

HIGH-LEVEL CHLORAMPHENICOL RESISTANCE IN *NEISSERIA MENINGITIDIS*

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## ABSTRACT

**Background** *Neisseria meningitidis* is nearly always susceptible to the penicillins, the cephalosporins, and chloramphenicol. Between 1987 and 1996, however, chloramphenicol-resistant strains were isolated from 11 patients in Vietnam and 1 in France.

**Methods** The minimal inhibitory concentration of chloramphenicol was determined for the 12 isolates. The isolates were analyzed by monoclonal-antibody-based serotyping and subtyping, pulsed-field gel electrophoresis, and multilocus enzyme electrophoresis. Bacterial DNA was analyzed by hybridization, the polymerase chain reaction, and sequencing to identify the resistance gene and determine the origin of the resistance.

**Results** The isolates were resistant to chloramphenicol (minimal inhibitory concentration,  $\geq 64$  mg per liter) and produced an active chloramphenicol acetyltransferase. All 12 strains belonged to serogroup B but had a high degree of diversity, and 10 could not be typed with the use of monoclonal antibodies. The nucleotide sequence of the resistance gene and the flanking regions was identical to that of an internal portion of transposon Tn4451 that carries the *catP* gene in *Clostridium perfringens*. Moreover, this gene was located in the same genomic site in the chloramphenicol-resistant isolates.

**Conclusions** The high-level chloramphenicol resistance that we describe in *N. meningitidis* isolates is of great concern, since in developing countries, chloramphenicol given intramuscularly is the standard therapy for meningococcal meningitis. The resistance to chloramphenicol is due to the presence of the *catP* gene on a truncated transposon that has lost mobility because of internal deletions, and the transformation of genetic material between strains of *N. meningitidis* probably played an important part in the dissemination of the gene. (N Engl J Med 1998;339:868-74.)

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**N**EISSERIA MENINGITIDIS can cause a spectrum of diseases ranging from transient fever and bacteremia to meningitis and fulminant septicemia. Meningitis is spread by airborne droplets or direct contact with discharge from the nose or throat of an infected person. Meningococcal infections are common in both temperate and subtropical climates, with sporadic cases throughout the year.

*N. meningitidis* is differentiated into serogroups on the basis of the composition of the polysaccharide capsule. Serogroup A causes widespread epidem-

ics in sub-Saharan Africa, where a 600-km-wide meningitis belt extends from the Gambia in the west to Ethiopia in the east. Outbreaks of serogroup A meningitis have occurred in the Middle East and in southern and eastern Asia. Serogroup B meningococci are the cause of most cases of meningococcal disease in developed countries, but the attack rates are substantially lower than those for serogroup A. During the late 1970s, a serogroup B clone (serotype 15, electrophoretic type 5) emerged in northwestern Europe. Intercontinental spread of this clone has been documented, with outbreaks in Cuba (in 1980), Chile (in 1985), and Brazil (in 1987). Strains of this type were also isolated from patients in the United States in 1988. Cases caused by serogroup B organisms began to decline slowly after 1988, whereas those caused by serogroup C organisms increased in several European countries and in the United States.<sup>1</sup> There are vaccines against serogroups A, C, W-135, and Y, but no effective vaccine against group B meningococci is available.

Among the bacteria that cause serious infections, *N. meningitidis* is one of the least problematic in terms of antibiotic resistance.<sup>2</sup> Resistance to penicillin G, sulfonamides, rifampin, and tetracyclines has been reported, but high-level resistance to penicillins through  $\beta$ -lactamase production is still extremely rare. Low-level resistance to penicillins due to an alteration in penicillin-binding protein-2 is widespread.<sup>2</sup> Sulfonamides have been used extensively, and resistance caused by a mutation in the chromosomal gene for the dihydropteroate synthase is common.<sup>3</sup> High-level resistance to rifampin is due to mutations in the gene for the  $\beta$  subunit of RNA polymerase (*rpoB*)<sup>4</sup> and low-level resistance to a decrease in membrane permeability.<sup>5</sup> The plasmid-borne *tet(M)* gene confers resistance to tetracyclines in genital and anorectal isolates of *N. meningitidis*.<sup>6,7</sup> In addition, the development of drug resistance in isolates of *N. gonorrhoeae*, a closely related organism, has led to concern about the almost inevitable progression of resistance in *N. meningitidis* isolates.

We report high-level resistance to chloramphenicol in epidemiologically unrelated clinical isolates of *N. meningitidis* through the production of a chloram-

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phenicol acetyltransferase. This observation is clinically important because intramuscular administration of chloramphenicol is standard therapy for meningococcal meningitis in developing countries.

## METHODS

### Patients and Strains

The properties of the chloramphenicol-resistant *N. meningitidis* strains isolated from cerebrospinal fluid are listed in Table 1. *N. meningitidis* LNP11890 was isolated from a two-year-old girl at the Hôpital de Metz (Metz, France) in July 1993. The patient, who had never been to Southeast Asia, presented with fever, vomiting, meningeal signs, and cloudy cerebrospinal fluid. She was treated with cefotaxime for eight days and recovered.

