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SHORT PAPER
Distribution of the meningococcal insertion sequence IS*1301* in clonal lineages of *Neisseria meningitidis*

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SUMMARY

The distribution of the meningococcal insertion sequence IS*1301* was analysed in 496 strains of different serogroups and clonal lineages of *Neisseria meningitidis*, and in 64 neisserial strains other than *N. meningitidis*. IS*1301* was found in meningococci, but not in apathogenic *Neisseria* sp. and *Neisseria gonorrhoeae*. The copy numbers of IS*1301* varied between 2 and 17 per genome. IS*1301* positive strains were mostly found among the serogroups 29E, W135, X, and Y. Clonal lineages of serogroup A, B, and C meningococci associated with epidemic meningococcal disease were rarely positive for IS*1301*.

We recently described a novel meningococcal insertion sequence, designated IS*1301*, which occurred in several copies in the serogroup B strain B1940 [1, 2]. IS*1301* consists of 844 bp and includes two overlapping open reading frames, which are flanked by inverted repeats of 19 bp. IS*1301* was classified as a member of the IS*5*-family, group IS*427* of insertion sequences [2]. It exhibits site-specificity for the target sequence 5′-AYTAG-3′, and its insertion results in duplication of the central AT [1]. Insertion into the meningococcal *siaA* gene was shown to be reversible and resulted in phase variation of the meningococcal capsule expression [3]. IS*1301* has been demonstrated to disrupt the *porA* gene in a serogroup C disease isolate [4]. Furthermore, it has been suggested that IS*1301* is involved in genomic rearrangements [5]. Our recent preliminary survey involving 118 neisserial strains of different species suggested that IS*1301* was restricted to meningococci and occurred with the highest frequency in serogroup Y strains [1]. In the present study, these findings were extended using a broad collection of meningococcal strains, which covered the genetic diversity of the species.

A total of 496 *N. meningitidis* strains, and 70 strains of other *Neisseriaceae*, including 8 strains of *N. gonorrhoeae*, 56 strains of apathogenic *Neisseriae* (14 species), and 6 strains of *Neisseriaceae* other than *Neisseria* spp. (5 genera) (Table 1) were chosen from the strain collections at the National Institute of Public Health (D. A. Caugant), Oslo; the Max-Planck-Institut für molekulare Genetik (M. Achtman), Berlin; the German Reference Centre for Meningococci (H.-G. Sonntag), University of Heidelberg; the Medical School Hannover, or purchased from the German type culture collection (DSMZ, Braunschweig, Germany). The strains were screened for the presence of IS*1301* by colony blot hybr-

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Table 1. *Distribution of IS*1301 *in neisseria strains*

Species	Serogroup	No. of strains	Positive for IS*1301* (%)
N. meningitidis	All serogroups	496	29
	A	47	19
	B	142	20
	C	188	13
	29E	27	89
	W135	15	46
	X	23	52
	Y	37	84
	Non-groupable	17	41
N. gonorrhoeae	—	8	0
Other*	—	63	0

* Including 25 strains of *N. lactamica*, 8 strains of *N. cinerea*, 6 strains of *N. sicca*, 3 strains of *N. flava*, and 2 strains each of *N. elongata*, *N. mucosa*, *N. perflava*, *N. subflava*, *Kingella kingei*, and 1 strain each of *N. canis*, *N. denitrificans*, *N. flavescens*, *N. ovis*, *N. polysaccharaea*, *N. weaverii*, *Acinetobacter baumanii*, *Moraxella cuniculi*, *M. catarrhalis*, *Eikenella corrodens* and *Haemophilus influenzae*.

Table 2. *Distribution of IS*1301 *in different lineages of* Neisseria meningitidis

Clonal lineage	Serogroup	No. of strains	Positive for IS*1301* (%)
ET-5 complex	B	23	4
	C	13	23
ET-37 complex	B	7	14
	C	103	3
A4	B	18	22
	C	14	0
Subgroups I–V	A	14	0
Subgroups VI	A	15	40
	B	2	100
	C	1	100
60 other ETs	A	4	75
	B	31	26
	C	17	29
	29E	26	92
	I	1	100
	W135	4	50
	X	14	86
	Y	22	82
Total		329	30

idizations and/or PCR. PCR was performed on whole bacteria from fresh cultures as template. Primers SH42 (5′-TTGAGCTAGGGTCATGG-3′) and SH46 (5′-AAATCAGGGTTAGGTTTCTT-3′) amplify a 444 bp product between positions 232 and 676 of IS*1301* (Genbank accession no. Z49092). Goldstar™-Polymerase was purchased from Eurogentec (Seraing, Belgium) and used under conditions recommended by the manufacturer, including 20 μM of each dNTP, 1·25 U polymerase and 20 pmol of each primer in a 50 μl reaction; cycle conditions: initial denaturation for 3 min at 94 °C, followed by 30 cycles of 94 °C/60 s, 45 °C/60 s, 72 °C/60 s. Colony blot and Southern blot hybridizations were performed as described recently [1]. For Southern blot analysis chromosomal meningococcal DNA was digested with *Hin*cII (New England Biolabs, Beverley, USA). An IS*1301*-specific probe was obtained by labelling a SH42/46 PCR

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product with digoxigenin using digoxigenin labelling and detection reagents purchased from Boehringer–Mannheim (Germany).

