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Discriminatory Power of Typing Schemes Based on Simpson's Index of Diversity for *Neisseria gonorrhoeae*

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Simpson's index of diversity was used to produce a single numerical value to compare the abilities of single or combined typing schemes to discriminate between unrelated isolates. This calculation was used to compare the discriminating power of auxotype and serovar determination and plasmid content analysis, either singly or in combination, for *Neisseria gonorrhoeae* isolates having different antimicrobial susceptibilities (i.e., antibiotic-susceptible isolates and those that produce penicillinase, carry plasmid-mediated resistance to tetracycline, have chromosomally mediated penicillin resistance, or both produce penicillinase and carry plasmid-mediated resistance to tetracycline). Plasmid content analysis and auxotype determination produced the lowest level of discrimination, while a combination of auxotype and serovar typing schemes generally provided higher levels of discrimination. Addition of plasmid content analysis to auxotype and serovar typing provided additional discrimination only with penicillinase-producing isolates. For isolates that carried plasmid-mediated resistance to tetracycline, isolates that were tetracycline resistant, isolates that both produced penicillinase and carried plasmid-mediated resistance to tetracycline, or isolates that had chromosomally mediated penicillin resistance, none of the typing methods produced high discriminatory indices, indicating that these isolates are probably derived from relatively few clones.

A number of typing schemes, including antibiotic resistance, nutritional requirement (auxotype [A]), plasmid content (P); and serovar (S; characterization of the major outer membrane protein, protein I, with monoclonal antibodies), are used to distinguish isolates of *Neisseria gonorrhoeae* (5). Less routinely used characterizations include restriction endonuclease analysis, immune characterization of lipooligosaccharides and, more recently, pulsed-field gel electrophoresis (4, 5). Because of the clinical importance of antibiotic-resistant isolates, gonococci are generally first classified on the basis of antibiotic resistance (e.g., penicillinase-producing *N. gonorrhoeae* [PPNG]). These isolates are subsequently typed for epidemiological purposes by using single or combined schemes generally based on A, S, and P.

The ability of a typing scheme to distinguish between unrelated isolates measures its discriminatory power. Gonococcal typing methods have been most often assessed subjectively by using the criteria of typeability, i.e., the percentage of isolates that can be assigned to a given typing group, and reproducibility, i.e., the ability to produce the same results after repeated testing. Although this permits some level of discrimination, the comparison between methods is difficult because several values, generally the size and number of groups, are compared. Hunter and Gaston (2) recently used a single numerical index of discrimination, based on Simpson's index of diversity, to compare the discriminating power of several typing methods for *Candida albicans* and some enteric species (2). This calculation is based on the probability that two unrelated isolates would be classified in the same strain type. In the present study, we used this index to compare the abilities of A, S, and P typing schemes, either singly or in combination, to discriminate between isolates of *N. gonorrhoeae* having different susceptibilities to antimicrobial agents.

The National Laboratory for Sexually Transmitted Diseases, Laboratory Centre for Disease Control (Ottawa, Ontario, Canada), has collected all PPNG and tetracycline-resistant (plasmid mediated) *N. gonorrhoeae* (TRNG) isolates in Canada since the first reported Canadian isolations (1976 and 1986, respectively) for national documentation, confirmation, and strain typing. In addition to collecting isolates with plasmid-mediated resistance, the National Laboratory for Sexually Transmitted Diseases has conducted periodic national surveillance of the antimicrobial susceptibilities and strain types of isolates which do not carry plasmid-mediated resistance. In this study, we calculated the indices of discrimination for various typing schemes for 5,150 PPNG and 606 TRNG isolates, 938 isolates that produce penicillinase and are TRNG (PP/TRNG), 285 isolates with chromosomally mediated resistance to penicillin (CMRNG; penicillin MIC, ≥2.0; some isolates were also resistant to combinations of tetracycline and erythromycin), and 5,036 isolates which were susceptible to penicillin and did not carry plasmid-mediated resistance to tetracycline (i.e., non-PPNG, non-TRNG).

Isolates were reconfirmed as *N. gonorrhoeae* by standard methods (1). β-Lactamase production was ascertained by using nitrocefin (Glaxo Research Group, Greenford, England). Auxotype, serovar, and plasmid content were determined as described previously (1). Confirmation of *tetM*-containing strains was carried out by DNA probe tests as described previously (1). Antimicrobial susceptibilities were determined by agar dilution, and the interpretive criteria used to determine resistance were those recommended by the National Committee for Clinical Laboratory Standards (3). All isolate information was entered into the biological-clinical data collection program developed in house. Data were sorted and analyzed with a SAS version of the system.

The index of discrimination of the isolates was determined as described by Hunter and Gaston (2) to compare the

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TABLE 1. Discrimination indices of gonococcal typing methods for penicillin-susceptible isolates and CMRNG

Typing method(s)	Penicillin-susceptible isolates[a]		CMRNG isolates[b]	
	No. of groups (size of largest group [%])	Index (%)	No. of groups (size of largest group [%])	Index (%)
P	8 (68.3)	48.2	4 (56.2)	50.1
A	46 (38.7)	80.3	10 (61.8)	51.6
S	40 (25.2)	81.8	11 (52.9)	61.3
A-P	76 (29.8)	85.8	13 (39.9)	78.1
A-S	211 (17.3)	93.4	21 (29.8)	78.7
A-S-P	280 (15.1)	94.0	27 (30.4)	84.5

[a] Number of isolates per sample: P, 4,505; A, 5,036; S, 4,795; A-P, 4,483; A-S, 4,794; A-S-P, 4,261.
[b] Penicillin resistant; number of isolates per sample: P, 276; A, 285; S, 191; A-P, 276; A-S, 191; A-S-P, 184.

discriminatory powers of single and combined methods for typing gonococci in accordance with the following formula:

$$D = 1 - \frac{1}{N(N-1)} \sum_{i=1}^{K} n_i (n_i - 1) \times 100$$

where K equals the number of distinct groups obtained with a given typing scheme, N equals the sum of all of the K groups, and n_i is the size of the ith group. D is expressed as a percentage.