We could obtain clinical information for only 4 of the 11 strains isolated between 1987 and 1996 in Vietnam and identified at the Institut Pasteur in Ho Chi Minh City. Strain LNP13943 was isolated in January 1987 from a 10-month-old girl in the Song Be region. The patient presented with high-grade fever but without vomiting or convulsions. The cerebrospinal fluid was cloudy. She was treated with penicillin G and chloramphenicol for 12 days and recovered.

Strain LNP14610 was isolated on May 10, 1996, from a nine-year-old boy living in Ho Chi Minh City. The patient presented with high-grade fever, vomiting, headache, meningeal signs, petechial lesions, and cloudy cerebrospinal fluid. He recovered after treatment with three daily intravenous injections of cefotaxime (6 g per day) for eight days, followed by penicillin G (1,250,000 units per day) for five days.

Strain LNP14608 was isolated on May 31, 1996, from a two-year-old girl living in the same district of Ho Chi Minh City where the nine-year-old boy lived. The strain was isolated at the same hospital. The patient presented with fever, vomiting, meningeal signs, and cloudy cerebrospinal fluid. Treatment included

intramuscular injections of gentamicin (0.08 g per day) twice a day for 7 days and intravenous injections of cephadrine (4 g per day) four times a day for 10 days. The patient recovered after two weeks.

Strain LNP14609 was isolated in June 1996 from a five-year-old girl living outside Ho Chi Minh City; the strain was isolated at a different hospital from that where strains LNP14610 and LNP14608 were isolated. The patient presented with fever, headache, vomiting, and cloudy cerebrospinal fluid. She was treated with intravenous injections of penicillin G (400,000 units per kilogram of body weight per day) for 10 days and recovered.

Nine chloramphenicol-susceptible strains (minimal inhibitory concentration, 1 mg per liter) with various antigenic characteristics were used as controls in polymerase-chain-reaction (PCR) studies: LNP14299, LNP14308, and LNP14371 in serogroup A; LNP15339, LNP15340, and LNP15341 in serogroup B; and LNP15345, LNP15346, and LNP15347 in serogroup C.

### Media and Resistance Studies

The strains were grown on GC-base agar (Difco) containing supplements, as described by Kellogg et al.,<sup>11</sup> and were incubated at 37°C with 5 percent carbon dioxide. The minimal inhibitory concentrations of antibiotics were determined by the E test on supplement G-containing agar medium (Sanofi Diagnostics Pasteur). Chloramphenicol acetyltransferase and aminoglycoside-modifying enzymes were assayed in supernatants (centrifuged at 100,000×g) after ultrasonic disintegration.<sup>12,13</sup> With *Escherichia coli* JM83, the following antibiotics were used: chloramphenicol (10 mg per liter) for cloning the resistance gene and kanamycin (20 mg per liter) for cloning the PCR product.<sup>14</sup> With neisseriae, the following antibiotics were used: chloramphenicol (10 mg per liter), nalidixic acid (20 mg per liter), and tetracycline (2 mg per liter). The transformation of *N. meningitidis* BM4376 (a nalidixic acid-resistant derivative of *N. meningitidis* 8013,<sup>15</sup> serogroup C, kindly provided by X. Nassif) and BM4377 (a *recA::tet(M)* tetra-

TABLE 1. PROPERTIES OF THE BACTERIAL STRAINS STUDIED.

STRAIN	YEAR OF ISOLATION	MIC OF CHLORAMPHENICOL*	ANTIGENIC CHARACTERIZATION†	RESULTS OF MULTILOCUS ENZYME ELECTROPHORESIS‡												
				ME	G6P	PEP	IDH	ACO	GD1	GD2	ADH	FUM	ALK	IP1	IP2	ADK
		mg/liter		no. of alleles												
LNP11890	1993	64	B:NT:P1.9	1	1	5	5	2	1	2	2	1	1	2	5	2
LNP13046	1994	96	B:NT:P1.9	1	1	5	5	2	1	2	2	1	1	2	5	2
LNP13047	1994	128	B:NT:P1.1	1	1	<b>4</b>	5	<b>0</b>	1	2	2	1	1	2	5	2
LNP13942	1992	96	B:NT:P1.15	1	1	7	5	<b>4</b>	1	2	2	1	1	2	5	2
LNP13943	1987	128	B:NT:NST	1	1	5	5	<b>4</b>	1	2	2	1	1	2	5	2
LNP13944	1992	64	B:NT:NST	1	1	<b>4</b>	7	<b>4</b>	1	2	2	1	1	2	5	2
LNP13946	1991	96	B:14:P1.1.7	1	1	5	5	<b>0</b>	1	2	2	1	1	2	5	2
LNP13947	1991	96	B:NT:P1.6	1	1	5	5	<b>4</b>	1	2	2	1	1	2	5	2
LNP13948	1991	192	B:NT:P1.9	1	1	5	5	2	1	2	2	1	1	2	5	2
LNP14608	1996	192	B:NT:P1.1	1	1	<b>4</b>	5	<b>0</b>	1	2	2	1	1	2	5	2
LNP14609	1996	128	B:4:P1.15	1	<b>3</b>	5	5	2	1	2	2	1	1	2	5	2
LNP14610	1996	64	B:NT:P1.5	1	1	5	<b>8</b>	2	1	2	2	1	1	2	5	2

\*MIC denotes minimal inhibitory concentration.

