PENINCILLIN-RESISTANT GONORRHOEA
A Report from University Hospital, Kuala Lumpur.

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INTRODUCTION

WHEN PENICILLIN was first introduced in 1943, it provided an easy cure for gonorrhoea. However, soon after it came into regular clinical use, treatment failures began to appear and minimum inhibitory concentration (M.I.C.) determinations showed increasing levels of resistance to penicillin among gonococcal isolates (Reyn, 1969). This type of low level or partial resistance is chromosomally-determined and is apparently related to a decreased permeability of the cell envelope (Sparling, 1972), and diminished binding of penicillin to the cell membrane (Rodriguez and Saz, 1975). To maintain effective therapy, increasingly high doses of penicillin had to be used with the addition of probenicid (Center for Disease Control, 1974).

Since early 1976, strains of gonococci totally resistant to penicillin have been reported from many countries (Ashford *et al.*, 1976; Phillips, 1976; WHO, 1977). These strains produce an enzyme, B-lactamase (penicillinase), which destroys the penicillin nucleus. This new type of resistance is mediated by plasmids (extrachromosomal loops of DNA) which can be transferred between gonococci and into other bacteria by transformation or by conjugation. These plasmids are genetically very similar to B-lactamase (TEM) plasmids of ampicillin resistant *Haemophilus influenzae* and a number of enteric bacteria (Percival *et al.*, 1976; Elwell *et al.*, 1977). The prevalence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) among gonococcal isolates appears to be very low (0-2%) in Europe and the United States (WHO, 1977; Siegel *et al.*, 1978). In the Philippines, however, the reported prevalence of PPNG among gonococcal isolates in certain areas was 20-40% (Sparling *et al.*, 1977).

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The PPNG infections found in Singapore have increased significantly in 1978 as they comprised 4.8% of total isolates up till the end of July 1978 as compared to 0.24% for the whole of 1977 (WHO, 1978a).

The Sexually-Transmitted Diseases Laboratory, University Hospital, Kuala Lumpur, initiated surveillance for cases of PPNG infections in February 1977. We report here some clinical and laboratory findings of 43 such cases diagnosed between February 1977 and November 1978.

MATERIALS AND METHODS

Isolation of Neisseria gonorrhoeae

Specimens were obtained from patients seen in the University Hospital, Kuala Lumpur (UHKL), general practitioners' clinics (GP clinics) and two sexually-transmitted diseases clinics (STD clinics). The specimens were collected on charcoal-impregnated cotton wool swabs and sent to the laboratory in Stuart's transport medium. In the laboratory, the swabs were examined microscopically for pus cells and intracellular Gram-negative diplococci and cultured on Modified Thayer Martin medium (BBL), which is selective for gonococci, as well as on chocolate agar (prepared using 10% ox blood in Oxoid Blood Agar Base). The culture plates were incubated in a candle jar at 36°C for 1 to 2 days. Colonies of *N. gonorrhoeae* were identified based on the Gram-stain, oxidase reaction and direct fluorescent antibody test, as well as sugar utilization tests. The gonococcal isolates were routinely tested for penicillin susceptibility on chocolate agar using discs containing 10 units of penicillin. If the zone of inhibition was less than 20 mm in diameter, a β-lactamase test was done using the rapid iodometric method (WHO, 1976). Strains were preserved for further tests by freezing nutrient broth suspensions at -70°C.

The first 10 strains of PPNG were sent to Dr. Clyde Thornsberry at the Center for Disease Control, Atlanta, U.S.A., who confirmed the β-lactamase

production and reported on their susceptibilities to several antibiotics.

Antibiotic susceptibility testing

The agar plate dilution method (Jaffe *et al.*, 1976) was used to test the susceptibilities of the first 19 penicillinase positive strains and 60 penicillinase negative strains to 9 antibiotics. A multiple inoculator was used to test 25 strains simultaneously.

RESULTS

A total of 2509 specimens were received in the 22-months study period. 71% of the specimens were high vaginal or endocervical swabs, 16% were urethral swabs, 7% were conjunctival swabs and 6% consisted of miscellaneous specimens like throat swabs, rectal swabs, swabs of genital ulcers, urine, bubo or joint aspirates and prostatic fluid. From these specimens, 211 cases of gonococcal infections were diagnosed out of which 43 were by penicillinase producers.

