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Pharyngeal carriage of *Neisseria meningitidis* in 2—19-year-old individuals in Uganda

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Summary In southern Uganda, only sporadic cases of serogroup A meningococcal disease have been reported since 2000. As part of an immunogenicity study of the tetravalent meningococcal polysaccharide vaccine, nasopharyngeal swab samples were collected twice, 4 weeks apart, from 2—19-year-old healthy individuals in Mbarara, Uganda. Only 15 (2.0%) of the 750 individuals carried meningococci asymptomatically. Most of the strains were non-serogroupable and none were serogroup A. However, two individuals carried a serogroup W135 strain, sequence type (ST)-11, similar to the clone that was responsible for the epidemic in Burkina Faso in 2002. Our study further demonstrates the geographical spread of serogroup W135 ST-11 strain and thus the potential epidemic risk.

1. Introduction

In the African meningitis belt, which includes 18 sub-Saharan countries, large epidemics of meningococcal meningitis occur during the dry season every 5—10 years (Lapeyssonnie, 1963). Until 2002 these epidemics have mostly been caused by *Neisseria meningitidis* serogroup A, but serogroup C has also occasionally been involved (Tikhomirov et al., 1997). To control the disease, the strategy of the WHO has been

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to implement reactive mass vaccination campaigns with the meningococcal A + C polysaccharide vaccine when the epidemic threshold is reached in a population (WHO, 1998).

In 2002, a large outbreak caused by serogroup W135 was reported in Burkina Faso (WHO, 2002). Mass vaccination with the A + C polysaccharide vaccine was stopped when serogroup W135 was shown to be the main capsular type involved in the epidemic. Because of a global shortage in supply of the meningococcal tetravalent ACYW polysaccharide vaccine, mass vaccination of the population could not be undertaken. The WHO then negotiated with GlaxoSmithKline the production of a trivalent ACW vaccine at a cost of US$1 per dose.

On the basis of the results of immunogenicity studies performed in the USA in the 1980s (Griffiss et al., 1985), we propose that another approach to remedy the global shortage of vaccine against serogroup W135 would be to use lesser doses of the tetravalent vaccine. To test the hypothesis that lesser doses might be as immunogenic as full doses of the tetravalent vaccine, we initiated an assay of the immunogenicity of a fractional dose of the ACYW vaccine in Uganda.

Uganda does not belong to the meningitis belt. However, large serogroup A outbreaks have been reported in the northern part of Uganda and in neighbouring countries, especially in refugee camps (Heyman et al., 1998; Ndihokubwayo et al., 1997; Santaniello-Newton and Hunter, 2000). In 2000, Mbarara in the south experienced an outbreak involving several hundred cases that started in the army barracks and then spread to the general population (Epicentre, unpublished data). In relation to the immunogenicity study, a survey of meningococcal carriage among the volunteers participating in the trial was performed and the results of this study are presented here.

2. Materials and methods

The study obtained ethical clearance from the Norwegian Regional Committee for Medical Research Ethics REK III, the Uganda National Council for Science and Technology and the Mbarara University of Science and Technology. A total of 750 individuals aged 2—19 years were enrolled in the study and were sampled twice 4 weeks apart. The individuals were vaccinated on the day of the first sampling.

Nasopharyngeal swab samples were plated directly in the field on Thayer—Martin medium with vancomycin, colistin and nystatin (Oxoid AS, Dardilly, France) and incubated in a candle jar at 37 °C within 3 h of sampling. Plates were incubated for up to 2 days. Bacterial growth was collected from the plate with a sterile loop, suspended in Greaves solution prepared by the Norwegian Institute of Public Health (NIPH), Oslo, Norway, as described previously (Craven et al., 1978) and frozen immediately at −20 °C. The samples were sent frozen in dry ice to the NIPH for further analyses.

Upon arrival, the samples were put in a freezer at −70 °C. Cultivation in Oslo (approximately 100 samples per week) was performed within a period of 6 months. One loop of the primary growth suspension was plated on chocolate agar with colimycin 7.5 mg/l, lincomycin 0.5 mg/l, amphotericin 1.0 mg/l and trimethoprim 5.0 mg/l. Plates were incubated at 35 °C in 10% CO_2 for 2 days and meningococci were identified by standard procedures (Riou and Guibourdenche, 1992). One colony of *N. meningitidis* from each positive sample was subcultured twice and preserved at −70 °C.

