Cotrimoxazole	2	25; 30	-
31 - 40	Cotrimoxazole	1	40	-
Over 40	Cotrimoxazole	1	47	-

Table 3
Age and sex distribution pattern of PPNG isolates resistant to a combination of two other antimicrobial agents tested

Age groups (in years)	Isolated PPNG Strains		Actual Ages of patients (in years)	
	Resistant to	Number	Males	Females
0 - 5	-	-	-	-
6 - 10	-	-	-	-
11 - 15	-	-	-	-
16 - 20	Cotrimoxazole and Tetracycline	1	-	19
21 - 30	-	-	-	-
31 - 40	Cotrimoxazole and Tetracycline	1	35	-
Over 40	Cotrimoxazole & Tetracycline	1	-	65
	Cotrimoxazole & Chloramphenicol	1	46	-

DISCUSSION

Before 1983, the prevalence rate of penicillin resistant gonococcal strains isolated in Kumasi, was less than 5% [Addy, 1983, unpublished data]. With the influx, in 1983 of Ghanaian "Returnees" from Nigeria, a three-day STD screening campaign, mounted in Kumasi, revealed that of 64 clinically obvious or suspected cases of gonorrhoeae examined, 60 (93.8%) of the isolates were PPNG. As a result of these findings a hospital-based study to detect PPNG was mounted. The findings from this study are presented in the report.

Our present findings show that from a low PPNG prevalence rate of less than 5% in 1983 [Addy, unpublished data], the prevalence rate of PPNG in Kumasi has now soared up to 60.1% in less than 10 years. This figure is higher than PPNG prevalence rates found in Accra, 36.4% ([5], Singapore, 35.5% [10], Malasia, 37.3% [11], Indonesia, 25% [12], Thailand, 42% [13], South Korea, 40% [14], Japan, 16.1 [15]) and the Philippines, 30-40% [6], but pretty close to that found in Nigeria [17]. Thus, there seems to be differences in penicillin susceptibility patterns of N. gonorrhoeae in different countries and even in different regions of the same country.

Sex distribution rates of PPNG in our study followed a universal pattern, with a preponderance of cases amongst the male population [21,22]. In both sexes, the highest prevalence rates of N. gonorrhoeae and PPNG were encountered with almost equal frequency among the 16-30 year olds, a finding which is in conformity with reports by Osoba, 1980 [5], Barnes and Holmes, 1984 [21] and Aral and Holmes, 1984 [23].

From personal observations we are convinced that factors that may account for the high prevalence of antibiotic resistant strains of the gonococcus in Kumasi, Ghana, could be:

(a) Inadequate control of the sale of antibiotics encourages self-medication, often with inadequate doses. Because of the fear of the stigma associated, in our community, with gonorrhoea and other sexually transmitted diseases, over-the-counter purchases of antibiotics is the order of the day.

(b) Absence of carefully standardized treatment regimen based on research on indigenous cases.

(c) Lack of medical control and counselling to practitioners of prostitution who serve as repositories for and sources of dissemination of the gonococcus.

(d) Lack of adequate supportive laboratory services.

The recommended (Ghana's Ministry of Health) first line treatment regimen for gonococcal infections for adults in Ghana is 4.8 mega-units of procaine penicillin intramuscularly preceded by 1.0 gm. of probenecid by mouth. In the case of treatment failures and infections due to PPNG, it is suggested that 2.0 gm. of spectimycin be given intramuscularly. An alternate suggestion is the use of cotrimoxazole, tetracycline, cefoxitin or cefurozine and the new cephalosporins.

From our antimicrobial sensitivity test results it becomes obvious that the above recommended first choice treatment regimen for gonococcal infections if followed religiously in Government Hospitals and clinics in Kumasi might have been effective against only 10.4% of the gonococcal strains isolated there and completely ineffective against all 95 PPNG strains. The penicillin treatment failure rate, therefore, is 89.6%.

Spectinomycin is not routinely used in our hospitals because of the high cost involved. Albeit, a preliminary report by Biddle et al. [24], revealed the existence of chromosomally mediated spectinomycin resistant non-PPNG isolates in Korea. Since 1981, however, spectinomycin resistant PPNG strains have been reported from many parts of the world [25, 26]. All the same, excellent treatment results with spectinomycin have been reported by Osoba [7] from Nigeria.

