# Annual Report of the National Center for Meningococci 2005

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## Introduction

Meningococcal infection is a major public health problem in European countries that causes a wide spectrum of diseases ranging from chronic meningococcemia and meningococcal meningitis to rapidly fatal septicemia. This remains a leading cause of bacterial meningitis and sepsis in infants and adolescents with a great emotional impact in the population. Preventive measures have been implemented to avoid the spread of the germ in certain populations at risk and new recommendations of vaccination have been published in November 2005 (1). The epidemiological surveillance by the National Center for Meningococci (NCM) contributes to implement these measures. The Central Bacteriology Laboratory of the University Hospital of Geneva has performed this task since 1990 in collaboration with the Swiss Federal Office of Public Health (SFOPH), Bern.

## Materials and methods:

During the year 2005, the NCM has received 60 strains of *Neisseria meningitidis* isolated from normally sterile specimens like blood samples (n= 38) and cerebrospinal fluid (CSF) (n=22) corresponding to 72% of severe meningococcal cases notified to the SFOPH (n=83). One additional strain of respiratory origin was also analysed because it was isolated from a child with a diagnosis of meningitis and

septicemia but where no culture was positive in these specimens. On the other hand, the analysis of blood and CSF was positive by the *N. meningitidis* specific PCR assay developed in our laboratory.

The serogroups (A, B, C, W135-Y) were determined with the latex agglutination kit (Biorad-Pasteur, Paris) . Furthermore these strains were also tested for their antimicrobial susceptibility (Minimal Inhibitory Concentration = MIC) to 9 antimicrobial agents (penicillin, cefuroxime, ceftriaxone, minocycline, rifampicin, erythromycin, azithromycin, ciprofloxacin, chloramphenicol) with the E-test method (AB Biodisk, Solna, Sweden) on Mueller-Hinton 5% sheep blood agar. The values for the determination of inhibitory concentrations were interpreted according to the criteria proposed by the British Society for Antimicrobial Chemotherapy (2) but for the penicillin, ceftriaxone, rifampicin, azithromycin, ciprofloxacin and chloramphenical, new values were applied according to the American CLSI/NCCLS recommendations (3). Serogroups, serotypes and subtypes were determined with a dot-ELISA technique based on a set of 27 monoclonal antibodies purchased from the National Institute for Biological Standards and Controls, Hertfordshire, Great Britain (4). In order to detect epidemic clones, the multilocus sequence typing method (MLST) was also performed on all strains. This genotyping method is based on the sequencing of seven housekeeping-genes abcZ (putative ABC transporter), adk (adenylate kinase), aroE (shikimate deshydrogenase), fumC (fumarate), gdh (glucose-6-phosphate deshydrogenase) pdhC (pyruvate déshydrogénase subunit) and pgm (phosphoglucomutase) (5). Determined sequences were subsequently introduced into the web site http://mlst.zoo.ox.ac.uk in order to obtain the corresponding MLST type number.

The NCM has implemented in 2004 a PCR assay to directly detect meningococci in specimens such as whole blood and CSF. Starting from two hundred microliters of sample, nucleic acids were extracted with the nucleic acid isolation kit I and the blood protocol of the MagNa Pure Compact system (Roche Diagnostic Ltd.) after a short pre-treatment using enzymatic and buffer lysis. A real-time PCR amplification was then performed to detect the *ctra* target gene (capsular transport gene of *Neisseria meningitidis*) (6). During the year 2005, a serogroup determination (A, B, C, Y-W135) was then developed using similar molecular methods (7). These molecular

techniques were evaluated with the precious help of a Swiss study in collaboration with the SFOPH and the PIGS (Pediatric Infectious Group of Switzerland) (8).

**Results:**

The 61 strains received in 2005 represented 72% of the 83 cases of invasive meningococcal diseases notified to the Swiss Federal Office of Public Health (Figure 1). The number of cases has decreased since 2000 with an incidence in 2005 close to 1 annual case per 100'000 inhabitants which is among those European countries with low incidence. Further epidemiological data are published on the web site of the Swiss Federal Office of Public Health http://www.bag.admin.ch/infreporting.

In 2005, 59 strains (96%) were serogrouped. Like in France, serogroup B strains were predominant with a small increase as compared to previous years representing 61% of strains, followed by serogroup C (21%), serogroup Y (8%) and W135 (6%) (Table 1, Figure 2). The epidemic peak related to serogroup C strains observed between 1999 to 2002 has continued to decline (Figure 3). In Switzerland, it is not clear whether the decline in group C-related meningitis and septicemiae is due to the availability of the MenC vaccine providing effective protection like the spectacular data of England. But public health authorities decided to recommend vaccination for children and teenagers based on the increase in incidence in this group of age (2.8 cases per 100 000 inhabitants), in the immunodeficiency population and for the laboratory workers (1).

