Acquisition of Beta-Lactamase by Neisseria meningitidis through Possible Horizontal Gene Transfer

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EPIDEMIOLOGY AND SURVEILLANCE

Acquisition of Beta-Lactamase by *Neisseria meningitidis* through Possible Horizontal Gene Transfer

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ABSTRACT We report the detection in France of a beta-lactamase-producing invasive meningococcal isolate. Whole-genome sequencing of the isolate revealed a ROB-1-type beta-lactamase gene that is frequently encountered in *Haemophilus influenzae*, suggesting horizontal transfer between isolates of these bacterial species. Beta-lactamases are exceptional in meningococci, with no reports for more than 2 decades. This report is worrying, as the expansion of such isolates may jeopardize the effective treatment against invasive meningococcal disease.

KEYWORDS *Neisseria meningitidis*, beta-lactamases

Beta-lactam agents are often used for the treatment of invasive meningococcal disease (IMD). Reduced susceptibilities to penicillin G, ampicillin, and amoxicillin have been increasingly reported worldwide. Isolates with reduced susceptibility to third-generation cephalosporins have been also described through *penA* gene transfer from gonococci to meningococci (1). However, beta-lactamase-producing meningococci remained exceptional, with no reported cases for more than 2 decades (2).

We identified in 2017 at the National Reference Center for Meningococci in Paris, France, an invasive isolate of *Neisseria meningitidis* that produced a beta-lactamase. The isolate was from a 23-year-old woman who was admitted to the hospital with fever and abdominal pain. *N. meningitidis* was isolated from blood culture. No respiratory samples were available to study any possible respiratory coinfection. Antibiotic susceptibility testing revealed resistances to penicillin G and amoxicillin, with MICs of 3 and 12 μg/ml, respectively. Clavulanic acid (an inhibitor of beta-lactamase) decreased the amoxicillin MIC to 0.5 μg/ml. The isolate was susceptible to third-generation cephalosporins (ceftriaxone and cefotaxime: MIC, 0.008 μg/ml), and the patient was successfully treated with ceftriaxone.

The isolate was of serogroup Y. Whole-genome sequencing (WGS) enabled genotyping of the isolate: group, Y; PorA variable region VR1, 5-2; PorA variable region VR2, 10-2; FetA variable region, F4-1; clonal complex (cc), 23; sequence type (ST), 3587. The data (FASTA format) are accessible on the PubMLST website (accession number 53820, isolate LNP29202) and at NCBI GenBank accession no. PRJNA454456. WGS also enabled the detection of a ROB-1-type beta-lactamase gene (bla_{ROB-1}) on the chromosome of the isolate that was 100% identical at both DNA and amino-acid levels to bla_{ROB-1} harbored by the pB1000 in *Haemophilus influenzae* but also found in other bacterial species, such as *Pasteurella* and *Moraxella* species (3).

The sequence analysis showed that the bla_{ROB-1} gene was inserted on the chromosome of the LNP29202 isolate upstream of the gene Neis0803 and downstream of the gene Neis0807. These two genes encode hypothetical proteins. The bla_{ROB-1} gene was present on the meningococcal chromosome with its promoter as well as the ribosome binding site, enabling the transcription and translation of the beta lactamase. The bla_{ROB-1} gene of the plasmid pB1000 was previously shown to be functional and responsible for the resistance to beta-lactam antibiotics (4). However, no additional