With regard to cotrimoxazole as an alternative treatment regimen in Ghana for gonorrhoea and related diseases, it was found that 12.6% of our PPNG isolates were resistant to it. A bigger treatment failure rate of 30% with this drug has, however, been reported [27] from Singapore.

With tetracycline, an in vitro inhibition rate of 91.6% was achieved. It should be noted, however, that a high failure rate with tetracycline has been recorded in Thailand [13]. It has also been claimed that patients find it difficult to take tetracycline, a phenomenon, which has not been observed in Kumasi. All the same, to date there have been no reports of N. gonorrhoeae strains exhibiting plasmid-mediated resistance to tetracycline. On the contrary, however, the level of chromosomally-mediated tetracycline resistance has been found to be on the increase all over the world [28]. This may explain the 8.4% in vitro resistance rate recorded by us.

With the emergence of these resistant gonococcal strains encountered in this survey, the treatment of diseases caused by the gonococcus has created formidable problems, particularly in pregnant women and neonates, for whom the choice of drugs is limited. It may further be stressed that even in the treatment of non-PPNG strains, Ghana, like most other developing countries, has only a limited number of inexpensive drugs available. This state of affairs naturally calls for a serious review of the

commended treatment regimen, vide supra, for both the acute condition and the various sequalae of chronic and sustained N. gonorrhoeae infections in our society.

The situation further calls for prompt tracing of sexual contacts, routine examination of pregnant women for gonococci and other sexually transmitted diseases, the necessity for epidemiological treatment [10], screening of high risk groups within the population, prompt use of spetinomycin (no resistant PPNG strains so far detected in in vitro tests by us) under medical supervision and strict control of the sale, by prescription, of all forms of antibiotics in the country.

In view of the high proportion of beta-lactamase producing N. gonorrhoeae in Kumasi and perhaps the whole country, and the possible escalation in the use of spectinomycin for the treatment of gonorrhoea, it is our opinion that the Ministry of Health should as a matter of urgency, mount a surveillance programme to determine the prevalence of spectinomycin resistance amongst strains of N. gonorrhoeae by presumptive disc sensitivity testing in our hospitals and clinics throughout the country.

It is being suggested that in view of the fact that N. gonorrhoeae strains have been isolated from sites other than the eye [16], gonococcal infections of the neonate, are to be promptly treated with the appropriate antimicrobial agent.