An index of 90% or greater is a desirable property of a typing scheme (2). This value is sensitive to both the number of groups the typing scheme defines and the size of the largest group. Isolates were divided into resistance groups because protocols for collecting these groups were different (i.e., >90% of isolates reported to carry plasmid-mediated resistance in Canada have been tested, whereas a representative sample of susceptible isolates or CMRNG was collected).

The discriminatory power of A, S, and P determinations, either alone or in combination, for penicillin-susceptible isolates is indicated in Table 1. For single typing methods, P analysis provided the least discrimination (i.e., eight P groups with 68.3% of the isolates in a single group; discriminatory index, 48.2%) while the A or S typing method was more discriminating (>40 groups; indices of 80.3 and 81.8%, respectively). Only A-S and A-S-P typing (>200 groups) produced indices of >90%, although addition of P determination to A-S typing did not significantly raise the level of discrimination of non-PPNG, non-TRNG isolates.

No typing scheme, including combined typing schemes, produced an index of greater than 90% for CMRNG isolates (Table 1). A-S-P typing was the most discriminatory for CMRNG (index, 84.5%), while single typing schemes failed to resolve over 50% of the isolates into different groups.

For isolates with plasmid-mediated resistance (i.e., PPNG, TRNG, and PP/TRNG), A and P typing, either alone or in combination, produced the lowest discriminatory indices (Table 2). For TRNG and PP/TRNG, the combination of A and P typing schemes was less discriminatory than S typing. For PPNG isolates, both A-S and A-S-P typing schemes were highly discriminatory and, unlike non-PPNG, non-TRNG isolates, addition of P analysis to the A-S typing scheme produced a higher index of discrimination than did A-S typing alone. No typing schemes produced an index of discrimination above 90% for TRNG or PP/TRNG (Table 2).

TABLE 2. Discrimination indices of various typing methods for PPNG, TRNG, and PP/TRNG isolates

Typing method(s)	PPNG[a]		TRNG[b]		PP/TRNG[c] index (%)
	No. of groups (size of largest group [%])	Index (%)	No. of groups (size of largest group [%])	Index (%)	
P	9 (38.4)	57.2	1 (100.0)	0	6.6
A	14 (47.6)	72.5	5 (85.5)	25.7	2.5
S	36 (26.4)	85.5	15 (29.7)	77.8	68.0
A-P	42 (25.1)	83.7	5 (85.5)	25.7	8.7
A-S	110 (19.4)	91.6	26 (28.5)	83.0	69.9
A-S-P	221 (15.5)	94.4	26 (28.5)	83.0	69.7

[a] Number of isolates per sample: P, 5,143; A, 5,150; S, 4,450; A-P, 5,141; A-S, 4,449; A-S-P, 4,446. Inclusion of the 940 PP/TRNG isolates in this group did not significantly alter the index of discrimination.
[b] Number of isolates per sample: P, 609; A, 606; S, 592; A-P, 606; A-S, 592; A-S-P, 592. If the 940 PP/TRNG isolates had been included in this group, the index of discrimination for P would have increased from 0 to 48.7%; the indices for A and A-P would have decreased to 14.6 and 54.9%, respectively.
[c] Number of isolates per sample: P, 940; A, 938; S, 936; A-P, 938; A-S, 935; A-S-P, 935.

As might be expected, these results indicate that the index of discrimination is sensitive to the size of the largest group defined by a particular typing scheme, as well as to the number of groups defined by the scheme. For example, only 15.5% of PPNG isolates composed the largest of 221 groups for A-S-P typing (index of discrimination, 94.4%), compared with 100% of TRNG, which made up a single group for P typing (index of discrimination, 0).

In conclusion, the present results show that certain single methods commonly used to classify gonococcal isolates, especially A and P determinations, are not very discriminatory, especially for isolates with plasmid-mediated resistance. Single typing schemes produced some of the lowest indices of discrimination, although of the three single typing methods evaluated, S was the most discriminatory. The scheme consistently producing the highest level of discrimination for all isolates was generally A-S typing. For certain isolates (i.e., CMRNG, TRNG, and PP/TRNG), even combined methods were not discriminatory enough. This may reflect the clonal nature of some of these isolates in Canada, since their occurrence is relatively recent.

The cost-benefit relationship for single typing schemes, especially for population studies to determine the molecular epidemiology of gonococcal isolates, should be evaluated, particularly if a single typing scheme produces only a few groups very large in size. This is especially true of the use of P analysis for TRNG isolates. Better discrimination of isolates such as TRNG or isolates from outbreaks with a common A-S-P class might be provided by using other typing methods, such as restriction endonuclease analysis, pulsed-field gel electrophoresis, or ribotyping, although these methods cannot be recommended for routine use.

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