

WGS analysis of a penicillin-resistant *Neisseria meningitidis* strain containing a chromosomal ROB-1 β -lactamase gene

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Objectives: *Neisseria meningitidis* is rarely penicillin resistant. We describe WGS analysis of a penicillin-resistant *N. meningitidis* collected from a case of invasive meningococcal disease.

Methods: Serogrouping, serotyping and serosubtyping were performed with specific antibodies. β -Lactamase was detected by nitrocefin. MICs were determined by Etest and agar dilution. Sequencing of *N. meningitidis* genomes was done on the Illumina MiSeq platform and genome data were analysed using the Bacterial Isolate Genome Sequence Database (BIGSdb) on the PubMLST *Neisseria* website (<https://pubmlst.org/neisseria/>). Transformation was used to confirm the genetic basis of the penicillin resistance.

Results: An *N. meningitidis* blood isolate from a female patient in her mid-50s with a painful and septic left shoulder was found to have penicillin MIC values of 3–12 mg/L. The isolate was typed as Y: 14, 19: P1.– and ST3587, and was weakly β -lactamase positive. WGS analysis identified a full-length copy of the β -lactamase gene *bla*_{ROB-1}, which was contained on a 1719 bp insert with a G + C content of 41.7% (versus a G + C content of *N. meningitidis* of 51.7%), suggesting that the *bla*_{ROB-1} gene came from a different bacterial species. A GenBank analysis of the *bla*_{ROB-1} gene insert found 99.77% identity with a DNA segment found in plasmid pB1000' from *Haemophilus influenzae*. Transformation of a penicillin-susceptible strain with the *bla*_{ROB-1} gene conferred β -lactamase activity and penicillin resistance.

Conclusions: *N. meningitidis* serogroup Y, ST3587 can carry and express the *bla*_{ROB-1} gene, leading to penicillin resistance. It is highly likely that the *N. meningitidis* isolate acquired the *bla*_{ROB-1} gene from *H. influenzae*.

Introduction

Invasive meningococcal disease (IMD) is a serious infection that may start off insidiously, but progress rapidly, leading to meningitis and/or septic shock.¹ The causative agent, *Neisseria meningitidis*, is divided into 12 serogroups and may be further typed based on their major outer-membrane protein antigens, PorB and PorA, into serotypes and serosubtypes, respectively. Antigenically *N. meningitidis* can be described as serogroup: serotype: serosubtype (e.g. B: 4: P1.4).² MLST can identify isolates as ST and related STs are grouped together as clonal complexes (CCs).³ Most invasive disease cases are caused by *N. meningitidis* belonging to the serogroups A, B, C, W, X and Y, and the relative frequency of these serogroups as causes of IMD vary geographically for reasons not totally understood.^{4,5} In addition, most invasive disease is caused

by strains belonging to several hypervirulent clones that can be represented by their CCs, such as ST11 CC, ST32 CC, ST41/44 CC, etc.^{6–8}

Resistance of *N. meningitidis* to some antibiotics for treatment or chemoprophylaxis has been described, most notably chloramphenicol, sulfonamides, rifampicin, ciprofloxacin and penicillin.^{9–12} The antibiotics of choice for treatment of confirmed IMD are third-generation cephalosporins such as cefotaxime or ceftriaxone, penicillin if the strain has been confirmed susceptible or chloramphenicol if the patient is allergic to β -lactam antibiotics.¹³ Although penicillin is not the first drug of choice for treatment, it is still recommended for use if there may be a delay in access to third-generation cephalosporins at a higher-level facility.¹⁴

