LETTERS TO THE EDITOR

Random amplification of Polymorphic DNA of penicillinase-producing *Neisseria gonorrhoeae* strains.

Recently, a new DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence has successfully been used for genome analysis and termed random amplification of polymorphic DNA (RAPD),¹ or arbitrarily primed polymerase chain reaction (AP-PCR),² or DNA amplification fingerprinting (DAF).³ We compare the RAPD-PCR technique and conventional typing methods in the differentiation of 22 penicillinase-producing strains of *N. gonorrhoeae* (PPNG) isolated in Valencia, Spain, between 1985 and 1991 from untreated patients with urogenital gonococcal infection.

PPNG strains were characterised by auxotyping, serotyping, plasmid profile and DNA amplification fingerprinting (RAPD-PCR). Chromosomal DNA was extracted and rapidly purified by the guanidium thiocyanate method of Pitcher *et al.*⁴ DNA amplification was performed using a kit from Operon Technologies (Alameda, CA USA) according to the manufacturer's instructions, with primers OPA-03 (AGTCAGCCAC) and OPA-13 (CAGCACCCAC). The method of unweighted pair-group average linkage was used for cluster analysis.

The characterisation of the 22 penicillinase-producing *N. gonorrhoeae* strains with conventional typing methods and the RAPD patterns is shown in the table.

DNA fingerprinting patterns amplified

with primers OPA-03 and OPA-13 produced well resolved patterns of 11 and 15 DNA fragments respectively. To determine strain similarities quantitatively the results obtained with both primers were combined. At the 86.5% similarity level, the 22 PPNG strains formed 10 clearly defined clusters. Cluster 4 had three prototrophic IB/rop strains with the 4.5 MDa plasmid that by phenotypic classification appeared to be identical. Cluster 7 comprised six strains harbouring the 3.2 MDa plasmid in which neither growth in NEDA medium nor serologic reactivity was observed.

The discriminating ability of the RAPD-PCR technique for penicillinase-producing *N. gonorrhoeae* strains was higher than that of conventional typing methods. Gonococcal strains with the same DNA amplification fingerprinting patterns could belong to different auxotypes and serovars or vice versa. The capacity of combined auxotyping/serotyping to discriminate among penicillinase-producing *N. gonorrhoeae* strains may be clearly diminished in isolates of non-requiring auxotype and non-typable. The outbreak of six non-typable penicillinase-producing *N. gonorrhoeae* strains that occurred in our urban area in 1990 is the first identified in the follow-up of all *N. gonorrhoeae* strains isolated in the Autonomous Community of Valencia since 1985. These strains were correctly grouped into cluster 7 by RAPD-PCR.

RAPD-AR-PCR is of great potential use for epidemiologic tracing in gonococcal disease. It provides some advantages over the use of restriction endonucleases because it is more simple and rapid to execute. Similar satisfactory results have been reported in the characterisation of other microorganisms.⁵ Although, up to the present time, RAPD-PCR has been mainly used for genome analysis,¹⁻³ it is of great potential use for epidemiologic tracing and clinical studies in gonococcal disease, particularly when non-typable strains are not characterised by conventional typing methods.

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Characterisation of the 22 penicillinase-producing *N. gonorrhoeae* strains with conventional typing methods and RAPD-patterns.

Strain	Source*	Plasmid† (MDa)	Auxotype‡	Serogroup/ serovar§	AP-RAPD pattern¶
020/85	EU	3.2	Arg-	Arst	C8
044/86	EU	4.5	Proto	Brop	C4
052/86	EU	4.5	Proto	Brop	C4
064/86	EU	3.2	Proto	Bx	C6
076/86	EU	3.2	Proto	Bo	C6
093/87	EU	4.5/24.5	Pro-	Brop	C9
099/87	EU	3.2	Proto	Bx	C7
115/87	EU	3.2	Pro-	Bpyvut	C2
136/87	EU	4.5	Proto	Brop	C4
171/88	EC	3.2	Proto	Brop	C7
219/90	EU	3.2	NG	NT	C7
220/90	EU	3.2	NG	NT	C7
221/90	EU	3.2	NG	NT	C7
222/90	EU	3.2	NG	NT	C7
228/90	EC	3.2	PAHU-	Bovt	C3
229/90	EU	3.2	Arg-	Av	C7
238/90	EU	3.2	NG	NT	C7
240/90	EU	3.2	NG	NT	C7
242/90	EU	3.2	PAHU-	Bust	C10
244/91	EU	3.2	PAH-	Av	C6
250/91	EU	3.2	Proto	Bovt	C1
004/91	EU	3.2	Proto	Bovt	C5

*EU: exudative urethritis in males; EC: exudative endocervicitis.

†The 2.6 MDa plasmid was present in all 22 strains.

