Serological ecology of *Neisseria gonorrhoeae* (PPNG and non-PPNG) strains: Canadian perspective

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SUMMARY One hundred and thirty eight penicillinase producing *Neisseria gonorrhoeae* (PPNG) and 239 non-PPNG strains were characterised serologically using a panel of seven monoclonal antibodies directed against protein 1A and seven against protein 1B. An association between serovar and susceptibility to antimicrobial agents, auxotype, and plasmid content was observed. Serogroup WI strains were more sensitive to penicillin, ampicillin, tetracycline, erythromycin, cefoxitin, and cefuroxime. Sixty five (82%) of the 79 WI strains were typed as being serovar Aedgkih, and 47 (72%) of these strains required arginine, uracil, and hypoxanthine for growth (AUH⁻). Seventy one (44%) of 160 WII/WIII strains were serovar Bacejk, and 42 (59%) of these required proline, citrulline, and uracil for growth (PCU⁻) and were plasmid free. Serovars Bcgk, Beghjk, Bacjk, and Bajk were associated with resistance to antimicrobial agents. Analysis of PPNG isolates showed a new serovar, Af, which was associated with strains imported from Malaysia and Singapore that required proline and ornithine for growth (Pro⁻Orn⁻) and carried the 24.5 megadalton transfer plasmid, the 2.6 megadalton cryptic plasmid, and the 4.5 megadalton penicillinase producing plasmid. Other associations between serovar and geographical location were noted.

Introduction

Differentiation between strains of *Neisseria gonorrhoeae* for epidemiological and clinical purposes has been accomplished by using a combination of several techniques: auxotyping,^{1,2} plasmid content analysis,^{3,4} and restriction endonuclease analysis.⁵ In characterising strains by nutritional requirement (auxotype), differences have been noted between geographical area of isolation, race of patient, and clinical syndrome.⁶⁻⁹ Correlation between auxotype and other phenotypes, such as plasmid carriage or susceptibility to antimicrobial agents has also been observed.^{4,6,10,12} For example, isolates requiring arginine, hypoxanthine, and uracil for growth

(AHU⁻) tend to be sensitive to β lactam antibiotics,^{6,10,11} the proline, citrulline, uracil requiring (PCU⁻) auxotype is plasmid free,³ and the carriage of certain plasmids in penicillinase producing strains of *N. gonorrhoeae* (PPNG) has been associated with specific auxotypes.^{4,11,12} These typing techniques have been invaluable in tracking microepidemics within countries as well as in monitoring the international dissemination of strains, particularly PPNG isolates.¹³⁻¹⁶

Serological methods based on the antigenic determinants of protein 1A or 1B have been used effectively to differentiate further between gonococcal strains.¹⁷⁻¹⁹ Serological classification is carried out using a modification of the coagglutination technique developed by Kronvall.²⁰ As a further refinement of the technique, which initially divided isolates into three groups using polyclonal antibodies, monoclonal antibodies directed against epitopes on protein 1 were produced, thereby further differentiating serogroups into serovars.²¹⁻²³ Gonococcal serovars have also been shown to vary according to geographical source, auxotype, and susceptibility to antibiotics.²⁴⁻²⁶ The international analysis of these variables is still preliminary, as are

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data pertaining to the relation between serovar and plasmid content.

The present work was undertaken to explore, in the Canadian context, the relation between serovar and other variables such as auxotype, susceptibility to antimicrobials, and plasmid content. Non-PPNG and PPNG strains were serologically classified to ascertain their serological diversity and whether particular serovars were peculiar to specific regions.