†Antigenic characterization was carried out according to the procedures described by Vedros<sup>8</sup> and Abdillahi and Poolman.<sup>9</sup> NT denotes not typable, and NST not subtypable.

‡Multilocus enzyme electrophoresis was performed according to the method of Caugant et al.<sup>10</sup> ME denotes malic enzyme, G6P glucose-6-phosphate dehydrogenase, PEP peptidase, IDH isocitrate dehydrogenase, ACO aconitase, GD1 nicotinamide adenine dinucleotide (NAD) phosphate-linked glutamate dehydrogenase, GD2 NAD-linked glutamate dehydrogenase, ADH alcohol dehydrogenase, FUM fumarase, ALK alkaline phosphatase, IP1 indophenol oxidase 1, IP2 indophenol oxidase 2, and ADK adenylate kinase. Each strain was characterized by a list of allele numbers for the different enzymes. Variant loci are in boldface type.

cycline-resistant derivative of *N. meningitidis* 8013) with total DNA from chloramphenicol-resistant *N. meningitidis* LNP13947 was performed as described elsewhere.<sup>15</sup>

#### Antigenic Classification of *N. meningitidis* Isolates

The strains were serogrouped with the use of monoclonal antibodies and the agglutination technique, as described elsewhere.<sup>8</sup> Isolates were typed and subtyped with the use of the whole-cell enzyme-linked immunosorbent assay of Abdillahi and Poolman.<sup>9</sup>

#### Multilocus Enzyme Electrophoresis

Protein extraction and selective enzyme staining were carried out as described by Caugant et al.<sup>10</sup> Every isolate was characterized according to the combination of its alleles at the 13 enzyme loci, and distinctive multilocus genotypes were designated as electrophoretic types.<sup>16</sup>

#### Nucleic-Acid Techniques

Isolation of DNA, cleavage by restriction endonucleases, and purification of DNA fragments from low-temperature-gelling agarose type VII (Sigma Chemical) were performed as described elsewhere.<sup>17</sup> Large restriction fragments were separated by pulsed-field gel electrophoresis in 0.5× TRIS–borate buffer in a CHEF-DRIII system (Bio-Rad). Purified DNA fragments to be used as probes were labeled with [ $\alpha$ -<sup>32</sup>P]deoxycytidine triphosphate by nick translation. Hybridization was carried out under highly stringent conditions.<sup>17</sup> The PCR assay was performed with a DNA thermal cycler (model 2400, Perkin–Elmer Cetus). Sequencing of double-stranded DNA was performed by the dideoxynucleotide chain-termination method<sup>18</sup> with a modified T7 DNA polymerase and [ $\alpha$ -<sup>35</sup>S]deoxyadenosine triphosphate.

#### Nucleotide-Sequence Accession Number

The nucleotide sequence of the *catP* gene and flanking regions from *N. meningitidis* LNP13947 has been deposited in the GenBank under accession number AF031037.

## RESULTS

#### Bacterial Identification

The minimal inhibitory concentrations of chloramphenicol against the 12 isolates, determined by the E test, ranged from 64 to 192 mg per liter (Table 1). Disk-agar diffusion tests showed that all 12 were also resistant to streptomycin and sulfonamides but remained susceptible to penicillins, cephalosporins, tetracyclines, macrolides, rifampin, and quinolones (data not shown). All strains belonged to serogroup B, and most could not be serotyped with the use of monoclonal antibodies. The antigenic characterization of each strain is shown in Table 1.

The strains were quite diverse. Pulsed-field gel electrophoresis after digestion with the *Bgl*II restriction endonuclease identified nine chromosomal profiles<sup>19</sup> (Fig. 1A), and multilocus enzyme electrophoresis identified eight electrophoretic types<sup>16</sup> (Table 1). Taken together, these results indicate that the chloramphenicol-resistant isolates were not clonally related. In particular, strains LNP14608, LNP14609, and LNP14610, which were isolated in 1996 and for which clinical information was available, differed from one another on the basis of both multilocus enzyme electrophoresis (Table 1) and pulsed-field gel electrophoresis (Fig. 1A, lanes 11, 12, and 13).