Penicillin resistance in *N. meningitidis* can be caused by the presence of *bla* genes encoding the production of β -lactamases, resulting in penicillin MIC values of >2 mg/L. Another mechanism of penicillin resistance is due to mutations in the *penA* gene, resulting in alterations in PBP2 and reduced susceptibility or resistance to penicillin, with penicillin MIC values of ≥ 0.5 mg/L. *N. meningitidis* resistant to penicillin is very rare.⁹ A search of the literature revealed only a handful of reports. Three cases of β -lactamase-positive and penicillin-resistant *N. meningitidis* causing IMD were reported in Canada in 1983¹⁵ and South Africa in 1987.¹⁶ In all three cases, the penicillin MICs were ≥ 256 mg/L. In addition, three cases of IMD due to β -lactamase-positive strains typed as B: 4: P1.15 were reported in Spain in 1986, 1991 and 1996, but no epidemiological links were reported by the authors.^{17,18} Also unusual for two of these three isolates were the lower penicillin MICs of 2–4 mg/L (the resistance breakpoint is 0.5 mg/L according to the CLSI¹⁹), although no MIC was reported for the third isolate. In 2006, a serogroup Y IMD case presenting with severe pneumonia and caused by a β -lactamase-negative strain with a penicillin MIC of 0.25–0.5 mg/L (depending on the method used to test for MIC) was reported in the USA.²⁰ In 2010, a β -lactamase-negative serogroup B strain with a penicillin MIC of 0.5–1.0 mg/L was reported to cause an IMD case in Australia.²¹ More recently, *N. meningitidis* strains with mutations in their *penA* genes that affect binding to β -lactam antibiotics and reduced susceptibility to penicillin are being increasingly reported.^{22,23} A recent clone of ST11 serogroup W meningococci resistant to penicillin and with significant *penA* gene mutations has been described to be expanding in Western Australia, and this strain has also been found in some European countries.²⁴ Mutations in the *penA* gene that led to decreased susceptibility to ceftriaxone were reported for the first time in 2017.²⁵

Surveillance of IMD including antibiotic susceptibility testing and genetic characterization of strains by WGS analysis using, for example, the *Neisseria* Bacterial Isolate Genome Sequence Database (BIGSdb)²⁶ can be very useful to monitor for these changes. In this report, we describe an invasive meningococcal isolate with resistance to penicillin caused by ROB-1 β -lactamase production encoded by a chromosomal *bla*_{ROB-1} gene that appears to be imported from a *Haemophilus influenzae* plasmid pB1000'. Genome comparison of our strain with isolates on the *Neisseria* MLST website (<https://pubmlst.org/neisseria/>) allowed us to identify another isolate with a chromosomal *bla*_{RIB-1} gene.

Materials and methods

Bacterial strains

Two serogroup Y *N. meningitidis* strains [National Microbiology Laboratory (NML) Y144 and NMLY048] recovered in 2016 from patients in the province of Alberta, Canada were studied by WGS analysis. For comparison, the draft genomes of two *N. meningitidis* strains with the same ST as NMLY144 and the complete genome of a serogroup W meningococcal strain M7124 were retrieved from the PubMLST *Neisseria* database (<https://pubmlst.org/neisseria/>) and included in the analysis. For transformation, a β -lactamase-negative and penicillin-susceptible serogroup W *N. meningitidis* strain NMLW082 from our culture collection was used as a recipient strain for the *bla* gene from NMLY144. NMLW082 has been typed as W: 2a: P1.2 and ST11, with a *penA* allele 1 and a penicillin MIC of 0.047 mg/L.

H. influenzae clinical isolates with or without expression of the TEM-1 and ROB-1 β -lactamases were used as positive and negative controls for

the phenotypic detection of β -lactamases and their genes and associated plasmids. A *Neisseria gonorrhoeae* clinical isolate was also used as a positive control for the detection of β -lactamase enzymes. All clinical isolates were from the NML culture collection.

Typing of *N. meningitidis*

Serogrouping, serotyping, serosubtyping, PorA genotyping and MLST were done by previously described standard methods.^{27,28}

Antibiotic susceptibility testing

β -Lactamase production was determined using DrySlide Nitrocefin (BBL, Becton Dickinson, Oakville, ON, Canada). To determine the MICs of penicillin, ceftriaxone, chloramphenicol, rifampicin and ciprofloxacin, Etest (AB Biodisk, Solna, Sweden) was performed on Mueller–Hinton agar with 5% blood according to CLSI guidelines.¹⁹ Penicillin MICs were also determined by the agar dilution method.²³

DNA purification and amplification

Genomic DNA was extracted from pure cultures using the Epicentre Masterpure Complete DNA and RNA Extraction Kit (Mandel Scientific, Guelph, ON, Canada) and following the kit protocol. Plasmid DNA was purified using a Qiagen Plasmid Mini Kit (Qiagen Canada, Toronto, ON, Canada). PCR amplifications of the *bla*_{TEM-1} and *bla*_{ROB-1} genes were carried out according to protocols described by Scriver *et al.*²⁹ The nucleotide sequences of the PCR amplicons were confirmed by Sanger sequencing using the DNA Analyzer 3730x1 (Applied Biosystems Inc., Foster City, CA, USA).