Materials and methods

STRAINS OF BACTERIA

Non-PPNG strains were selected from the national collection (1973-84) of the antimicrobials and molecular biology division of the Laboratory Centre for Disease Control, Ottawa. They had been stored either by lyophilisation in 2% skimmed milk medium or by freezing (at -70°C) in growth medium containing 20% glycerol.²⁷ The strains for testing were chosen to reflect the distribution of auxotypes seen in Canada¹¹ and to represent most geographical regions in the country (33 strains from the Atlantic regions, 25 from the province of Quebec, 84 from Ontario, 13 from Manitoba and Saskatchewan, 43 from Alberta, and 40 from British Columbia). In addition, 138 PPNG strains, 114 of which had been isolated in Canada, 20 in Chile (from Dr J Garcia Moreno of Santiago), and four in Argentina (from Dr F Marcenac of Buenos Aires), were typed serologically.

SEROLOGICAL CHARACTERISATION

The strains were serologically characterised by coagglutination using monoclonal antibodies to protein 1 as described by Tam *et al*²¹ and Bygdeman *et al*.²³ Isolates were identified as belonging to serogroups designated WI or WII/III. Serogroups were subdivided into different serovars using monoclonal antibodies specific for protein 1A or 1B (table I).²¹

Two different nomenclatures have been used to designate serovars in published reports, though almost identical panels of monoclonal antibodies have been used.²² In the present study, all serovars beginning with A belonged to serogroup WI, whereas all beginning with B were serogroup WII/III. The present study also included several monoclonal antibodies (6G 9/Af, 5C 2/Ak, 2H 7/Be, and 3B10/Bj) that were not used in nomenclature published by Knapp *et al*.²²

The 239 non-PPNG strains were typed at the Karolinska Institute. All other serological analyses were completed in Ottawa with serum samples and control strains sent from Sweden.

MINIMUM INHIBITORY CONCENTRATION (MIC) MEASUREMENT, AUXOTYPING, AND PLASMID CONTENT ANALYSIS

The MICs of penicillin, ampicillin, tetracycline, spectinomycin, erythromycin, cefoxitin, and cefuroxime for the isolates were measured by an agar dilution technique.²⁷ Isolates were auxotyped using the method of Hendry and Stewart,² as modified by Hendry,²⁸ and their plasmid contents were analysed as described previously.²⁹

STATISTICAL ANALYSIS

Associations between the variables (plasmid content, serovar, and auxotype) were examined using the likelihood ratio statistic that was estimated by the maximum likelihood method based on log linear models.³⁰

Results

SEROGROUP AND SUSCEPTIBILITY TO ANTIMICROBIALS OF NON-PPNG STRAINS

Most (160, 67%) of the 239 non-PPNG strains were serologically typed as being serogroup WII/III, whereas 79 (33%) were of the WI serogroup. Strains

TABLE I Monoclonal antibodies used in coagglutination tests to classify gonococcal isolates into serogroups WI and WII/III and subgroup serogroups into serovars

Serogroup WI strains tested with monoclonal antibodies specific for protein 1A:							WII/III strains tested with monoclonal antibodies specific for protein 1B:							Examples of serovar designation*
6G9 Af	4G5 Ae	2F12 Ad	6D9 Ag	5C2 Ak	5G9 Ai	5D1 Ah	3C8 Ba	2D6 Bc	2H7 Be	2G2 Bg	2D4 Bh	3B10 Bj	2H1 Bk	
+	+	+	+	+	+	+								Afedgkih
	+	+			+	+								Aedih
	+						+	+	+			+	+	Ae
								+					+	Bacejk
													+	Bck
									+	+	+	+	+	Beghjk

* = represents only a limited number of possible designations, depending on agglutination pattern.

+ = positive test reaction.