#### Characterization of the Chloramphenicol-Resistance Gene

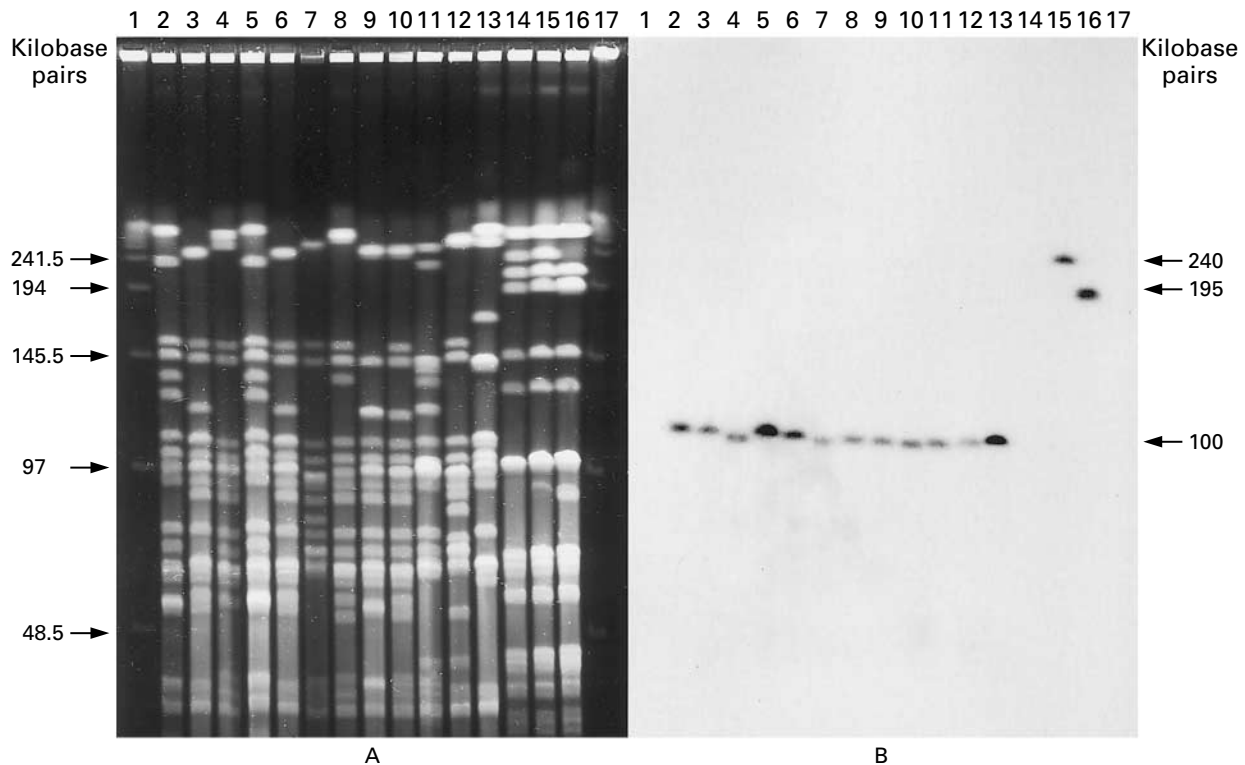
Total DNA from *N. meningitidis* strain LNP13947 and pUC18 DNA digested with *Sau*3A and *Bam*HI, respectively, were mixed, ligated, and introduced into *E. coli* JM83 (minimal inhibitory concentration of chloramphenicol, 2 mg per liter). The resulting transformed isolates (transformants) were selected on the basis of their resistance to chloramphenicol, and the smallest hybrid plasmid, pAT447, was found to contain a 6.5-kb insert. Subcloning was accomplished by introducing the 4.6-kb *Eco*RI–*Hind*III fragment of the insert into pUC18, which generated pAT448. The latter construct conferred high levels of resistance to the new host (minimal inhibitory concentration, >256 mg per liter) by synthesis of a chloramphenicol acetyltransferase, as determined by an enzyme assay (data not shown).

The sequence of a 1.5-kb DNA segment in the cloned fragment from *N. meningitidis* LNP13947 was determined. The sequence of the resistance gene and of the flanking regions was identical to approximately 1 kb of DNA (nucleotides 257 to 1243; numbering according to GenBank accession number AF031037) present within the 6.3-kb plasmid-borne transposon Tn4451 from *Clostridium perfringens* (nucleotides 2721 to 3707; numbering according to GenBank accession number U15027), which carries the *catP* gene.<sup>20,21</sup> Two primers (primer A, 5'ATTC-AGAGTTTAGGACGG3', and primer B, 5'ATCAA-ATAATCAATCC3') designed from the sequence of *catP* allowed amplification of a fragment of total DNA with the predicted size of 300 bp from all the resistant isolates but not from chloramphenicol-susceptible strains LNP14299 (serogroup A), LNP15339 (serogroup B), and LNP15345 (serogroup C) (data not shown).

After susceptible strains of *N. meningitidis* had been independently transformed with chromosomal DNA from *N. meningitidis* LNP13947, four randomly selected chloramphenicol-resistant transformants were studied further: BM4378 and BM4379, obtained from BM4376, and BM4380 and BM4381, obtained from BM4377.

#### Genomic Region of the *catP* Gene

The probe made from the 300-bp fragment internal to *catP* from *N. meningitidis* LNP13947 hybridized to a *Bgl*II fragment of approximately 100 kb in the 12 chloramphenicol-resistant isolates (Fig. 1B, lanes 2 through 13). The 45-kb difference in the size of the *Bgl*II fragment in transformants BM4378 and BM4380 (Fig. 1B, lanes 15 and 16, respectively) was due to the presence of a *Bgl*II site within the tetracycline-resistance determinant<sup>15</sup> in BM4377. To investigate the junctions between the DNA of the truncated transposon and that of the host, two primers were designed on the basis of the 5' and 3' meningococcal sequences flanking the Tn4451-like



**Figure 1.** Analysis of Genomic DNA from Clinical Isolates of *N. meningitidis* by Pulsed-Field Gel Electrophoresis (Panel A) and Hybridization (Panel B).

