PLASMIDS IN PENICILLINASE-PRODUCING *NEISSERIA GONORRHOEAE* IN PENINSULAR MALAYSIA

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SUMMARY

The plasmid profiles of 66 strains of penicillinase-producing Neisseria gonorrhoeae *(PPNG), isolated in Peninsular Malaysia, were determined by agarose gel electrophoresis. All the isolates harboured two common plasmid species, a 4.4 x 10^6 Far-East type R plasmid associated with β-lactamase production and a 2.6 x 10^6 plasmid of unknown function. In addition to these two plasmids, 51 (77%) PPNG isolates also carried a 24.5 x 10^6 conjugative plasmid.*

INTRODUCTION

Neisseria gonorrhoeae, the causative agent of gonorrhoea, is normally controlled by penicillin. However, in 1976,[1,2] highly penicillin-resistant penicillinase-producing *N. gonorrhoeae* (PPNG) strains appeared. Since then, these PPNG strains, causing treatment failures and epidemic outbreaks, have been isolated in many countries with increasing frequency.[3]

The genetic determinant of penicillin resistance in these PPNG strains has been found to be plasmid-borne. All PPNG strains studied so far possess either a 4.4 x 10^6 or a 3.2 x 10^6 non-auto-transmissible β-lactamase specifying R plasmid.[4,5] The 4.4 x 10^6 R plasmid has been epidemiologically associated with isolates of PPNG of Far-East origin and the 3.2 x 10^6 R plasmid has been epidemiologically linked with PPNG isolates from West Africa. In addition to these R plasmids, PPNG strains may also possess a small 2.6 x 10^6 multicopy cryptic plasmid,[6] and a large 24.5 x 10^6 conjugative plasmid that can promote transfer of itself and the nonconjugative R plasmid.[7]

In Malaysia, PPNG strains have been isolated since 1977.[8] This paper describes the plasmid contents of 66 PPNG strains isolated in Peninsular Malaysia.

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MATERIALS AND METHODS

Bacterial strains and growth conditions

The gonococcal strains were isolated from genito-urinary specimens obtained from both male and female patients attending private sexually transmitted diseases (STD) clinics or the STD clinic at University Hospital, Kuala Lumpur.

The specimens were first inoculated on modified Thayer-Martin agar (Gibco Laboratories). All cultures were identified as *N. gonorrhoeae* by colony morphology, Gram stain, oxidase reaction, and the Phadebact gonococcus test (Pharmacia Diagnostics).

All isolates were examined for β-lactamase production with chromogenic cephalosporin

(Nitrocefin, Glaxo)[9] or β-lactamase detection papers (Oxoid).

Reference PPNG strains CDC 76-073389, harbouring the 2.6 x 10^6 cryptic plasmid, the 4.4 x 10^6 penicillinase-plasmid and the 24.5 x 10^6 conjugative plasmid, and CDC 77-083718, harbouring the 2.6 x 10^6 cryptic plasmid and the 3.2 x 10^6 penicillinase-plasmid, were used to provide reference plasmids of known molecular weight.

For plasmid DNA extraction, PPNG strains were grown on chocolate agar plates at 36°C for 24 hours in an incubator containing 6% CO_2 – 94% air (Forma Scientific).

Isolation of plasmid DNA and agarose gel electrophoresis

Plasmid DNA was extracted from PPNG cells using a rapid alkaline-sodium dodecyl sulphate lysis method adapted from that of Birnboim.[10] The detail of this rapid microprocedure will be published elsewhere.

Plasmids were then resolved by electrophoresis in horizontal submerged 0.5% agarose slab gels in Tris-borate buffer (90 mM Tris, 90 mM boric acid, 2.5 mM EDTA, pH 8.3), and photographed as described previously.[11] Plasmid molecular weight were determined by coelectrophoresis with plasmids of known size from the reference PPNG strains.

RESULTS

A total of 66 PPNG strains were examined. On the basis of their plasmid profiles (Fig. 1), these PPNG strains were divided into two plasmid groups (Table I). All of them harboured two common plasmid species, the 4.4 x 10^6 Far-East type R plasmid and the small 2.6 x 10^6 cryptic plasmid. In addition to these two plasmids, 51 (77%) PPNG isolates also harboured the large 24.5 x 10^6 conjugative plasmid.

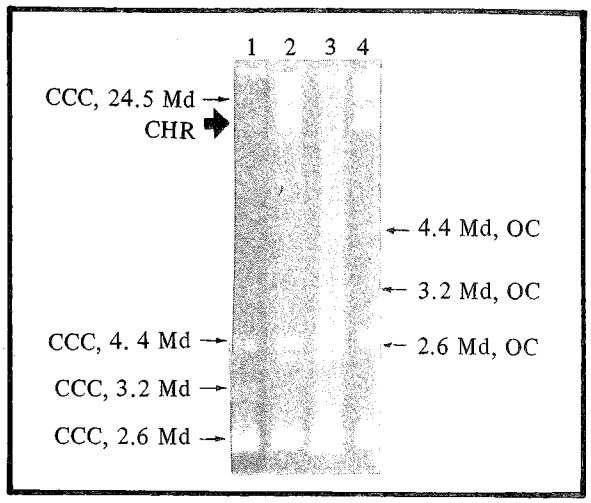

Fig. 1 Agarose (0.5%) gel electrophoresis of plasmid DNA extracted from PPNG strains. Lane 1, CDC 77-083718; lane 2, CDC 76-073389; lane 3, PPNG plasmid group I; and lane 4, PPNG plasmid group II. (CCC = covalently closed circular plasmid DNA; OC = open circular plasmid DNA; CHR = chromosomal DNA).

TABLE I
PLASMID PROFILES OF 66 PPNG STRAINS

Plasmid group	Plasmid complement (x10^6)	No. of strains	(%)
I	2.4, 4.4	12	(23)
II	2.6, 4.4, 24.5	51	(77)

DISCUSSION

Our present findings show that in all 66 PPNG isolates examined, the production of β-lactamase was associated with the 4.4 x 10^6 R plasmid that is typical of PPNG from the Far-East. All of them also carried the 2.6 x 10^6 multicopy cryptic plasmid. In addition, most (77%) isolates also harboured the large 24.5 x 10^6 conjugative plasmid.

These findings are in accord with previous reports[12-14] that PPNG strains harbouring the Far-East type R plasmid also frequently carry the 24.5 x 10^6 transfer plasmid. In contrast, most of the PPNG strains harbouring the West Africa type R plasmid do not carry the large conjugative plasmid,[15] although in recent years, more and more of these PPNG strains have been shown to carry this large transfer plasmid,[14,16-18]

The 24.5 x 10^6 conjugative plasmid is self transmissible and is also capable of conjugally

mobilizing the penicillinase plasmids into other gonococci, *Neisseria* and *Escherichia coli.*[19] The high incidence (77%) of this large plasmid among the PPNG strains studied may account for the significant increase in the number of PPNG isolated in Malaysia since 1977.[8]

Our present findings therefore underline the importance of increased vigilance and surveillance to prevent rapid dissemination of PPNG in Malaysia.

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