TABLE II Minimum inhibitory concentration (MIC) of antibiotic related to serogroup

Antibiotic	MICs (mg/l) for 79 WI serogroup strains			MICs (mg/l) for 160 WII/III serogroup strains		
	MIC 50%	MIC 90%	Range of MICs	MIC 50%	MIC 90%	Range of MICs
Penicillin	0.016	0.25	≥ 0.008 – 1.0	0.25	2.0	≥ 0.008 – 32.0
Ampicillin	0.032	0.25	≥ 0.008 – 1.0	0.5	2.0	≥ 0.008 – 32.0
Spectinomycin	16.0	16.0	≥ 4.0 – 32.0	16.0	16.0	≥ 4.0 – 32.0
Tetracycline	0.25	0.5	≥ 0.032 – 1.0	0.5	2.0	≥ 0.063 – 4.0
Erythromycin*	0.25	0.5	≥ 0.032 – 1.0	0.25	2.0	≥ 0.032 – 4.0
Cefoxitin	0.25	0.5	≥ 0.008 – 2.0	0.5	4.0	≥ 0.001 – 4.0
Cefuroxime	0.008	0.063	≥ 0.002 – 0.25	0.125	0.5	≥ 0.001 – 2.0

* MICs of erythromycin for 70 WI isolates and 124 WII/III strains.

of the WI serogroup (table II) were generally more sensitive to antimicrobial agents than strains of serogroup WII/III. Except for susceptibility to spectinomycin, which was identical in both groups, the MIC 90% end points of penicillin, ampicillin, tetracycline, erythromycin, cefoxitin, and cefuroxime for WI strains were four to eight times lower than those for WII/III isolates. The MIC 50% end points of all antimicrobials except erythromycin and spectinomycin were also two to 16 times lower for WI strains than for WII/III strains. In addition, the upper range of MICs for WII/III strains was appreciably higher than for WI strains.

SEROVAR, AUXOTYPE, AND SUSCEPTIBILITY TO ANTIMICROBIALS OF NON-PPNG STRAINS

When serogroups were subtyped into serovars, the WI serogroup was notably less heterogeneous than the WII/III serogroup. WI strains were differentiated into eight serovars, and 65 (82%) of the 79 strains typed were in serovar Aedgkih (table III). This serovar

proved to be remarkably sensitive to antimicrobials, particularly to β lactam antibiotics, such as penicillin and cefuroxime (data not shown). A large percentage (89–91%, 70–72) of these isolates were susceptible to ≤ 0.032 mg/l of penicillin, ampicillin, and cefuroxime; 54% (43) were sensitive to ≤ 0.125 mg/l of tetracycline.

The 79 serogroup WI strains comprised 10 auxotypes (table III). The ornithine, uracil, and hypoxanthine requiring (OUH⁻) auxotype predominated (in 42, 53%), followed by prototrophic (NR (wild or non-requiring)) strains (in 11, 14%) and strains requiring citrulline, uracil and hypoxanthine (CUH⁻) (in nine, 11%). The remaining 17 strains consisted of seven other auxotypes. Of the 65 strains of serovar Aedgkih, 39 (60%) were OUH⁻ auxotype and a further eight (12%) were CUH⁻ auxotype (table III). (As citrulline and ornithine are part of the arginine biosynthetic pathway, these two auxotypes may be classified as arginine, uracil, and hypoxanthine requiring (AUH⁻) strains).

The 160 strains of the WII/III serogroup were differentiated into 18 serovars (table IV) with 133 (83%) comprising five serovars and with serovar Bacejk accounting for 71 (44%) of the strains. In addition, WII/III isolates were typed into 15 auxotypes; five auxotypes comprised 147 (92%) strains (table IV). The NR auxotype predominated (in 61, 38%) followed by PCU⁻ in 45 (28%). Of the 71 serovar Bacejk strains, 42 (59%) were PCU⁻, and most (42, 93%) of the 45 PCU⁻ strains were serovar Bacejk. The three other PCU⁻ strains were typed as being serovars Baej, Bcejk, and Beghk. Similarly, PCUH⁻ strains were largely serovar Bacejk. The NR auxotype strains were differentiated into 18 serovars, but serovar Bcgk was exclusively NR.