Similarly to 2004, the MLST 11 has been most frequently identified (20%) followed by various and new other MLST types (Table 2). The MLST type 8, corresponding almost always to C:2b:P1.2,5 strains, has disappeared. No epidemic clone has been detected with this typing method.

Of the 61 strains tested in 2005, only 51% were susceptible to penicillin, 2% were resistant and 47% were intermediate with the new proposed breakpoints by CLSI/NCCLS. The range of Miniminal Inhibitory Concentrations was not different than the values observed during the previous years (range 0.03 to 0.5) and of the data published in the literature (Table 3) (9). Other tested antimicrobial agents were active against meningococci, with the exception of erythromycin (31% of susceptible

strains). No resistance against azithromycin or ciprofloxacin was detected in the isolates.

During nine months, 34 suspected clinical specimens of 27 different patients were prospectively analysed. The CSF and/or blood specimens were positive for 9 patients. The two patients with a positive culture were detected by PCR. Two patients with a Gram stain evidence of a meningococcal infection but culture negative were also positive for *N. meningitidis* using the *ctrA* gene. Molecular diagnostic methods have also increased the number of suspected infection in 5 additional cases (suspected infection but no confirmation by gram stain and culture). Altogether, the implementation of this molecular assay yielded in increase in detection sensitivity of 55%. All samples positive for *N. meningitidis* by PCR could also be rapidly tested for direct molecular serogroups. All paediatric cases were infected with serogroup B strains.

**Discussion:**

The repartition of strains (serogroups and MLST types) has weakly varied in 2005, as compared to 2004. Serogroup C strains were less represented than the year before, corresponding to even less than a quarter of all strains analysed. This serogroup determination is essential for vaccine recommendations from a public health standpoint and for the secondary prophylaxis of contacts. The determination of serotypes and subtypes remains valuable allowing a reliable comparison of inter-laboratory results. The MLST 8 which is representative of a part of the serogroup C strains has disappeared. New MLST types have appeared but often representative of a unique strain (19 different MLST type with only one strain).

The proportion of strains with a reduced sensitivity to penicillin has increased independently from the modifications of breakpoints. Forty nine strains with these new values were resistant or intermediate to this antimicrobial agent. Strains with a CMI of > 0.125mg/L show a polymorphism in the *penA* gene suggesting a direct correlation between a change in the expression of this gene and the reduced sensitivity. In 2005, thirteen strains corresponded to these criteria and their *penA* gene could be sequenced. For 2006, we have planed to develop the sequence

determination of the *penA* gene to measure and document this trend of increased penicillin-resistance in the Swiss isolates.

Direct detection of meningococci by real-time PCR was performed in the Swiss pediatric population and yielded a good sensitivity. Adult cases of our hospital were also tested. This approach has allowed the confirmation of suspected cases of invasive meningococcal disease (IMD). The major advance was the molecular genogroup determination because it provides a rational for to a rapid decision concerning the contact vaccination and/or the administration of a prophylactic antibiotic.

**References:**


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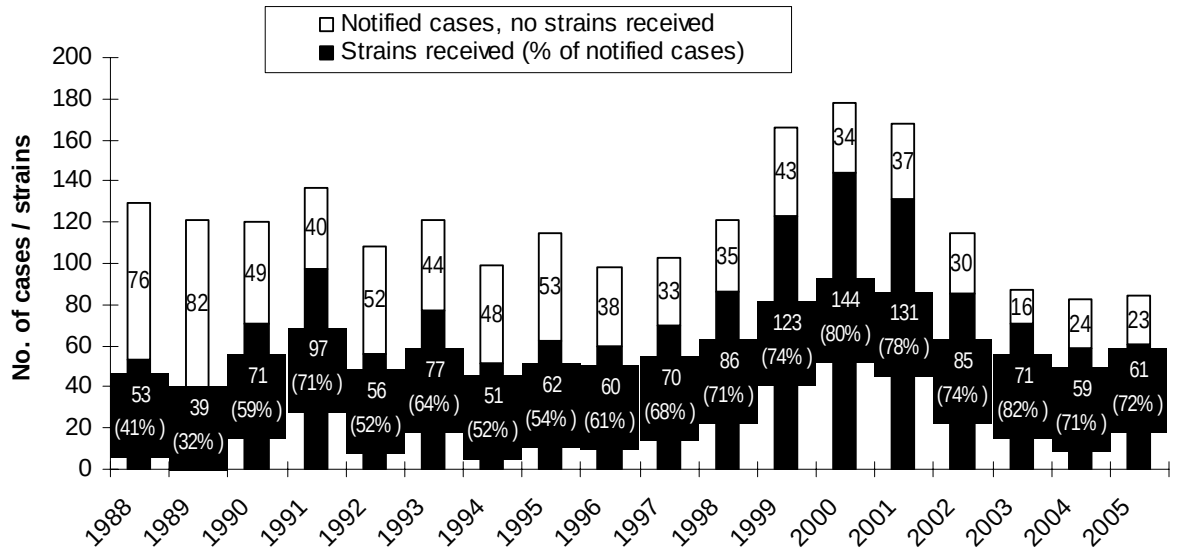