Figure 1 shows the temporal distribution of all cases of gonococcal infections and Fig. 2 shows the temporal distribution of cases seen in UHKL only. The first two cases of infection by PPNG were diagnosed in October 1977 in UHKL (Ngeow and Chong, 1978). A 5-month interval lapsed before the next 2 cases were detected in April 1978. Since then, there has been a continuous isolation of PPNG strains. From August 1978 special efforts were made to obtain specimens from private clinics and the increase in number of specimens received resulted in a larger number of isolations of penicillinase negative and positive strains over the last 4 months of the study. As shown in Fig. 2, there were 43 cases of gonococcal infections from UHKL in the 11 months of 1977 with only 2 cases due to PPNG while 9 out of the 62 cases in the 11 months of 1978 were due to PPNG i.e. an increase in the proportion of PPNG cases from 4.7% in 1977 to 14.5% in 1978. A similar analysis is not made for cases from GP clinics and STD clinics because GP clinics contributed a rather small number of cases and most of the cases from STD clinics were seen after July 1978.

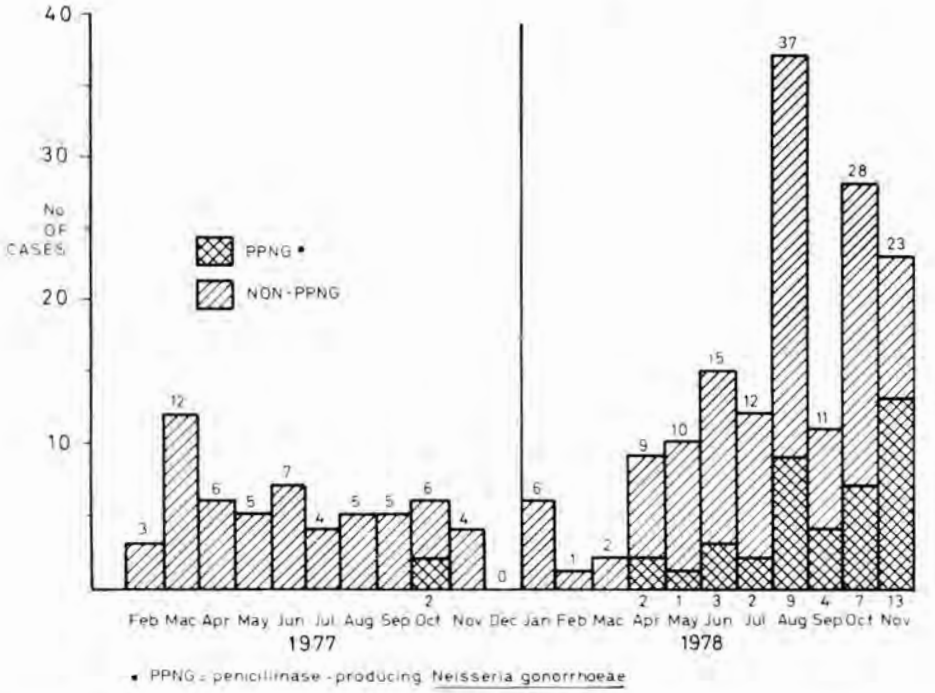

Fig. 1. Total number of cases of Gonococcal Infection by month from UHKL, GP Clinics and STD Clinics.

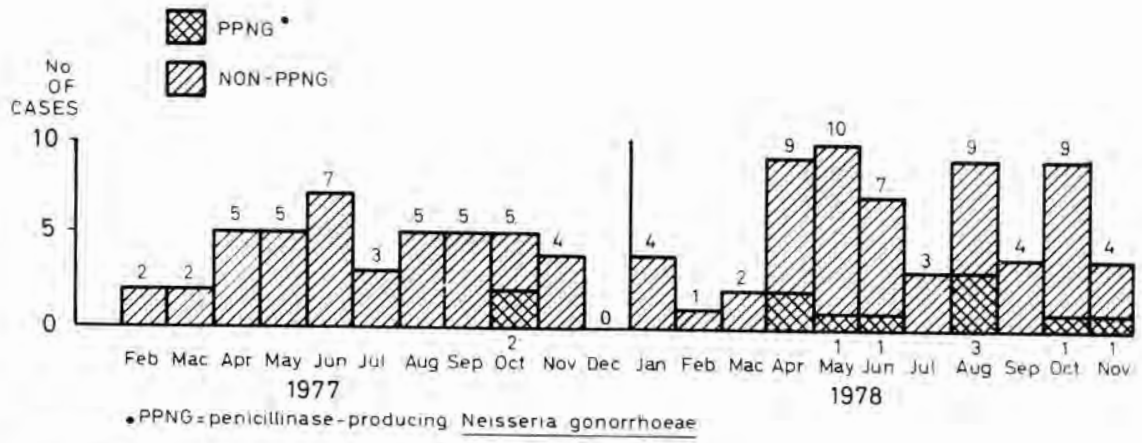

Fig. 2. Number of cases of Gonococcal Infection by month from UHKL

TABLE I

Distribution of PPNG infections from 3 sources

Sources	February 1977 - November 1978 (22 months)		August 1978 - November 1978 (4 months)	
	Total number of gonococcal infections	Total number of PPNG infections	Number of gonococcal infections	Number of PPNG infections
UHKL	105	11 (11%)	26	5 (19%)
GP clinics	14	2 (14%)	3	1 (33%)
STD clinics	92	30 (33%)	70	27 (39%)
Total	211	43 (20%)	99	33 (33%)