Statistical analysis of the data about serovars and auxotypes in tables III and IV showed a significant degree of dependence between serovar and auxotype for WI non-PPNG strains ($p = 0.02$) and a highly significant degree of dependence between these same

TABLE III Serovars and auxotypes of 79 non-PPNG WI isolates

Serovars	No	No with auxotype †				
		OUH ⁻	CUH ⁻	NR	Orn ⁻	Other ‡
Aedgkih	65	39	8	6	2	10
Aedgk	4	1	1	1	1	
Aedih	3			3		
Afedgkih	2	1				1
Ae	2			1		1
Others	3	1			1	1
Total	79	42	9	11	4	13

One each of Adgk, Adgkih, Aed.

† Nutritional requirement: ornithine (O⁻ or Orn⁻), uracil (U⁻), hypoxanthine (H⁻), citrulline (C⁻), proline (P⁻ or Pro⁻), or methionine (M⁻ or Meth⁻); NR = non-requiring, wild type, or prototrophic.

‡ PCUH⁻(1), POUH⁻(5), OH⁻(2), OUHM⁻(1), CUHM⁻(1), Pro⁻(2).

TABLE IV Serovars, auxotypes, and susceptibility to antimicrobials of 160 WII/III non-PPNG strains

Serovar	No	No with auxotype †						No (%) resistant‡ to:				
		NR	PCU ⁻	Pro ⁻	Orn ⁻	PCUH ⁻	Other ‡	Penicillin	Ampicillin	Cefoxitin	Cefuroxime	Tetracycline
Bacejk	71	10	42	8	2	4	5			60 (85)		
Bajk	21	13		3	2		3		11 (52)	15 (71)		
Bacjk	18	3		13		1	1	14 (78)	16 (89)	14 (78)		12 (67)
Beghjk	15	10	1	1	1		2	9 (60)	9 (60)	12 (80)		
Bcgk	8	8						6 (75)	6 (75)	6 (75)	4 (50)	4 (50)
Others*	27	17	2	6			2			19 (70)		
Total	160	61	45	31	5	5	13					

*Four Bak; three each of Bck, Bacek, and Baejk; two each of Bcej, Back, Bahjk, Baj, and Bcejk; and one each of Bacej, Bachjk, Baej, and Bcgk.

†See Table III for auxotype designations.

‡Other auxotypes: OUH⁻(1), PO⁻(2), POUH⁻(1), OH⁻(2), PCUM⁻(1), PH⁻(1), M⁻(1), PM⁻(1), CU⁻(2), and PCUHM⁻(1).

§Resistance noted only if number ≥ 50%. Resistance to penicillin, ampicillin, cefoxitin, and cefuroxime defined by MIC 0.5 mg/l, to tetracycline by MIC 1.0 mg/l.

variables for WII/III non-PPNG strains ($p = 0.0001$).

When we compared the serovar and auxotype variables related to susceptibility to antibiotics, it became evident that certain combinations of serovar and auxotype that seemed to be clonal had a wide range of MICs of certain antimicrobial agents. For example, PCU⁻ auxotype strains (serovar Bacejk) showed a wide range of MICs of penicillin (table V), and most isolates had reduced susceptibility (MIC 0.125–0.5 mg/l). On the other hand, the range of MICs of cefoxitin for Bacejk/PCU⁻ isolates (which resembled that of tetracycline) was quite narrow. Similarly, NR strains of serovar Bacejk showed a wide range of MICs of penicillin and a narrow range of MICs of

cefoxitin (which also resembled the range of MICs of tetracycline). In comparing serovar Aedgkih/OUH⁻ and CUH⁻ strains, the OUH⁻ strains were largely homogeneous, though a few isolates had higher MICs of penicillin; both groups showed narrow ranges of MICs of cefoxitin.