Figure 1 : Comparison of annual number of *N. meningitidis* strains received at the national center for meningococci in Geneva and the number of invasive meningococcal infections notified to the Swiss Federal Office of Public Health from 1988 to 2005

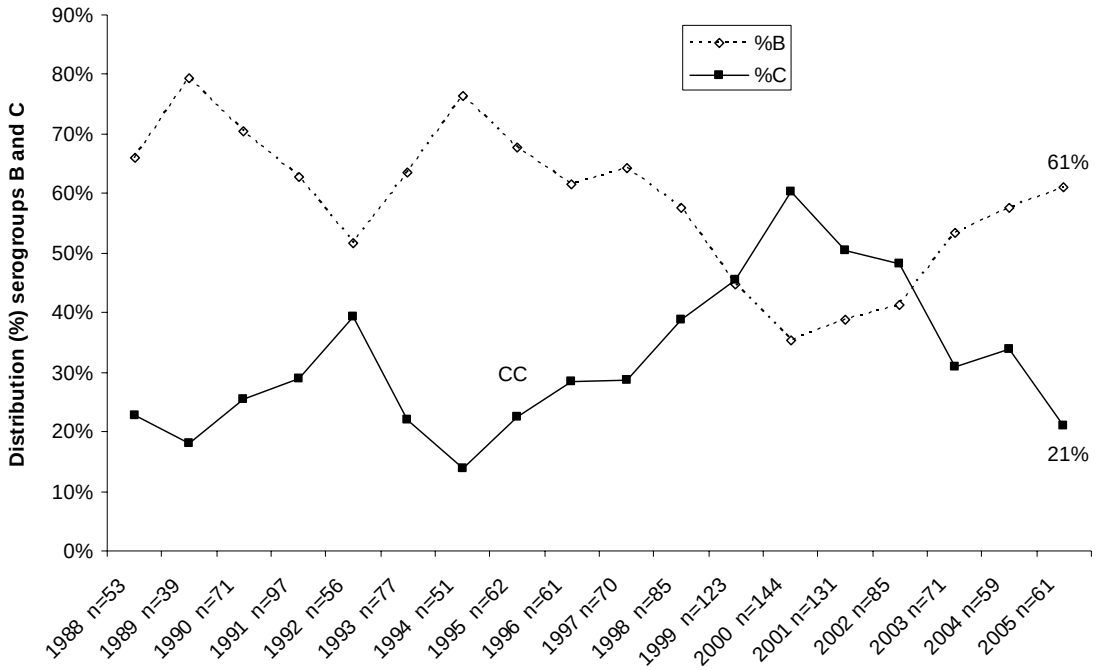


Figure 2 : Distribution of serogroups B and C of *N. meningitidis* from 1988 to 2005