Twenty-nine per cent of 496 *N. meningitidis* strains from different global sources (Australia, North and South America, Western and Southern Africa, Eastern and Western Europe) contained IS*1301* (Table 1). IS*1301* was found in all included serogroups (A, B, C, 29E, I, W135, X, Y) and among non-groupable isolates. However, the distribution was biased between different serogroups: IS*1301* was detected in only 13–20% of serogroup A, B, and C strains, which are the serogroups causing more than 90% of the disease worldwide, but in 46–89% of serogroup W135, 29E, X, and Y strains (Table 1). IS*1301* did not occur in 56 strains of apathogenic *Neisseria* spp. (14 species) and in 8 strains of *N. gonorrhoeae* (Table 1).

Multilocus enzyme electrophoresis data were available for 339 of 496 *N. meningitidis* strains [6–9]. Of these 339 strains, 30% contained IS*1301*. The collection included strains of subgroups I, III, IV-1, IV-2, V, and VI, the ET (electrophoretic type)-5 complex, the ET-37 complex, the cluster A4, and strains from 60 other ETs (Table 2). In serogroups B and C, the presence of IS*1301* was remarkably low (7%) among lineages associated with epidemic disease (ET-37 complex, ET-5 complex, cluster A4). In contrast, IS*1301* was found in 31% of other serogroup B and C strains representing 43 ETs associated with carrier status or sporadic cases of meningococcal disease. The case/carrier status was known for 274 of the strains (166 case isolates, 108 carrier isolates). Of the case isolates, 25·3% harboured IS*1301*, whereas of the carrier isolates, 41·7% harboured IS*1301*. However, since the clonal lineages comprising the case isolates or the carrier isolates were not comparable, we cannot draw the conclusion from these data that, e.g. an ET-5 complex strain tends to be less virulent, if it harbours IS*1301*.

Eight subgroup VI strains and three Scottish isolates exhibiting two new ETs were the only serogroup A meningococci positive for IS*1301*. Five serogroup A strains of subgroup I isolated in Australia, Egypt, the USA or Austria were negative, as were 3 subgroup III strains (isolated in Nepal, China, Finland), 3 subgroup IV strains (isolated in Niger, USA, The Gambia), and 2 subgroup V strains (isolated in China). Subgroup VI of the serogroup A is exceptional in that it includes a number of strains exhibiting the serogroup B and C capsular polysaccharides. Among the subgroup VI, all strains (8/8) isolated in the GDR (1985 and 1986 [10]) were positive for IS*1301*, but only 1 out of 4 strains from the USSR (1989) and none out of 6 strains from Czechoslovakia (1980–3). Interestingly, we could also isolate a novel small meningococcal plasmid of 1986 bp length from the subgroup VI strains isolated in the GDR between 1985 and 1987 [10]. We could not detect this plasmid designated pJS-A (EMBL database accession no. AJ238491) in any other meningococcal lineage until now. Therefore, the subgroup VI strains isolated in the GDR were unique in that they acquired both IS*1301* and a novel plasmid, whose function, however, remains obscure until now.

In order to determine the copy numbers of IS*1301* in strains derived from different lineages, Southern blot hybridizations were preformed with *Hin*cII digested chromosomal DNA of 17 IS*1301* positive meningococcal strains comprising the clusters A3 and A4, the ET-37 complex, the ET-5 complex, and the subgroup VI. The copy numbers of IS*1301* in these strains exhibited extensive variation with numbers ranging from 2 to 17 (data not shown).

The following three hypotheses might explain the low occurrence of IS*1301* in clones associated with epidemic disease: (i) in epidemic clones, IS*1301* negative descendants exhibit a selective advantage, because the disruption of genes essential for systemic infection by IS*1301* insertion is avoided. However, we could previously demonstrate *in vitro* that reversible switch of encapsulation due to reversible insertion of IS*1301* into capsule synthesis genes might faciliate the entry of meningococci into the bloodstream after colonization of the nasopharynx [3]. (ii) IS*1301* destablizes clonal lineages by major chromosomal rearrangements following intragenomic recombination. Such IS*1301* mediated chromosomal rearrangements with consecutive loss of genes required for encapsulation have been demonstrated for non-groupable meningococcal strains [5]. According to this scenario, carriage of IS*1301* would be responsible for the sporadic occurrence and rapid disappearance from the human population of a high number of unusual meningococcal clones. (iii) Ancestors of epidemic clones were initially negative for IS*1301* and did not acquire the system by horizontal gene transfer, either because there is some sort of transformation barrier restricting horizontal gene exchange in these lineages, or because horizontal gene transfer is *per se* a rarely occurring event in epidemic clones.

In conclusion, IS*1301* occurs most frequently in meningococcal serogroups other than A, B, and C. Its

prevalence in clonal lineages frequently associated with meningococcal disease is very low. Therefore, unlike other bacterial insertion sequences [11, 12], IS*1301* is no candidate for epidemiological analysis. IS*1301* and a novel plasmid entered subgroup VI meningococci isolated in the former GDR either simultaneously or in two independent events, again illustrating the tremendous genomic plasticity of meningococci.

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