Just as certain serovars and auxotypes of the WI serogroup were responsible for the overall sensitivity to antimicrobial agents observed with this serogroup, certain serovars and auxotypes of the WII/III serogroup showed a high degree of resistance to antimicrobial agents (table IV). Group WII/III serovars Bacjk, Beghjk, and Bcgk mostly comprised resistant isolates (table IV). Over half of those isolates were resistant to penicillin, ampicillin, cefoxitin, and

TABLE V Minimum inhibitory concentrations (MICs) of penicillin and cefoxitin for several combinations of serovar and auxotype

Serovar/auxotype	No of strains requiring MICs (mg/l) of penicillin of:										Total
	0.008	0.016	0.032	0.063	0.126	0.25	0.5	1	2	4	
Bacejk/PCU ⁻	1		1	1	10	15	9	1	3	1	42
NR	1		1		1	5	2				10
Pro ⁻	1	3	1		1	1	1				8
Bacjk/Pro ⁻							1	1	2	9	13
Aedgkih/OUH ⁻	23	7	8			1		1			39
CUH ⁻	6	2									8
Serovar/auxotype	No of strains requiring MICs (mg/l) of cefoxitin of:										Total
	0.008	0.016	0.032	0.063	0.126	0.25	0.5	1	2	4	
Bacejk/PCU ⁻					1	1	26	14			42
NR							9	1			10
Pro ⁻			1		2	2	1	1	1		8
Bacjk/Pro ⁻	2							3		8	13
Aedgkih/OUH ⁻	1		1	2	12	19	4				39
CUH ⁻	3			1	2	2					8

NR = non-requiring, wild type, or prototrophic auxotype; Pro⁻ = requiring proline; PCU⁻ = requiring proline, citrulline, and uracil, OUH⁻ = requiring ornithine, uracil, and hypoxanthine; CUH⁻ = requiring citrulline, uracil, and hypoxanthine.

cefuroxime ($\text{MIC} \geq 0.5 \text{ mg/l}$) and to 1.0 mg/l tetracycline. The WII/III serovars generally had reduced sensitivity to cefoxitin. The relation between serovar and MIC is clearly outlined when the MICs for Pro^- strains with either Bacejk or Bacjk serovars are compared (table V). Bacjk Pro^- strains were very resistant to penicillin and cefoxitin and the range of MICs was relatively narrow for penicillin. The range of MICs for Bacejk/ Pro^- strains, however, which were more sensitive, was wide.

SEROVAR AND PLASMID CONTENT OF NON-PPNG AND PPNG STRAINS

Most (77, 98%) of the 79 non-PPNG WI strains carried only the 2.6 megadalton cryptic plasmid (table VI); the other two WI strains harboured the 24.5 megadalton transfer plasmid as well. None of the WI strains was plasmid free. By contrast, only 107/160 (70%) of the WII/III strains harboured the 2.6 megadalton plasmid and 15% (16/107) of these strains also carried a 24.5 Md plasmid; 33% (53/160) carried no plasmids. The relation between carriage of the transfer plasmid and serogroup WII/III serovars was appreciable. Though most (79%, 42/53) of the plasmid free strains were PCU^- auxotype, it was notable that 49/53 (93%) plasmid free strains were serovar Bacejk irrespective of auxotype. The 24.5 megadalton transfer plasmid was present in strains that were either NR or Pro^- , but these strains comprised nine serovars, seven of which were WII/III. Statistical

analysis of the data in table VI showed a significantly high degree of dependence between the variables serovar and plasmid content ($p = 0.001$), and between plasmid content and auxotype ($p = 0.001$).

ANALYSIS OF PPNG STRAINS

PPNG strains comprised 14 serovars, five from the WI serogroup and nine from the WII/III serogroup (table VII). Though most of the PPNG strains characterised (74/138, 54%) were WI serogroup, 46 of these strains (of serovar Ae) were associated with a single microepidemic.¹⁴ Of the 69 WII/III serogroup strains, 20 (of serovar Bacjk) had been collected from a microepidemic in Toronto and were distinguishable only by their serovar and unique plasmid content,³¹ and a further 19 strains (of serovar Bajk) had been isolated in Santiago, Chile and appeared to be homogeneous (in the absence of contact tracing data).³² Interestingly, strains contracted in Africa comprised 10 of the 14 serovars, whereas isolates contracted in Asia were represented by five serovars (Ae, Af, Back, Bajk, and Bacjk). Though the data are limited, certain serovars (Ae and Bacjk) of PPNG strains are found throughout the world, whereas others seem to be more associated with African (Beghjk, Bcegjk, Bcgjk, and Aedgkih) or Asian (Af) origins. Serovars Aedgkih harboured only African type penicillinase producing plasmids but these plasmids were associated with several auxotypes.