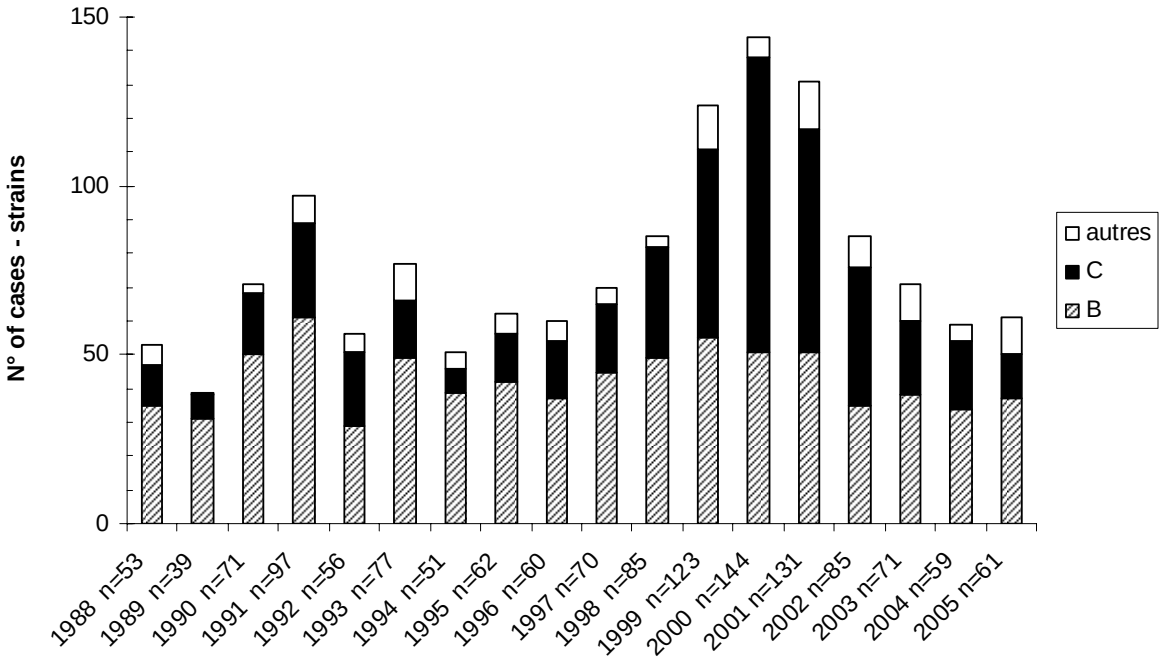


Figure 3 : Serogroup distribution of invasive meningococcal isolates 1988-2005

| | Serotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Serogroup | 1 | 2a | 4 | 14 | 15 | 21 | unknown | total | |
| A | | | | | | | | | 0% |
| B | 2 | | 13 | 3 | 5 | | 14 | 37 | 61% |
| C | | 12 | | | 1 | | | 13 | 21% |
| W135 | | | | | | | 4 | 4 | 7% |
| Y | | | | 4 | 1 | | | 5 | 8% |
| unknown | | | 1 | | | 1 | | 2 | 3% |
| Total | 2 | 12 | 14 | 7 | 7 | 1 | 18 | 61 | 100% |
| | 3% | 20% | 23% | 11% | 11% | 2% | 30% | 100% | |

Table 1 : Distribution of serogroups and serotypes of *N. meningitidis* strains isolated in Switzerland 2005

Table 2: Distribution of most MLST types of meningococci in Switzerland 2005.

| | MLST | | | | | | |
|---|---|---|---|---|---|---|---|
| | 11 | ND* | 41 | 23 | 1127 | Other MLST** | Total |
| Number of strains | 12 | 9 | 4 | 4 | 3 | 29 | 61 |
| | 20% | 14% | 7% | 7% | 5% | 47% | 100% |

*: Not defined MLST

**:Other MLST: 23 different MLST

Table 3:

Inhibitory activity of 9 antimicrobial agents on 61 meningococci isolated in Switzerland in 2005

| Agent | Minimal Inhibitory Concentration (µg / ml) range | 50% | 90% | Breakpoint sensitive ≤ µg/ml | % sensitive |
|---|---|---|---|---|---|
| Penicillin | 0.03-0.5 | 0.06 | 0.25 | 0.06* | 51% |
| | | | | 0.12** | 87% |
| Cefuroxime | 0.03-0.38 | 0.06 | 0.38 | 1** | 100% |
| Ceftriaxone | <0.01 | <0.01 | <0.01 | 0.1* | 100% |
| Minocycline | 0.125-0.5 | 0.25 | 0.5 | 4** | 100% |
| Rifampicin | 0.01-0.0.063 | 0.01 | 0.09 | 0.5* | 100% |
| Erythromycin | 0.38-3 | 1 | 1 | 0.5** | 31% |
| Azithromycin | 0.38-2 | 1 | 2 | 2* | 100% |
| Ciprofloxacin | <0.01 | <0.01 | <0.01 | <0.03* | 100% |
| Chloramphenicol | 0.5-1 | 1 | 1 | 2* | 100% |

* CLSI/NCCLS 2005 (3)
**According to the British Society for Antimicrobial Chemotherapy (5).