Reference

1. *Phillips, I. Beta-lactamase - producing penicillin - resistant gonococci. Lancet, ii: 656-657 (1976).*
2. *Ashford, W.A., Golash, R.G. and Hamming, V.A. Penicillinase-producing Neisseria gonorrhoeae. Lancet, ii: 657-658 (1976).*
3. *World Health Organization, Neisseria gonorrhoeae and gonococcal Infections. Techn. Rep. Ser., No. 616 Geneva. WHO, 1978.*
4. *Poit, P. Resistance of gonococcus from the Ivory Coast. Lancet, i: 857 (1977).*
5. *Osoba, A.O., Afoakwa, S.N., Twum-Danso, K. and Ochei, J. Penicillinase - producing Neisseria gonorrhoeae in West Africa. 30th General Assembly of the International Union Against the Veneral Diseases and the Treponematoses, Berlin, German Democratic Republic, 6-12, June 1980.*
6. *Addy, P.A-K, Adjei, O., Agbemadzo, T.M. and Aikins-Bekoe, P. Ophthalmia neonatorum caused by penicillinase-producing Neisseria gonorrhoeae in Kumasi, Ghana., E. Afri. Med. J., 65/2: 28-30 (1988).*
7. *Osoba, A.O. Sexually Transmitted Diseases in Tropical Africa - A review of the present situation. Brit. J. Vener. Dis. 1981: 57: 89-94 (1981).*
8. *Thin, R.N., Barlow, D., Eytkyn, S. and Phillips, I. Imported penicillinase-producing Neisseria gonorrhoeae becomes endemic in London. Brit. J. Vener. Dis., 59:364-368 (1983).*
9. *Flyn, J. and Watkins, S.A. A serum-free medium for testing fermentation reactions in Neisseria gonorrhoeae. J. Clin. Path., 25: 525 (1972).*
10. *Sng, E.H., Lim. A.I. and Yeo, K.L. Susceptibility to antimicrobials of Neisseria gonorrhoeae isolated in Singapore: Implications on the need for more effective treatment regimens and control strategies. Brit. J. Vener. Dis. 60: 374-379 (1974).*
11. *Ngeow, Y.F. and Thong, M.L. Penicillin-resistant gonorrhoea A report from University Hospital, Kuala Lumpur. Med. J. Malaysia.*
12. *Josodivondo, S. Penicillinase-producing Neisseria gonorrhoeae in Jakarta, Indonesia (letter). Brit. J. Vener. Dis., 58: 408 (1982).*
13. *Brown, S., Biddle, J., Warnnissorn, T., Panikabutra, K. and Traisupa, A. Antimicrobial resistance of Neisseria gonorrhoeae in Bangkok: single-drug treatment passe? Lancet, ii: 1366-1368 (1982).*
14. *Joong, H.K, Jahang, W.J., Jong, H.C. and Sung, W.H. Sudden spread of PPNG (Penicillinase-producing N. gonorrhoeae) in Korea.. Journal of the Korean Medical Association, 25:163-167 (1982).*
15. *Yoshida, S-I., Urabe, S., and Mizuguchi, Y. Antibiotic sensitivity patterns of penicillin - positive and penicillin - negative strains of Neisseria gonorrhoeae isolated in Fukuoka, Japan. Brit. J. Vener. Dis., 58:305-307 (1982).*
16. *Perine, P.L., Morton, R.S., Poit, P., Siegel, M.S. and Antal, G.M. Epidemiology and treatment of penicillinase-producing Neisseria gonorrhoeae. Sex. Transm. Dis., 1979; 6:152-158 (1979).*
17. *Odugbemi, T. Antimicrobial Susceptibility and Plasmid content patterns of Gonococcal Isolates from Africa (Abstract). Presented at the 4th Congress of the African Union Against Veneral Diseases and Treponematoses, Libreville, Gabon, 1-5 April, 1985.*
18. *Nsanze, H. Sexually transmitted diseases. Africa Health, 6/3: 29-30 (1984).*
19. *Hira, S.K., Seth, J., Khosla, A., Bhat, S. Balaji, M. and Nkandwire, O. Ophthalmia neonatorum in Zambia (Abstract). Presented at the 4th Congress of the African Union Against Venereal Diseases and Treponematoses, Libreville, Gabon, 1-5 April, 1985.*
20. *Frost, E., Yvert, F., Ndong, J.Z. and Ivanoff, B. Neonatal Conjunctivitis in Franceville, Gabon (Abstract). Presented at the 4th Congress of the African Union Against Veneral Diseases and Treponematoses, Libreville, Gabon, 1-5 April, 1985.*
21. *Barnes, R.C. and Holmes, K.K. Epidemiology of gonorrhoea: current perspectives. Epidemiologic Reviews, 6: 1-30 (1984).*
22. *Nsan, E., The epidemiology of STD - a Nigerian perspective. Africa Health, 6/4: 25 - 26 (1984).*
23. *Aral, S.O. and Holmes, K.K. Epidemiology of sexually transmitted diseases. In: Holmes, K.K., Mardh, P.A., Sparling, P.F. et al., eds. Sexually transmitted diseases. New York: McGraw-Hill, (1984).*
24. *Biddle, J.W., Grundy, P., Whittington, W.L. et.al. Spectinomycin resistant Neisseria gonorrhoeae in Korea (Abstract). Presented at the International Society for STD Research, 5th International Meeting, August, 1-3, 1983, Seattle, W.A. (1983).*
25. *Adams, H., Ashford, W., Potts, D.W., San Diego, A., Ghong, V. and Grant, D. Spectinomycin resistant*

penicillinase-producing Neisseria gonorrhoeae in California. M.M. W.R., 30: 221-22 (1981).

26. *Easmon, C.S., Ison, C.A., Bellinger, C.M. and Harris, J.W. Emergence of resistance after spectinomycin treatment for gonorrhoea due to beta-lactamase-producing strains of Neisseria gonorrhoeae. Br. Med. J., 284: 1604-1605 (1982).*
27. *Rajan, V.S., Tan, T., Khoo, R., Sng. E.H., and Pang, C.P. and Tan, N.J. Treatment of gonorrhoea: the Singapore experience. Infect. Dis. 1: 71-74 (1977).*
28. *Jordan, W.C. Doxycycline vs. tetracycline in the treatment of men with gonorrhoea: the compliance factor. Sex. Transm. Dis.18 (Suppot.5): 5105 (1981).*