DNA from the chloramphenicol-resistant *N. meningitidis* strains is shown in lanes 2 through 13, as follows: LNP11890, lane 2; LNP13046, lane 3; LNP13047, lane 4; LNP13942, lane 5; LNP13943, lane 6; LNP13944, lane 7; LNP13946, lane 8; LNP13947, lane 9; LNP13948, lane 10; LNP14608, lane 11; LNP14609, lane 12; and LNP14610, lane 13. DNA from chloramphenicol-susceptible *N. meningitidis* BM4376, used as a recipient in transformation experiments, is shown in lane 14. DNA from two chloramphenicol-resistant transformants, BM4378 and BM4380, from two independent experiments is shown in lanes 15 and 16, respectively. DNA was digested by *Bgl*III and fractionated by pulsed-field gel electrophoresis (under the following conditions: initial pulse, 1 second; final pulse, 10 seconds; voltage, 6 V per centimeter; duration of electrophoresis, 30 hours; included angle, 120 degrees; and temperature, 17°C) (Panel A), transferred to a nitrocellulose sheet, and hybridized to the <sup>32</sup>P-labeled *catP* PCR product (Panel B). Lanes 1 and 17 show concatemers of bacteriophage lambda used as standards for molecular size.

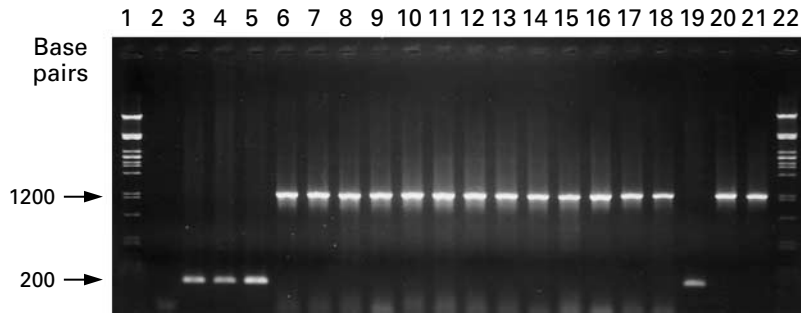
insertion (primer C, 5'CTAAATCAATAATAATAT-TCCC3', and primer D, 5'ACCCAGGTAGAAATCAATGAA3').

These primers, which allow amplification of a 1206-bp fragment with the insertion of pAT448 as a template (Fig. 2, lane 6), gave rise to products of approximately 1200 bp in the chloramphenicol-resistant isolates (Fig. 2, lanes 7 through 18) and to products of approximately 200 bp in the susceptible *N. meningitidis* strains belonging to serogroup A (three strains), B (three strains), and C (four strains) (Fig. 2, lanes 3, 4, and 5). The predicted size of the PCR product corresponding to the target site before the acquisition of transposon sequences was 218 bp, on the assumption that there was no deletion in the adjacent meningococcal DNA. Moreover, with the use of the same primers, a 1200-bp product was also obtained from the four independent resistant transformants (Fig. 2, lanes 20 and 21)

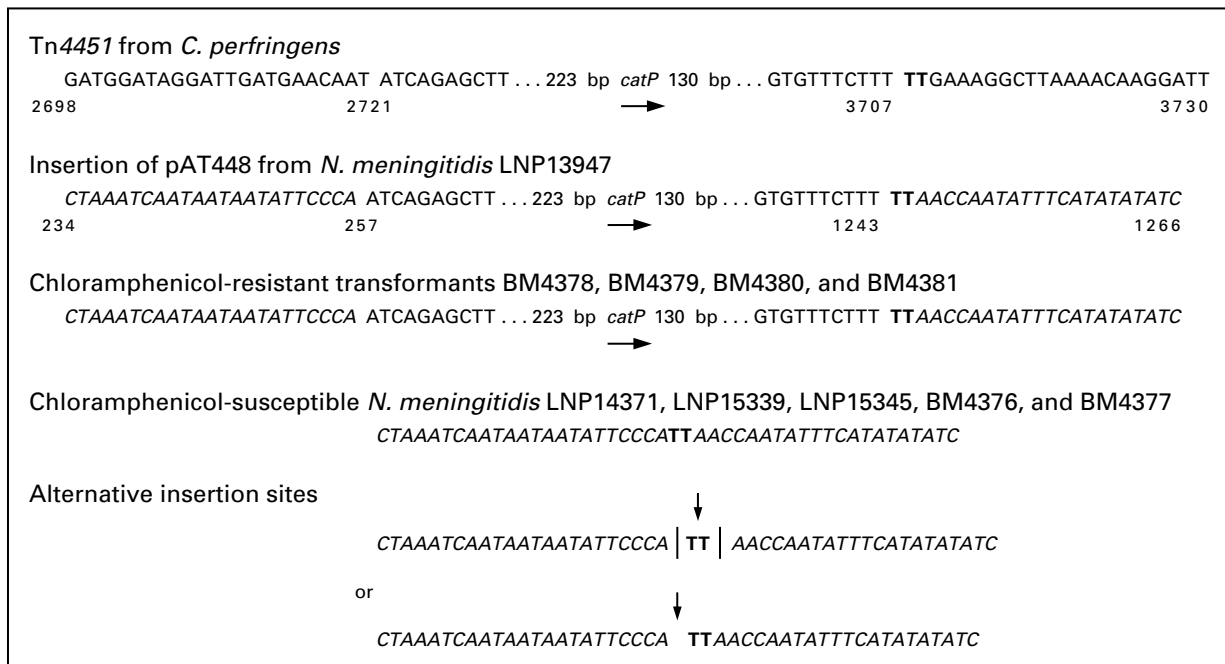
as opposed to a 200-bp product from the chloramphenicol-susceptible corresponding strains (Fig. 2, lane 19). Taken together, these results indicate that the *catP* gene was located in the same genomic region in all the resistant *N. meningitidis* strains.