Table I shows the distribution of gonococcal infections in the 3 sources. Out of 211 cases of gonorrhoea, 105 (49.8%) were from UHKL while various GP clinics contributed 14 (6.6%) and the remaining 92 (43.6%) came from 2 STD clinics in Kuala Lumpur. For UHKL, an overall 11% of gonococcal infections were by penicillinase positive strains while the corresponding figures for GP clinics and STD clinics were 14% and 33% respectively. Because of the increase in number of specimens received from August 1978 onwards with most of the specimens coming from STD clinics, a separate analysis was made for the 4-month period August to November 1978. During this period about a year after the first appearance of PPNG, the percentage of gonococcal infections by PPNG was 19% for UHKL, 33% for GP clinics and 39% for STD clinics.

Some clinical features associated with infections by penicillinase-producing gonococci were gathered from culture request forms (Table II). Most of the cases appeared to be uncomplicated genital tract infections occuring in young males in the 20-29 years age group.

Table III shows the distribution of MICs of 9 antibiotics for both penicillinase positive and penicillinase negative strains. The positive strains were much more resistant to penicillin and ampicillin compared to the negative strains. For penicillin, the positive strains had MICs ranging from 4 to 64 mg/L with a median between 8-16 mg/L while the negative strains were all inhibited by 2 mg/L or less with a median MIC between 0.25 — 0.5 mg/L. Among the negative strains, 36% were fully sensitive to pencillin (MIC 0.06 mg/L or less) while the remaining 64% were less sensitive to penicillin. Similarly for ampicillin, the MICs for the positive strains were much higher than those for the negative strains. For tetracycline, 90% of the positive strains and 40% of the negative strains were considered less sensitive (MIC more than 1 mg/L). Although all strains were inhibited by 8 mg/L of tetracycline, the median MIC for the positive strains was 4-fold higher than that for the negative strains. Hence the PPNG strains were more resistant to tetracycline than the non-PPNG strains. For the other 6 antibiotics, however, both types of strains were not markedly different in their susceptibilities. The median MICs for kanamycin, gentamicin and spectinomycin were the same, and those for erythromycin, chloramphenicol and cotrimoxazole were two-fold higher for the positive strains.

DISCUSSION

In July 1977 the Ministry of Health, Malaysia reported the appearance of PPNG for the first time in this country. The source of the infection was believed to be from Thailand. From our surveillance, it is apparent that following their introduction into the country around the middle of 1977, these strains have spread rapidly and have become firmly established in our population.

The different isolation rates of PPNG among gonococci from UHKL, GP clinics and STD clinics may be a reflection of different populations at risk, and other problems associated with the collection of epidemiologic data. We would expect every patient seen at UHKL with signs and symptoms of gonorrhoea to have a cultural examination. General practitioners who do not have such easy access to laboratory facilities may have sent us specimens only from problem cases or cases resistant to treatment with the usual penicillin regime. Patients seen at STD clinics are believed to be highly promiscuous and thus constitute a high risk group.

The MICs of most of our gonococcal isolates conform to the general pattern of antibiotic sensitivity of gonococci isolated from Southeast Asia (Reyn, 1969). The proportion of non-PPNG strains less sensitive to penicillin is considerably lower than that reported from neighbouring countries like Thailand where in 1975 92% of gonococci isolated in Bangkok were less sensitive to penicillin (Suvanamalik, 1977) and Singapore where 96% of isolates in 1977 were less sensitive to penicillin (WHO, 1978a). However, the isolates from these 2 countries were obtained mainly from STD clinics whereas most of the non-PPNG strains we tested were from outpatients of UHKL who probably represent a less promiscuous population. It is well known that antibiotic resistance tends to build up most rapidly in areas where large groups of promiscuous males consort with relatively closed groups of promiscuous females, where antibiotics are freely available or where following treatment no follow up tests are done (Willcox, 1977).

