
SHORT PAPER

Distribution of the meningococcal insertion sequence *IS1301* in clonal lineages of *Neisseria meningitidis*

R. HILSE†¹, J. STOEVE SANDT³, D. A. CAUGANT², H. CLAUS³, M. FROSCH³
AND U. VOGEL^{3*}

¹ Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

² WHO Collaborating Centre for Reference and Research on Meningococci, National Institute of Public Health, P.O. Box 4404 Torshov, N-0403 Oslo, Norway

³ Institut für Hygiene und Mikrobiologie, Universität Würzburg, Josef-Schneider-Strasse 2, 97080 Würzburg, Germany

(Accepted 26 October 1999)

SUMMARY

The distribution of the meningococcal insertion sequence *IS1301* was analysed in 496 strains of different serogroups and clonal lineages of *Neisseria meningitidis*, and in 64 neisserial strains other than *N. meningitidis*. *IS1301* was found in meningococci, but not in apathogenic *Neisseria* sp. and *Neisseria gonorrhoeae*. The copy numbers of *IS1301* varied between 2 and 17 per genome. *IS1301* 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 *IS1301*.

We recently described a novel meningococcal insertion sequence, designated *IS1301*, which occurred in several copies in the serogroup B strain B1940 [1, 2]. *IS1301* consists of 844 bp and includes two overlapping open reading frames, which are flanked by inverted repeats of 19 bp. *IS1301* was classified as a member of the IS5-family, group IS427 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]. *IS1301* has been demonstrated to disrupt the *porA* gene in a serogroup C disease isolate [4]. Furthermore, it has been suggested that *IS1301* is involved in genomic rearrangements [5]. Our recent preliminary survey involving 118 neisserial

strains of different species suggested that *IS1301* 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 *IS1301* by colony blot hybr-

* Author for correspondence.

† Present address: Neurologische Klinik, Medizinische Hochschule Hannover, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany.

Table 1. *Distribution of IS1301 in neisseria strains*

Species	Serogroup	No. of strains	Positive for IS1301 (%)
<i>N. meningitidis</i>	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
<i>N. gonorrhoeae</i>	—	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 baumannii*, *Moraxella cuniculi*, *M. catarrhalis*, *Eikenella corrodens* and *Haemophilus influenzae*.

Table 2. *Distribution of IS1301 in different lineages of Neisseria meningitidis*

Clonal lineage	Serogroup	No. of strains	Positive for IS1301 (%)
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 IS1301 (Genbank accession no. Z49092). GoldstarTM-Polymerase was purchased from Eurogentec (Seraing, Belgium) and used under conditions recommended by the manufacturer, including 20 μ M of each dNTP, 1.25

U polymerase and 20 pmol of each primer in a 50 μ l 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 *HincII* (New England Biolabs, Beverly, USA). An IS1301-specific probe was obtained by labelling a SH42/46 PCR

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 *IS1301* (Table 1). *IS1301* 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: *IS1301* 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). *IS1301* 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 *IS1301*. 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 *IS1301* was remarkably low (7%) among lineages associated with epidemic disease (ET-37 complex, ET-5 complex, cluster A4). In contrast, *IS1301* 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 *IS1301*, whereas of the carrier isolates, 41.7% harboured *IS1301*. 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 *IS1301*.

Eight subgroup VI strains and three Scottish isolates exhibiting two new ETs were the only serogroup A meningococci positive for *IS1301*. 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 *IS1301*, 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 *IS1301* and a novel plasmid, whose function, however, remains obscure until now.

In order to determine the copy numbers of *IS1301* in strains derived from different lineages, Southern blot hybridizations were performed with *HincII* digested chromosomal DNA of 17 *IS1301* 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 *IS1301* 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 *IS1301* in clones associated with epidemic disease: (i) in epidemic clones, *IS1301* negative descendants exhibit a selective advantage, because the disruption of genes essential for systemic infection by *IS1301* insertion is avoided. However, we could previously demonstrate *in vitro* that reversible switch of encapsulation due to reversible insertion of *IS1301* into capsule synthesis genes might facilitate the entry of meningococci into the bloodstream after colonization of the nasopharynx [3]. (ii) *IS1301* destabilizes clonal lineages by major chromosomal rearrangements following intragenomic recombination. Such *IS1301* 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 *IS1301* 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 *IS1301* 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, *IS1301* 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], *IS1301* is no candidate for epidemiological analysis. *IS1301* 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.