#### Insertion Site of the *catP* Gene into the *N. meningitidis* Chromosome

The sequence of the *catP* gene and flanking regions from transposon Tn4451, from the pAT448 insert, and from the 1200-bp PCR products obtained from the transformants was compared with that of the 200-bp product obtained from susceptible *N. meningitidis* strains (Fig. 3). Insertion of the *catP* gene occurred at the same site in a region with a large proportion of adenosine and thymidine (77 percent, as compared with 50 percent under normal conditions).<sup>22</sup> Two thymidines are present in the meningococcal DNA and at the 3' end of the trun-



**Figure 2.** Analysis of the Genomic Region of the *catP* Gene in *N. meningitidis* by PCR. Lane 2 shows a negative control. Chloramphenicol-susceptible *N. meningitidis* strains belonging to serogroup A (LNP14371), B (LNP15339), and C (LNP15345) are shown in lanes 3, 4, and 5, respectively. Plasmid pAT448 DNA, which was used as a positive control, is shown in lane 6. Chloramphenicol-resistant *N. meningitidis* strains are shown in lanes 7 through 18, as follows: LNP11890, lane 7; LNP13046, lane 8; LNP13047, lane 9; LNP13942, lane 10; LNP13943, lane 11; LNP13944, lane 12; LNP13946, lane 13; LNP13947, lane 14; LNP13948, lane 15; LNP14608, lane 16; LNP14609, lane 17; and LNP14610, lane 18. Chloramphenicol-susceptible *N. meningitidis* BM4376, used as a recipient in transformation experiments, is shown in lane 19. Chloramphenicol-resistant transformants BM4378 and BM4380 from two independent experiments are shown in lanes 20 and 21, respectively. Lanes 1 and 22 show fragments obtained by digestion of bacteriophage lambda DNA by *Pst*I and used as standards for molecular size.



**Figure 3.** Site of Insertion of the *catP* Gene into Chromosomal DNA of *N. meningitidis*. The nucleotide sequence of the flanking regions of transposon Tn4451, at the 5' upstream and 3' downstream transposon–chromosome junctions in plasmid pAT448, from transformants BM4378, BM4379, BM4380, and BM4381, was aligned with that of chloramphenicol-susceptible *N. meningitidis* strains LNP14371, LNP15339, LNP15345, BM4376, and BM4377. The distance between the 5' and 3' end of the Tn4451-like element and the N- and C-terminals of the *catP* gene is indicated in base pairs. The arrow underneath the *catP* gene indicates the direction of transcription. The coordinates of certain nucleotides are indicated under the corresponding sequence. The nucleotide sequence of chromosomal DNA from *N. meningitidis* is shown in italics. Two alternative insertion sites are indicated by vertical arrows. The two thymidines present in chloramphenicol-susceptible meningococci (strains LNP14371, LNP15339, LNP15345, BM4376, and BM4377), in the transformants, and at the 3' end of the truncated transposon are shown in boldface type.

cated transposon, indicating that there is a 1-bp or 2-bp deletion in the target DNA or no loss of meningococcal DNA in the transformants (Fig. 3).

#### Resistance to Sulfonamides and Streptomycin

The entire gene for the dihydropteroate synthase from two sulfonamide-susceptible strains (LNP14299 and LNP15339) and two sulfonamide-resistant strains (LNP13943 and LNP14610) was amplified by PCR with the use of specific primers<sup>23</sup> and sequenced. The resistant strains had a substitution of guanosine for thymidine at position 92 (*N. meningitidis* numbering), resulting in the replacement of the conserved phenylalanine by a leucine at position 31 and a 6-bp insertion (TCCGGC) resulting in two extra amino acids (serine at position 195 and glycine at position 196) in a highly conserved region of the enzyme.<sup>23</sup> Each of these mutations has been shown to be associated with sulfonamide resistance in *N. meningitidis* strains.<sup>23</sup>

