Complete Sequence of a β-Lactamase-Encoding Plasmid in Neisseria meningitidis

ANDERS BACKMAN,1* PAULA ORVELID,1 JULIO A. VAZQUEZ,2 OLA SKÖLD,3 AND PER OLČEN1

Department of Clinical Microbiology and Immunology, Örebro Medical Center Hospital, SE-701 85 Örebro, 1 and
Department of Pharmaceutical Bioscience, Biomedical Centre, Uppsala University, SE-751 23 Uppsala, 2 Sweden, and
Servicio de Bacteriología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid, Spain2

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Identical β-lactamase-encoding (TEM-1) plasmids were found in two different clinical Neisseria meningitidis strains. They were completely sequenced (5,597 bp) and designated pAB6. The plasmid is almost identical to Neisseria gonorrhoeae plasmid pJD5 (5,599 kb) and may have been picked up from a gonococcus in vivo.

Plasmids in Neisseria meningitidis are found at various frequencies in different strain collections (1, 10, 12). Their presence is sometimes correlated with β-lactamase-mediated resistance to penicillin (5, 6).

N. meningitidis is basically highly susceptible to penicillin, with MICs of ≤0.05 mg/liter, and this drug is still the major antimicrobial agent used for the treatment of meningococcal disease. However, increasing numbers of N. meningitidis strains with decreased susceptibility to penicillin G (MICs of >0.1 mg/liter) have been reported in recent years from many countries (11), such as Spain (15); D. Fontanals, V. Pineda, I. Pons, and R. C. Rojo, Letter, Eur. J. Clin. Microbiol. Infect. Dis. 8:90–91, 1989), Italy (17), Greece (18), the United Kingdom (E. M. Sutcliffe, D. M. Jones, S. el-Sheikh, and A. Percival, Letter, Lancet i:657–658, 1988), the United States (20), Canada (2), Israel (C. Block, Y. Davidson, E. Melamed, and N. Kelier, Letter, J. Antimicrob. Chemother. 32:166–168, 1993), The Netherlands (8), and Sweden (1).

A few isolates of N. meningitidis resistant to penicillin via plasmid-encoded β-lactamase production have been found in Canada, South Africa, and Spain (6, 19; P. Botha, Letter, Lancet i:54, 1988; D. Fontanals, V. Pineda, I. Pons, and J. C. Rojo, Letter, Eur. J. Clin. Microbiol. Infect. Dis. 8:90–91, 1989). Two of these strains were shown to carry a plasmid-borne β-lactamase (6, 19), but it was not further characterized. The other isolates probably also contained β-lactamase-encoding plasmids, since both species occasionally coexist in the genitourinary tract (6). The spread of penicillin resistance among strains of N. meningitidis can be monitored by examining the characteristics of the types of β-lactamase-encoding plasmids and the β-lactamase genes.

The aim of the present study was to sequence and identify the type of β-lactamase plasmids found in two strains of N. meningitidis isolated in Spain (19). The β-lactamase-producing N. meningitidis strains MC9690-129 and MC9690-130 were isolated from the blood of a patient with meningitis and from the throat of a contact person, respectively. Both strains were B4:P1.15 (19). The strains were cultured on GC agar plates (3% GC medium base; Difco Laboratories) with 1% supplements (0.4% D-glucose, 0.01% L-glutamine, 0.1% cocarboxylase, and 0.5% ferric nitrate), 0.5% IsoVitalex enrichment (BBL), and 0.5 mg of penicillin G/liter at 37°C in an atmosphere with 5% CO2, for 18 to 20 h.

Antibiograms for the N. meningitidis strains were established with the E-test (Biodisk, Solna, Sweden) on chocolate Mueller-Hinton agar (9). The MICs (in micrograms per milliliter) of penicillin G (1.0), penicillin V (3.0), ampicillin (3.0), piperacillin (0.032), oxacillin (24), cefuroxime (0.38), ceftriaxone (<0.002), ceftazidime (0.023), imipenem (0.19), ciprofloxacin (0.004), rifampin (0.008), and chloramphenicol (0.75) for the two plasmid-carrying N. meningitidis strains were identical. The strains were tested for β-lactamase production by the chromogenic cephalosporin method (16), using nitrocefin discs (Biodisk, Solna, Sweden). The tests were performed with aqueous suspensions of bacteria at room temperature, and the results were noted after 10 to 15 min and compared with those of positive and negative controls.

Both strains were confirmed as β-lactamase producers.

The Wizard Plus Midiprep DNA purification system (Promega) and the QIAprep Spin Miniprep kit (Qiagen GmbH, Hilden, Germany) were used to prepare plasmid DNA. Plasmid DNA was digested with XbaI and PvuII, and the TEM fragment was ligated into the M13mp18/19 vectors (Pharmacia, Biotech, Uppsala, Sweden) according to the manufacturer’s instructions. The recombinant DNA was transformed into E. coli JM105, which was cultured according to the manufacturer’s instructions (Pharmacia Biotech).

Sequencing was performed with the ABI PRISM BigDye Terminator cycle sequencing system, using a Ready Reaction
kit, on an ABI PRISM 310 genetic analyzer according to the manufacturer's instructions (Perkin-Elmer Applied Biosystems). The TEM gene sequence was determined by direct sequencing of the clones. The complete primer sequence was then determined by primer walking.

The nucleotide sequences of the two plasmids were determined and compared to each other and to registered sequences in the international databases GenBank, European Molecular Biology Laboratory (EMBL), DNA Data Bank of Japan (DDBJ), and Protein Data Bank (PDB) by BLAST search (http://www.ncbi.nlm.nih.gov). The comparisons between the two plasmids showed that they had identical DNA sequences of 5,597 bp. Thus, the same plasmid was found in the two strains, and it was designated pAB6.

Conclusions. In the present study, two β-lactamase plasmids isolated from N. meningitidis were completely sequenced for the first time; they were found to be identical and designated pAB6 (Fig. 1). The sizes and sequences of pAB6 and pJD5 (5, 7) are almost identical. pAB6 could thus be a variant of pJD5 picked up from a gonococcus in vivo. If these types of pathogenic meningococcal strains harboring plasmids like pAB6 become common, they will cause problems...
for the treatment of meningococcal meningitis and septicemia. Therefore, it appears to be important to evaluate N. meningitidis strains regularly for any changes in susceptibility patterns and for the introduction of β-lactamase plasmids.

Nucleotide sequence accession number. The nucleotide sequence of pAB6 has been submitted to the GenBank database under accession no. AF126482.

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REFERENCES