TABLE VI Relation between plasmid content, serovar, and auxotype of 239 non-PPNG strains

Plasmid content (megadaltons)	Serogroup	Serovar	No with auxotype*								Total
			NR	PCU^-	Pro^-	Orm^-	PCUH^-	OUH^-	CUH^-	Other	
2.6	WI	Aedgkih	6			2		39	8	10	65
		Others	4			2		3	1	2	12
	WII/III	Bacejk	8		7	2	1			3	21
		Bajk	11		1		2			2	16
		Bacjk	2		10		1			1	14
		Beghjk	10		1	1				2	14
		Others	20		4					2	26
2.6 + 24.5	WI	Aed	1								1
		Aedih			1						1
	WII/III	Bacejk			1						1
		Bajk	2		2						4
		Bacjk	1		3						4
		Bcgk	4								4
		Others	1		2						3
No plasmids	WII/III	Bacejk	2	42			3			2	49
		Bajk								1	1
		Beghjk		1							1
		Others		2							2

* Auxotype designations as for Tables III, IV, and V.

TABLE VII Serology and molecular epidemiology of 138 PPNG isolates

Serovars (No)	No of isolates with indicated auxotype* (plasmid content) [†] from:										
	Africa			Asia			North America			Other areas §	
	NR	Pro ⁻	Other*	NR	Pro ⁻	PO ⁻	NR	Pro ⁻	PO ⁻	NR	Other*
Ae (52)	3 (Afr ⁻)	4 (Afr ⁻)			1 (Asia ⁻)	2 (Asia ⁺)			45 (Asia ⁺)	1 (Afr ⁻)	
Aedgkth (7)	1 (Asia ⁺)	2 (Asia ⁻)	1 (Afr ⁻)				1 (Asia ⁺)		1 (Afr ⁻)		1 (Afr ⁻)
Aedth (10)	1 (Afr ⁺)								2 (Afr ⁻)	1 (Afr ⁺)	1 (Asia ⁻)
Af (4)											
Afe (1)							2 (Asia ⁺)				
Bacjk (26)	2 (Asia ⁺)	2 (Asia ⁺)				1 (Asia ⁺)					1 (Afr ⁺)
Baek (21)							20 (Tor ⁺)				1 (Asia ⁺)
Baek (7)							1 (Afr ⁻)				19 (Asia ⁺)
Beghjk (3)	1 (Afr ⁻)	1 (Afr ⁺)				1 (Asia ⁻)			1 (Asia ⁻)		3 (Asia ⁺)
		1 (Asia ⁺)									
Other (7)‡	3 (Afr ⁻)	1 (Asia ⁻)									
	1 (Asia ⁺)								1 (Asia ⁻)		1 (Afr ⁻)

* Other auxotypes: Om⁻(2) and Meth⁻(1). Auxotype designations as for tables III, IV, V, and VI.

† Afr⁺ = 2·6, 3·2, and 24·5 megadalton; Afr⁻ = 2·6, and 3·2 megadalton, Asia⁺ = 2·6, 4·5, and 24·5 megadalton, Asia⁻ = 2·6 and 4·5 megadalton, Tor⁺ = 2·6, 3·05, and 24·5 megadalton plasmids.

‡ Other serovars: Bak(1), Baejk(1), Baejk(2), Bcgjk(2), and Bcgjk(1).

§ Other areas: Chile, Argentina, Brazil, Portugal, and Jamaica.