ACKNOWLEDGEMENTS

We thank H.-G. Sonntag and I. Ehrhard, University of Heidelberg, for providing us with the collection of German meningococcal case- and carrier-strains. We thank M. Achtman, Max-Planck-Institut für molekulare Genetik, Berlin, for generously providing us with strains, and for inviting R. H. to perform part of the work in his laboratory. We gratefully acknowledge the help of G. Morelli during the examination of strains from M. Achtman's collection. This work was supported by grants from the Deutsche Forschungsgemeinschaft to M. F.

REFERENCES

- Hilse R, Hammerschmidt S, Bautsch W, Frosch M. Site-specific insertion of *IS1301* and distribution in *Neisseria meningitidis* strains. *J Bacteriol* 1996; **178**: 2527–32.
- Mahillon J, Chandler M. Insertion sequences. *Microbiol Mol Biol Rev* 1998; **62**: 725–74.
- Hammerschmidt S, Hilse R, van Putten JP, Gerardy Schahn R, Unkmeir A, Frosch M. Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. *EMBO J* 1996; **15**: 192–8.
- Newcombe J, Cartwright K, Dyer S, McFadden J. Naturally occurring insertional inactivation of the *porA* gene of *Neisseria meningitidis* by integration of *IS1301*. *Mol Microbiol* 1998; **30**: 453–4.
- Dolan JM, Miller YK, Kahler CM, Ajello G, Stephens DS. *IS1301*-dependent and -independent recombinational events resulting in unencapsulated *Neisseria meningitidis*. In Nassif X, Quentin M-J, Taha M-K, eds. *Proceedings of the 11th International Pathogenic Neisseria conference (EDK, Paris), 1998*; 351.
- Caugant DA, Mocca LF, Frascch CE, Froholm LO, Zollinger WD, Selander RK. Genetic structure of *Neisseria meningitidis* populations in relation to serogroup, serotype, and outer membrane protein pattern. *J Bacteriol* 1987; **169**: 2781–92.
- Crowe BA, Wall RA, Kusecek B, et al. Clonal and variable properties of *Neisseria meningitidis* isolated from cases and carriers during and after an epidemic in The Gambia, West Africa. *J Infect Dis* 1989; **159**: 686–700.
- Wang JF, Caugant DA, Morelli G, Koumare B, Achtman M. Antigenic and epidemiologic properties of the ET-37 complex of *Neisseria meningitidis*. *J Infect Dis* 1993; **167**: 1320–9.
- Wang JF, Caugant DA, Li X, Hu X, Poolman JT, Crowe BA, Achtman M. Clonal and antigenic analysis of serogroup A *Neisseria meningitidis* with particular reference to epidemiological features of epidemic meningitis in the people's republic of China. *Infect Immun* 1992; **60**: 5267–82.
- Grahlow WD, Caugant DA, Hoiby EA, Selander RK. Occurrence of clones of the ET-5 complex of *Neisseria meningitidis* in the German Democratic Republic. *Z Klin Med* 1990; **45**: 947–50.
- Baquer N, Burnens A, Stanley J. Comparative evaluation of molecular typing of strains from a national epidemic due to *Salmonella brandenburg* by rRNA gene and *IS200* probes and pulsed-field gel electrophoresis. *J Clin Microbiol* 1994; **32**: 1876–80.
- Bik EM, Gouw RD, Mooi Fr. DNA fingerprinting of *Vibrio cholerae* strains with a novel insertion sequence element: a tool to identify epidemic strains. *J Clin Microbiol* 1996; **34**: 1453–61.