Serogroups, serotypes and serosubtypes were determined using a dot-blot method with polyclonal antisera and monoclonal antibodies, as described previously (Caugant et al., 1994; Wedege et al., 1990).

The strains were further analysed by multilocus sequence typing (MLST), which identifies allelic variation from the nucleotide sequences of internal fragments of seven housekeeping genes (Maiden et al., 1998). Each strain was characterised by its sequence type (ST). New alleles and STs were deposited in the MLST database. In addition, *porA* and *fetA* sequencing was performed as described at http://pubmlst.org/neisseria/, to determine genetic variation in these two important surface antigens (Russell et al., 2004; Thompson et al., 2003).

3. Results

Neisseria meningitidis was recovered from 15 of the 750 individuals, giving an overall carriage rate of 2.0%. Ten individuals were carriers at the first sampling and 14 at the second sampling performed 4 weeks later. Nine of the 15 carriers were positive for meningococci in both samples. The youngest carrier was 5 years old; the highest carriage rate (4.7%) was found in the 16—19 years age group (Table 1).

In accordance with the results from carrier surveys performed elsewhere (Yazdankhah and Caugant, 2004), the isolates were mainly non-serogroupable (17/24; 71%). Two carriers harboured a serogroup 29E strain, two individuals acquired a serogroup W135 strain during the 4-week period and one carrier had a serogroup B strain in the first sample and a non-serogroupable strain 4 weeks later (Table 2).

Serotyping, serosubtyping, *fetA* and *porA* genotyping, together with the MLST results showed that the nine individuals who were positive on both occasions kept the same strain (Table 2). The 15 *N. meningitidis* strains were classified into eight distinct STs, of which five had not been identified elsewhere (MLST database, 10 November 2005). Two strains assigned to ST-437 and ST-4890, respectively, belonged to the hypervirulent ST-41/44 complex (Maiden et al., 1998). ST-437 has been previously identified both from carriers and cases in the Czech Republic, Spain, Japan, Taiwan and Morocco, whilst ST-4890 was new to our study.

Four individuals harboured a ST-192 strain and one harboured ST-4795, a single-locus variant of ST-192. All these isolates were negative for the *fetA* gene by PCR (Table 2)

Table 1 Meningococcal carriage in 750 individuals in Mbarara, Uganda

Age group (years)	No. of participants	No. of carriers	Carriage rate (%)
2—5	193	1	0.52
6—10	248	5	2.02
11—15	223	5	2.24
16—19	86	4	4.65

Table 2 Strains of *Neisseria meningitidis* carried by 750 individuals in Mbarara, Uganda, 4 weeks apart

Volunteer no.	Strains from sample no. 1					Strains from sample no. 2				
	Serogroup	Serotype:Subtype	ST	*porA* genotype	*fetA* genotype[a]	Serogroup	Serotype:Subtype	ST	*porA* genotype	*fetA* genotype[a]
21	29E	NT:P1.19,6	4794	18-1,3	F5-5	29E	NT:P1.19,6	4794	18-1,3	F5-5
112	—	—	—	—	—	W135	2a:P1.5,2	11	5,2	F1-1
115	—	—	—	—	—	NG	7:NST	192	18-11,42-2	Neg.
134	NG	7:NST	4795	18-11,42-2	Neg.	NG	7:NST	4795	18-11,42-2	Neg.
161	NG	7:NST	192	18-11,42-2	Neg.	NG	7:NST	192	18-11,42-2	Neg.
165	NG	7:NST	192	18-11,42-2	Neg.	NG	7:NST	192	18-11,42-2	Neg.
232	—	—	—	—	—	W135	2a:NST	11	5,2	F1-1
238	B	14,19:P1.13	437	17,13-9	F5-2	NG	14,19:P1.13,6	437	17,13-9	F5-2
262	—	—	—	—	—	NG	7:NST	192	18-11,42-2	Neg.
497	NG	NT:P1.19,6	4794	18-1,3	F5-5	NG	NT:P1.19,6	4794	18-1,3	F5-5
500	29E	NT:P1.19,6	4794	18-1,3	F5-5	29E	NT:P1.19,6	4794	18-1,3	F5-5
501	NG	NT:P1.19,6	4794	18-1,3	F5-5	—	—	—	—	—
589	—	—	—	—	—	NG	8,10,19:P1.7,1	4890	7-1,1	F1-7
682	NG	4,7:P1.14,22a	4958	22-10,14	F3-1	NG	4,7:P1.14,22a	4958	22-10,14	F3-1
683	NG	7,17:NST	4959	21-3,3-9	F2-14	NG	7,17:NST	4959	21-3,3-9	F2-14