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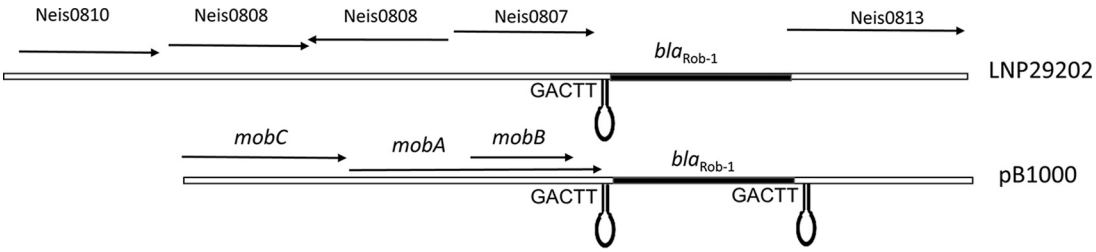
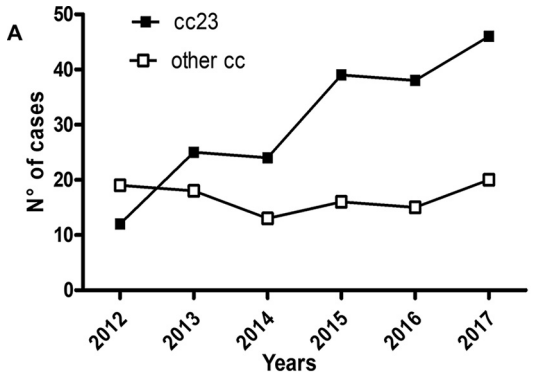


FIG 1 Schematic representation of the genetic organization of the chromosomal region of the isolate LNP29202 that received the insertion of the bla_{ROB-1} gene. The organization of the pB1000 plasmid harboring the bla_{ROB-1} gene is also shown. The arrows indicate the genes (names are above the arrows). The GACTT sequences and the transcription terminator (the hairpin structure) are also indicated.

genes from pB1000 (such as the *mobABC* genes) were transferred with the bla_{ROB-1} gene on the chromosome of the LNP29202 isolate (Fig. 1).

It was previously suggested that the flanking sequences of the bla_{ROB-1} gene on the pB1000 plasmid mediate the insertion of this gene. Indeed, a perfect two repeats of the sequence GACTT that are linked to a transcriptional terminator are located upstream and downstream of bla_{ROB-1} on the pB1000 plasmid (4). The bla_{ROB-1} in the LNP29202 isolate is preceded by only one copy of the GACTT that was linked to a transcriptional terminator, as in the plasmid pB1000 (Fig. 1). A similar organization was also reported in the plasmid pHS-Tet encoding tetracycline resistance in *Haemophilus parasuis* strain HS1543 (5). The acquisition of the bla_{ROB-1} gene may have occurred through a respiratory coinfection of *N. meningitidis* and isolates of these bacterial species.

The isolate reported here belonged to *N. meningitidis* serogroup Y (NmY) of the cc23. IMD cases due to NmY cc23 have been increasing in France and in other European countries since 2010 (6). NmY (of cc23 and other cc) are frequently observed in the elderly and are involved in pneumonia (7). However, many NmY/cc23 cases are now occurring in adolescents and young adults who have a higher prevalence of meningococcal carriage (8), thereby perhaps providing an opportunity for concurrently carried bacterial species to exchange genetic material (Fig. 2). The acquisition of



B

	N° of cases	Median age	Q1-Q3	p value
cc23 cases	184	42	17-76	0.0003
Other cc cases	101	64	29-87	
All	285	54	19-81	

FIG 2 (A) Evolution of cases (numbers) of group Y invasive meningococcal disease in France per year during the period 2012 to 2017 according to clonal complexes (cc23 versus other cc). (B) Evolution of cases (numbers) of IMD for the whole period according to median age and to the clonal complexes (cc23 versus other cc). A chi-square test was performed to calculate the *P* value (cc23 versus other cc). The cases represent all the cases that were received at the National Reference Center for Meningococci, which types all the isolates as part of its mission of surveillance of IMD in France.

bla$_{ROB-1}$ among meningococcal isolates is worrying, as it may jeopardize the use of beta-lactams that are first-line antibiotics for the empirical treatment of IMD. Preventive strategies (vaccination) should be enhanced to reduce the incidence of the disease and the circulation of the isolates. These measures should reduce the use of antibiotics and the selection of resistance.

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