Discussion

The differentiation between gonococcal isolates based on combinations of plasmids, serogroups and serovars, and auxotypes has been a powerful tool for defining the epidemiology of outbreaks of infections with PPNG strains,^{5 13-16 26 31 33} in monitoring regional and temporal changes in the phenotype of the gonococcus,^{18 26 33} and even in forensic studies.²⁶ The characterisation of gonococci in this way not only permits the development of a global perspective on the spread of these strains, it also further enhances the biological characterisation of the strains regarding properties such as resistance to antibiotics,^{18 25 26} site of infection,³⁴ expression of disease,⁹ and production of immunoglobulin A1 proteases.³⁵

The study published here extends previous observations regarding the serology of gonococci from North America.^{9 15 26 33} Some 67% of isolates in this survey were serogroup WII/III. In a recent study from Winnipeg, Manitoba, 67% of 325 consecutive isolates were also WII/III.⁹ Thus in Canada, as in Thailand, Singapore, Korea, areas of Europe, the United States of America, Australia, and Africa, the predominant serogroup in non-PPNG strains was WII/III.^{15 33 36-39} The most common WI serovar in Canada and other areas was Aedgkih. This serovar, as shown in the present study, was rarely associated with infections with PPNG strains.³⁶ The same serovar was rarely detected in strains from Bangkok, Korea, Singapore, or Gabon.³⁶ Thus, as Bygdeman has previously stated, North America may, like Europe, be one of the focuses for this serovar in non-PPNG strains.³⁶ The greater diversity of WII/III serovars compared with WI serovars has also been observed by other workers.³⁶ The serovar Bacejk, which was the most prevalent in the present collection of Canadian strains (44% of WII/III strains), was one of the more prevalent serovars globally.³⁶ Though it was an uncommon serovar in South East Asia (1-3%), it was fairly prevalent in Nordic countries (7-29%) and Sydney, Australia (30%).³⁶

Differences in susceptibility to antimicrobial agents between serogroups have been noted previously.^{18 36 41} WI serovars tend to be more sensitive to antibiotics, particularly to β lactam antibiotics, than WII/III serovars. The present study shows that strains with serovars of the WII/III serogroup are heterogeneous in susceptibility to antimicrobials and that certain serovars correlated with resistance to antimicrobial agents. Differences between the serogroups were most pronounced for MICs of penicillin, ampicillin, and cefuroxime, whereas MICs of spectinomycin were identical for both groups. The MICs of tetracycline, erythromycin, and cefoxitin were slightly higher for WII/III serogroups. The overall sensitivity of the WI

serogroup may be attributed to the predominance of serovar Aedgkih, which comprises the AUH⁻ auxotype, an auxotype known for its extreme sensitivity to penicillin.^{11 41} Serovar Bcjk strains were significantly resistant to antimicrobials. The homogeneity or heterogeneity of MICs for different auxotypes and serovars varied with each antibiotic (table V). Strains that are heterogeneous (such as Pro⁻/Bacjk/cefoxitin MICs) but have disparate MICs might be further subtyped with the development of different monoclonal antibody reagents.

It is intriguing to speculate that serovar may be a more stable genetic property than auxotype. That is, older serovars may comprise several auxotypes, whereas the opposite may be true of newer serovars. For example, serovar Bacejk comprises over six auxotypes, whereas serovar Bcjk (possibly a newer serovar) includes only one auxotype. Furthermore, if auxotype were the more stable genetic property one would expect to see several serovars in one auxogroup. The reverse is true. For example, only 59% of Bacejk strains were PCU⁻, though 93% of the PCU⁻ strains were of serovar Bacejk. PCU⁻ is the most prevalent auxotype in Canada and is distinguished by the absence of the 2.6 megadalton plasmid that is characteristic of other gonococcal auxotypes.^{2 3} Interestingly, Brunham *et al* found that this auxotype was significantly associated with asymptomatic urethral infection in men.⁹

The presence of the 24.5 megadalton transfer plasmid in non-PPNG isolates correlated appreciably with the WII/III serovar. This explains why Asian type PPNG strains are more often found with the transfer plasmid, as strains endogenous in Africa tend to be serogroup WI and free of transfer plasmids.