Current therapeutic regimes for uncomplicated gonorrhoea recommend the use of penicillin or ampicillin as first-line drugs. However, in areas where a large proportion of gonococcal infections are due to PPNG, which would not respond to any of the penicillin regimes, the continuous use of penicillin would help to select out the resistant strains. Hence, it may be necessary to use other antimicrobials effective against these resistant strains as first line drugs. The choice of an alternative drug would be influenced by its efficacy on local strains, activity on other STD agents like treponema and chlamydia, side effects, cost, ease of administration and the readiness with which antibiotic resistance develops. Spectinomycin given 2 grams intramuscularly is the best alternative single-dose regime currently available (Siegel *et al.*, 1978). Unfortunately resistant strains have been encountered (Reyn, 1973) and increased usage of this drug as initial treatment could result in the widespread emergence of spectinomycin-

TABLE II

Distribution of 43 PPNG infections by Sex, Age, Ethnic group and Clinical Presentation

Clinical Presentation	No.cases	Sex		*Age (years)							Ethnic group		
		M	F	<1	1-9	10-19	20-29	30-39	40-49	50-59	Chinese	Malay	Indian
Urethritis	31	31					15	13	2		25	4	2
Cervicitis	6		6			1	3			1	5	1	
Conjunctivitis	2	2		2							1	1	
Pelvic Inflammatory Disease	2		2				1	1			2		
Spouse of case	2	1	1				1	1			1	1	
Total	43	34	9	2		1	20	15	2	1	34	7	2

* The ages of 2 cases, 1 male and 1 female, were not available.

TABLE III

Antibiotic susceptibility of Neisseria Gonorrhoeae

		MIC mg/l									
Antibiotic		≤0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
		cumulative %									
Penicillin	–	42	48	72	88	100					
	+						5	10	73	84	100
Ampicillin	–	12	32	47	70	92	100				
	+							5	37	90	100
Erythromycin	–	28	45	63	100						
	+	5	10	26	95	100					
Tetracycline	–		3	42	60	87	92	100			
	+			10	10	26	84	100			
Kanamycin	–				3	12	43	90	100		
	+							53	100		
Gentamicin	–			2	8	27	93	100			
	+				5	21	84	100			
Chloramphenicol	–	13	15	23	50	63	92	98	100		
	+			6	28	45	78	100			
Spectinomycin	–					5	13	40	97	100	
	+						16	26	100		

		0.125/0.025	0.25/0.05	0.5/0.1	1/0.2	2/0.4	4/0.8	8/1.6	16/3.2
Cotrimoxazole*	–			3	15	28	47	73	100
	+							16	100

– 60 non-PPNG

\+ 19 PPNG

* 5 parts sulfamethoxazole + 1 part trimethoprim

resistant strains. Therapeutic trials conducted in Singapore showed that tetracycline, thiamphenicol and cotrimoxazole gave treatment failure rates above 11% and were not recommended for routine use in gonococcal infections but that kanamycin 2 grams intramuscularly is useful as a second-line drug (Rajan *et al.*, 1977). Gentamicin has also been found effective by some investigators (Bowie *et al.*, 1974) but is generally not used for treating uncomplicated gonococcal infections. Erythromycin may be considered for the treatment of pregnant females with penicillin allergy but is ineffective for gonococcal urethritis in the male (WHO, 1978b). At present PPNGs are expected to respond to the same alternative antibiotic regimes as the non-PPNGs as their in vitro sensitivities are similar. Nevertheless, well-controlled therapeutic trials need to be conducted on PPNG infections to correlate in vitro sensitivities with clinical response.

The control of PPNG infections rests on intensive surveillance of high risk populations, effective treatment of cases, re-examination after therapy to detect treatment failures and rapid contact tracing with epidemiologic treatment of exposed partners (WHO, 1978b). This requires the collaborative efforts of responsible health authorities, private and hospital practitioners, social workers and the population at risk. Medical practitioners can contribute vastly just by taking relevant social histories, encouraging patients to return for post-therapy follow up and taking specimens from various sites as urethra, cervix, rectum and pharynx for re-examination to ensure the eradication of *N. gonorrhoeae*.

SUMMARY

Surveillance for cases of penicillin resistant gonorrhoea was initiated at the University Hospital, Kuala Lumpur in February 1977. Clinical specimens examined were from patients seen at the University Hospital, various general practitioners' clinics and two sexually-transmitted diseases clinics. Out of 211 cases of gonococcal infections diagnosed by the end of November 1978, 43 cases were due to penicillinase-producing *Neisseria gonorrhoeae*. For the University Hospital, an overall 11% of gonococcal infections were by penicillinase positive strains, while the corresponding figures for general practitioners' clinics and the sexually-transmitted diseases clinics were 14% and 33% respectively. Laboratory methods for the isolation, identification and antibiotic susceptibility testing of penicillinase-producing gonococci are described and some clinical features associated with infections by these strains are presented.

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