ST: sequence type; NG: non-serogroupable; NT: non-serotypeable; NST: non-serosubtypeable.
[a] Neg.: negative PCR reaction for *fetA* using the conditions described at http://pubmlst.org/neisseria/.

under the conditions described at http://pubmlst.org/neisseria/. ST-192 has previously been found in carriers in The Gambia and Niger.

ST-4794 was found in four individuals; this was a new ST, but single-locus and double-locus variants of ST-4794 have been identified in carriers in the Czech Republic and the UK. These isolates of ST-4794 reacted with the P1.19 monoclonal antibody, although sequencing of PorA demonstrated a variable region 1 that was of the P1.18 family.

Two carriers harboured two new STs (ST-4958 and ST-4959) that were not closely related to any other ST in the database. Finally, two individuals, aged 7 and 16 years, respectively, acquired a serogroup W135 strain that belonged to the hypervirulent clone ST-11, PorA 5,2, similar to the strain responsible for the Hajj outbreaks in 2000 and 2001 and the epidemic in Burkina Faso in 2002 (Mayer et al., 2002; Nicolas et al., 2005a).

4. Discussion

The overall meningococcal carriage rate (2.0%) was low in the sampled Ugandan population compared with estimates of approximately 10% obtained in European populations (Caugant et al., 1994; Yazdankhah and Caugant, 2004). However, this rate was comparable with those reported in northern Nigeria in the 1970s (Hassan-King et al., 1979), in western Zaire in the 1990s (Cheesbrough et al., 1995) and more recently in Morocco and Oman after the Hajj outbreak in 2000 (Nicolas et al., 2005b). In contrast, MacLennan et al. (2000) found a carriage rate of 8.4% among 510 Gambian children aged 5 years old, which is much higher than that obtained for European children of that age (Caugant et al., 1994).

Sampling technique and culture conditions are factors greatly influencing the results of carriage studies. The technicians who carried out the study had all received suitable training, and the method of freezing the primary growth from a selective plate before isolation of meningococci had been successfully evaluated in Norway prior to its utilisation in this study. The fact that 9 of the 15 carriers harboured meningococci in both samples taken 4 weeks apart also supports the validity of the method. Thus, our protocol should permit a study of meningococcal carriage to be undertaken basically anywhere in the world without requiring microbiologists on site.

There was limited phenotypic and genotypic diversity of the carried isolates, with two STs recovered from four individuals each. None of the volunteers harboured serogroup A meningococci, which is the predominant disease-causing serogroup in Uganda, or serogroup X strains that have recently been associated with carriage, endemic cases and outbreaks in Ghana, Burkina Faso and Niger (Djibo et al., 2004; Gagneux et al., 2002; Nicolas et al., 2005a). Two of the carriers acquired a serogroup W135 strain belonging to ST-11, similar to that responsible for the epidemic in Burkina Faso and identified in many carriers in the African continent (MacLennan et al., 2000; Nicolas et al., 2005b). The common occurrence of ST-11 when associated with serogroup W135 in the carriage state is in contrast to its low ability to establish a commensal relationship with the host when associated with the serogroup C capsule (Caugant et al., 1994; Nicolas et al., 2005b). As far as we know, this is the first report of the W135 strain in Uganda. Thus, our study further demonstrates the geographical spread of the serogroup W135 ST-11 strain and therefore the potential epidemic risk. It also highlights the need for laboratory capacity building for accurate identification of serogroups in endemic countries. An affordable vaccine that also protects against the W135 serogroup remains a requirement in order to be able to face future outbreaks.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

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