The importation of PPNG strains into Canada is readily discernable when serovars are analysed, as none of the most prevalent non-PPNG serovars, except Bacjk, were also the most prevalent PPNG serovars. In addition, WI serovars were more prevalent than WII/III serovars in the PPNG strains characterised. Serovar Ae, which was the most prevalent serovar in PPNG strains in this study, has also been detected in Sweden and Holland.^{13 36} Though the PPNG strains with this serovar that were isolated in Sweden had been imported from "Africa", the serovar had been imported into Canada from around the world. Furthermore, serovar Ae strains that harboured the cryptic plasmid, the 3.2 megadalton penicillinase producing plasmid, and the transfer plasmid have been characterised in Amsterdam, though they were not isolated in the present study.³⁶

The serological analysis of PPNG strains, coupled with auxotyping and plasmid content analysis, has been highly efficacious in tracing the intercontinental spread of PPNG strains. Several workers have observed

that serogroup WI PPNG strains that were typed auxanographically as NR, Pro⁻, or Arg⁻ could be traced to African origins, whereas certain WII/III serovars were characteristic of PPNG strains isolated on the Asian mainland.^{15 18 36-40} Studies of the prevalence of PPNG strains in Africa, which have shown WII/III strains with 4.5 megadalton penicillinase producing (Asian type) plasmid, have generally attributed their presence to the importation of these strains from Thailand, Singapore, or Hong Kong.¹⁶ Conversely, PPNG strains with WI serovars were considered to be endogenous.^{16 36} The present study indicates that a diversity of serovars have been exported from Africa. Serovar Aedgkih strains that are Pro⁻ auxotype and carry the cryptic plasmid and the 3.2 megadalton penicillinase producing plasmid (African type) are undoubtedly African in origin, as has been observed by other workers.^{16 36} The present study documents the presence of this combination of serovars and plasmids in an Orn⁻ strain isolated in Argentina and in Pro⁻Orn⁻ strains imported into Canada from Jamaica and the United States of America. Data from the present study also support the hypothesis that PPNG strains that are serovar Ae, NR auxotype, and harbour the cryptic African type plasmid probably originated in Africa, whereas isolates of the Ae serovar and Pro⁻ or Pro⁻Orn⁻ auxotype that harbour Asian type penicillinase producing plasmids with (Asia⁺) or without (Asia⁻) the transfer plasmid may have originated in South East Asia (Singapore, Malaysia, or Hong Kong). An Asia⁺ strain was responsible for a major epidemic of infections with PPNG strains in Canada.¹⁴

Serovar Af PPNG strains have not been observed outside Canada, though two of the strains had been imported from the Philippines and Malaysia.¹⁴ Af non-PPNG strains have, however, been isolated in Singapore.³⁶ Similarly, though serovar Afe/Pro⁻/Asia⁺ strains have been isolated in Amsterdam,³⁶ the Afe/NR/Africa⁺ strain characterised in the present study was imported into Canada from Portugal. Several PPNG serovars that have been associated with Asian origins include Back, Bak, and Bcejk.³⁶ Though PPNG strains of these serovars were imported into Canada from Africa, their origins probably rest in the Far East. The PPNG strains recovered from Chile (Bajk/NR/Asia⁺) have been observed in both Asia and Africa. Similarly three PPNG strains from Argentina (Bac/NR/Asia⁺) have also been observed in Asia.³⁶

In conclusion, this study has shown that certain WII/III serovars correlate highly with resistance to antibiotics, that the presence or absence of the cryptic and transfer plasmids correlate highly with both serovar and auxotype, and that the global distribution of PPNG strains can be characterised, in part, by

analysing serovar, auxotype, and plasmid content.

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