‡Arg: arginine requiring; Proto: prototrophic; Pro: proline requiring; NG: no growth in NEDA medium; PAHU: proline, arginine, hypoxanthine, and uracil requiring; PAH: proline, arginine, and hypoxanthine requiring.

§NT: non-typable.

¶Random amplified polymorphic DNA using a combination of primers OPA-03 and OPA-013.

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Direct culture of gonococci in the patient care area

Suspected gonococcal cervicitis or urethritis is usually confirmed by culture. Direct culture onto a selective medium in the patient care area followed by immediate incubation of the plates at the right atmosphere is considered to be the most sensitive method in the diagnosis of gonococcal infections.¹ If direct plating is not possible, the samples may be either transported in nonnutritive holding media such as Stuart or Amies medium, or in nutritive transport systems. We tested the ability of a commercial nutritive transport system, Biocult-CC^R (Orion Diagnostica, Espoo, Finland) to support the survival and growth of gonococci.

The urethral and cervical samples were taken at the Department of Sexually Transmitted Diseases (STD), Aurora Hospital, Helsinki, by two STD specialists, and inoculated directly at the patient's side first onto a modified Thayer-Martin (TM) medium (BBL GCII) agar base with 1.7% of haemin, 5% chocolate horse blood, 1% Iso-Vitalex, vancomycin 1 mg/l, mycostatin 50 × 10⁵ IU/l, colimycin 0.6 mg/l, trimethoprim 2 mg/l and clindamycin 0.2 mg/l) and next, using the same swab, onto the agar surfaces of the Biocult-GC transport system. The TM plates were immediately placed in a 35°C incubator in a humidified atmosphere of 5% CO₂ in air. The Biocult-GC transport device was transferred into its container, CO₂ generating tablet added, the container closed tightly and placed at 35°C. The TM plates were transferred to the microbiology laboratory on the next floor the following morning, and Biocult-GC transported to another microbiology laboratory after 15 to 48 h incubation at the STD Department. The TM plates and Biocult-GC were examined for growth of gonococci after 48 hours of culture by two independent microbiologists. *N gonorrhoeae* growing on TM plates were identified

by colony morphology, Gram stain, positive oxidase test, agglutination by monoclonal antibody (Phadebact Monoclonal GC Test 50, KaroBio Diagnostics Ab, Huddinge, Sweden), and production of acid from glucose but not from maltose or lactose. The sensitivity to penicillin (by E-test^R, Biotest, Stockholm, Sweden) and ciprofloxacin (by disc diffusion test, Rosco, Taastrup, Denmark) as well as beta-lactamase production were also determined. *N gonorrhoeae* on Biocult-GC were likewise identified by colony morphology, Gram stain, oxidase positivity and growth characteristics.

First, 199 consecutive samples were studied. Smears of four patients revealed intracellular diplococci in methylene blue staining. Growth of *N gonorrhoeae* was detected in three samples (1.5%) on TM plates and on Biocult-GC (Table, group 1). One of the samples grew only on TM plates, another on Biocult-GC only.

In the second part of the study, samples from patients with clinically suspected *N gonorrhoeae* infection and from two patients not suspected to have gonococcal infection but visiting the STD Department on the same day as the index patient were studied. Intracellular diplococci were found in 33 smears from the 102 patients. *N gonorrhoeae* was detected by culture in 33 samples from 30 patients. Cultures from 29 patients were positive on TM plates and from 26 on Biocult-GC (Table 1, group II). Biocult-GC failed to show growth of *N gonorrhoeae* in samples of four patients growing on TM and TM from one growing on Biocult-GC. The difference between the yields on TM and Biocult-GC was statistically not significant.

Although the overall agreement between TM and Biocult-GC cultures was good, five samples failed to grow on Biocult-GC and two on TM. This result may be biased as Biocult-GC was always inoculated after the TM plate (but using the same swab for both), and might have had a smaller inoculum than TM.

Gonococci are fastidious organisms and may survive in nonnutritive transport tubes for less than 24 hours.¹ In this study Biocult-GC supported the survival and growth of *N gonorrhoeae* to the same extent as direct plating onto TM plates in the patient care area and may thus be an alternative to nonnutritive transport tubes if direct inoculation of samples onto TM plates in the patient care area is not possible, but inoculation, incubation and safe transport of cultured Biocult GC is. Presumptive identification of

Table Detection of *N. gonorrhoeae* on Thayer-Martin (TM) plates and Biocult-GC^R

Group	n	Growth of gonococci			p [†]
		TM	Biocult-GC	Total	
I	199 (153)*	3 (3)	3 (3)	4 (4)	0
II	131 (102)	31 (29)	28 (26)	33 (30)	0.1969
All	330 (255)	34 (32)	31 (29)	37 (34)	0.1536

66% (102) of the patients were females in group 1, and 43% (43) in group II.
*Number of samples (patients) studied.
†Yield on Thayer-Martin plates and Biocult-GC compared by Chi square test; all p-values not significant.