DNA from the 12 streptomycin-resistant isolates did not hybridize with probes specific for the streptomycin-adenylyltransferase *ant(3'')*(9) gene and the streptomycin-phosphotransferase *aph(3'')-I* and *aph(6)-I* genes. No streptomycin adenylyltransferase or phosphotransferase activity was detected in crude extracts from these strains. In other bacterial genera, streptomycin resistance can also result from mutations in the gene for ribosomal protein S12 (*rpsL*), leading to the substitution of lysine at positions 43 and 88 (*E. coli* numbering),<sup>24</sup> or in the portions of the *rrs* gene that correspond to the highly conserved 530-loop and 912 region of 16S ribosomal RNA.<sup>25</sup> Sequencing of the *rpsL* gene (from position 75 to position 300) and the *rrs* gene (from 368 to 979) in susceptible strains (LNP14299 and LNP15399) and resistant strains (LNP13943 and LNP14610) did not reveal any mutation.

#### DISCUSSION

*N. meningitidis* is one of the few bacterial species that causes serious infections for which penicillin G and chloramphenicol are still routinely recommended in developing countries. Strains of *N. meningitidis* with high levels of resistance to chloramphenicol were isolated from cerebrospinal fluid in Vietnam and in France between 1987 and 1996. In Vietnam, chloramphenicol and penicillin G were used for the treatment of meningococcal meningitis in the 1980s, and penicillin G and cephalosporins were used in the 1990s, whereas cephalosporins were used in France during that period. The isolates were also resistant to streptomycin and sulfonamides but remained susceptible to penicillins, cephalosporins, tetracyclines, rifampin, and quinolones; quinolones are used for the treatment of meningococcal disease and also as prophylaxis to reduce nasopharyngeal carriage of meningococci.<sup>26</sup> The resistance of the *N. meningiti-*

*dis* isolates to sulfonamides was due to the production of an altered dihydropteroate synthase, but the mechanism of streptomycin resistance remains unknown.

The resistance of the *N. meningitidis* isolates to chloramphenicol was due to the presence, at a unique site, of the *catP* gene as part of a truncated copy of transposon Tn4451 from *C. perfringens*. Although it has been shown that excision of Tn4451 from *C. perfringens* and from the *E. coli* chromosome is precise,<sup>27</sup> no intact copy of the transposon was found in *N. meningitidis*. Deletions within transposons have been reported, in particular in *N. meningitidis*, *N. gonorrhoeae*,<sup>28</sup> and commensal neisseria species.<sup>29</sup> In the chloramphenicol-resistant strains, there were large deletions in Tn4451, accounting for approximately 80 percent of the transposon, that included the genes responsible for excision and made the transposon immobile.

The initial acquisition of chloramphenicol resistance by *N. meningitidis* could have occurred by transformation or conjugation, raising the possibility of a transfer of genetic information from a strict anaerobe to a strict aerobe. For conjugation, a meeting point between *C. perfringens* and *N. meningitidis* is not mandatory, since — although it was first detected in the former species — a Tn4451-related element could also be present in an aerobic-anaerobic bacterium that acted as a donor. Unlike conjugation, transformation does not require physical contact between the donor and recipient cells and can therefore occur between microorganisms in different ecosystems.

Even if these two possible mechanisms cannot be inferred with certainty from the primary transfer event, the subsequent spread of chloramphenicol resistance in *N. meningitidis* strains can be envisioned, given the extreme transformability of this species. Transformation has been shown to be responsible for the dissemination of other genetic traits<sup>30,31</sup> in this naturally competent species and can easily be reproduced under laboratory conditions. As mentioned above, because of deletions, the truncated transposon is a stable component of the *N. meningitidis* genome. Resistance can therefore easily be transferred by transformation and integration of the incoming DNA through homologous recombination between the flanking regions and the corresponding portion of the chromosome of the recipient and insertion of the heterologous *catP* gene. This process would account for the observation that an identical fragment of foreign DNA was inserted at the same site in all the transformants.

The emergence of high-level chloramphenicol resistance in *N. meningitidis* isolates is of great concern, since intramuscular administration of chloramphenicol in oil is the standard treatment for meningococcal meningitis in developing countries.

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## REFERENCES

1. Achtman M. Global epidemiology of meningococcal disease. In: Cartwright K, ed. Meningococcal disease. Chichester, England: John Wiley, 1995:159-76.
2. Oppenheim BA. Antibiotic resistance in *Neisseria meningitidis*. Clin Infect Dis 1997;24:Suppl 1:S98-S101.
3. Kristiansen B-E, Rådström P, Jenkins A, Ask E, Facinelli B, Sköld O. Cloning and characterization of a DNA fragment that confers sulfonamide resistance in a serogroup B, serotype 15 strain of *Neisseria meningitidis*. Antimicrob Agents Chemother 1990;34:2277-9.
4. Nolte O. Rifampicin resistance in *Neisseria meningitidis*: evidence from a study of sibling strains, description of new mutations and notes on population genetics. J Antimicrob Chemother 1997;39:747-55.
5. Abadi FJ, Carter PE, Cash P, Pennington TH. Rifampin resistance in *Neisseria meningitidis* due to alterations in membrane permeability. Antimicrob Agents Chemother 1996;40:646-51.
6. Gascoyne-Binzi DM, Heritage J, Hawkey PM, Sprott MS. Characterization of a *tet(M)*-carrying plasmid from *Neisseria meningitidis*. J Antimicrob Chemother 1994;34:1015-23.
7. Winterscheid KK, Whittington WL, Roberts MC, Schwabke JR, Holmes KK. Decreased susceptibility to penicillin G and Tet M plasmids in genital and anorectal isolates of *Neisseria meningitidis*. Antimicrob Agents Chemother 1994;38:1661-3.
8. Vedros NA. Serology of the meningococcus. In: Bergan T, Norris JR, eds. Methods in microbiology. Vol. 10. London: Academic Press, 1978: 293-314.
9. Abdillahi H, Poolman JT. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. FEMS Microbiol Lett 1987;48:367-71.
10. Caugant DA, Frøholm LO, Bøvre K, et al. Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. Proc Natl Acad Sci U S A 1986;83:4927-31.
11. Kellogg DS Jr, Peacock WL, Deacon WE, Brown L, Pirkle CI. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J Bacteriol 1963;85:1274-9.
12. Shaw WV. Chloramphenicol acetyltransferase from chloramphenicol-resistant bacteria. Methods Enzymol 1975;43:737-55.
13. Haas MJ, Dowding JE. Aminoglycoside-modifying enzymes. Methods Enzymol 1975;43:611-28.
14. Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 1985;33:103-19.
15. Nassif X, Puaoli D, So M. Transposition of the Tn1545-Δ3 in the pathogenic Neisseriae: a genetic tool for mutagenesis. J Bacteriol 1991;173: 2147-54.
16. Caugant DA, Mocca LF, Frasch CE, Frøholm 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.
17. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. Plainview, N.Y.: Cold Spring Harbor Laboratory Press, 1989.
18. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977;74:5463-7.
19. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
20. Steffen C, Matzura H. Nucleotide sequence analysis and expression studies of a chloramphenicol-acetyltransferase-coding gene from *Clostridium perfringens*. Gene 1989;75:349-54.
21. Bannam TL, Crellin PK, Rood JI. Molecular genetics of the chloramphenicol-resistance transposon Tn4451 from *Clostridium perfringens*: the TnpX site-specific recombinase excises a circular transposon molecule. Mol Microbiol 1995;16:535-51.
22. Hoke C, Vedros NA. Taxonomy of the Neisseriae: deoxyribonucleic acid base composition, interspecific transformation, and deoxyribonucleic acid hybridization. Int J Syst Bacteriol 1982;32:57-66.
23. Fermér C, Kristiansen B-E, Sköld O, Swedberg G. Sulfonamide resistance in *Neisseria meningitidis* as defined by site-directed mutagenesis could have its origin in other species. J Bacteriol 1995;177:4669-75.
24. Funatsu G, Wittmann HG. Location of amino acid replacements in protein S12 isolated from *Escherichia coli* mutants resistant to streptomycin. J Mol Biol 1975;68:547-50.
25. Moazed D, Noller HF. Interaction of antibiotics with functional sites in 16S ribosomal RNA. Nature 1987;327:389-94.
26. Knapp JS, Rice RJ. *Neisseria* and *Branhamella*. In: Murray PR, ed. Manual of clinical microbiology. 6th ed. Washington, D.C.: American Society for Microbiology Press, 1995:324-40.
27. Abraham LJ, Rood JI. The *Clostridium perfringens* chloramphenicol resistance transposon Tn4451 excises precisely in *Escherichia coli*. Plasmid 1988;19:164-8.
28. Swartley JS, McAllister CF, Hajjeh RA, Heinrich DW, Stephens DS. Deletions of Tn916-like transposons are implicated in *tetM*-mediated resistance in pathogenic *Neisseria*. Mol Microbiol 1993;10:299-310.
29. Roberts MC. Characterization of the Tet M determinants in urogenital and respiratory bacteria. Antimicrob Agents Chemother 1990;34:476-8.
30. Bowler LD, Zhang QY, Riou JY, Spratt BG. Interspecies recombination between the *penA* genes of *Neisseria meningitidis* and commensal *Neisseria* species during the emergence of penicillin resistance in *N. meningitidis*: natural events and laboratory simulation. J Bacteriol 1994;176:333-7.
31. Rådström P, Fermér C, Kristiansen B-E, Jenkins A, Sköld O, Swedberg G. Transformational exchanges in the dihydropteroate synthase gene of *Neisseria meningitidis*: a novel mechanism for acquisition of sulfonamide resistance. J Bacteriol 1992;174:6386-93.

**CORRECTION**

**High-Level Chloramphenicol Resistance in *Neisseria meningitidis***

High-Level Chloramphenicol Resistance in *Neisseria meningitidis*. On page 870, in the second paragraph in the right-hand column, the sentence that begins on line 11 should have read, "Two primers (primer A, 5'ATTCAGAGTTTAGGACGG3', and primer B, 5'ATCAAATAATCA~~T~~ATCC3')," not "5'ATCAAATAATCAATCC3'," as printed.