WGS, data assembly and analysis

Using genomic DNA samples, multiplexed libraries were created with TruSeq sample preparation kits (Illumina, San Diego, CA, USA) and paired-end, 300 bp indexed reads were generated on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Genome sequence data were assembled using SPAdes³⁰ and annotated using the Prokaryotic Genome Annotation System (PROKKA).³¹ The assembled genomes were uploaded to the PubMLST *Neisseria* database (<https://pubmlst.org/neisseria/>), which runs the BIGSdb platform.²⁶ Canadian isolates NMLY144 and NMLY048 were compared by core genome MLST (cgMLST) with two other isolates, M17 240005 and 2816 (both ST3587), isolated in the UK and Italy, respectively [available in the PubMLST *Neisseria* database (accessed in February 2018); their features are described in Table S1 (available as [Supplementary data](#) at *JAC* Online)], using the BIGSdb genome comparator tool.³² For cgMLST, a distance matrix based on the number of variable alleles was generated by the genome comparator tool, and a neighbour-net phylogenetic network figure was generated using SplitsTree4 (version 4.14.5).^{32,33} The genome of a serogroup W meningococcal strain M7124 (GenBank accession number CP009419.1) was included as an outgroup in the cgMLST analysis. These five genomes were also compared and a pan-genome map was generated using GView.³⁴ The Oxford Nanopore Technology (ONT) MinION sequencer was also employed to produce long reads of the NMLY144 strain. ONT libraries were prepared using the rapid sequencing kit (RAD-0002) and an R9.4 flow cell.

The surface protein antigen genes *porA*, *porB*, *fetA*, *nadA*, *nhba* and *fhbp* [encoding, respectively, class 1 outer membrane porin protein (PorA), class 2/3 outer membrane porin protein (PorB), iron-regulated *Neisseria* outer membrane protein (FetA), *Neisseria* adhesin A (NadA), *Neisseria* heparin binding antigen (NHBA) and factor H binding protein (fhbp)] of isolate NMLY144 as well as its *penA* allele were also determined from its WGS data using the PubMLST *Neisseria* database (<https://pubmlst.org/neisseria/>).

BLAST search (<https://blast.ncbi.nlm.nih.gov>) was performed using GenBank sequence data of the *bla*_{ROB-1} gene (GenBank accession number AF022114.1) to locate this gene in the draft genome of isolate NMLY144.

Transformation of penicillin-susceptible *N. meningitidis* strain NMLW082 with the *bla*_{ROB-1} gene from NMLY144

To verify that insertion of the *bla*_{ROB-1} gene was responsible for the β -lactamase activity and resistance to penicillin in NMLY144, the 1719 bp insert (non-coding sequence plus the *bla*_{ROB-1} gene) from NMLY144 was transferred into NMLW082 via transformation. This insert was first PCR amplified from genomic DNA prepared from NMLY144 with primers that targeted the flanking regions (NEIS0807 and NEIS0803) of the insert. The PCR primers were NEIS0807F (5'-GCCACACCTTCCTTAAAGACGA-3') and NEIS0803R [5'-**TTCAGACGGCAT**CCTCAGTCTCCTTCATCTCC-3' (the bold letters indicated a neisserial DNA uptake sequence)]. Subsequently, transformation was carried out as described by Bogdan et al.³⁵ Briefly, colonies of the recipient strain were grown overnight on blood agar, and then resuspended in tryptic soy broth to an OD of 0.6 at 600 nm. The cells were harvested by centrifugation at 3000 g for 10 min at 4°C and gently resuspended in 1.5 mL of ice-cold transformation solution, consisting of LB broth with 10% (w/v) PEG 8000, 5% (v/v) DMSO and 50 mM MgCl₂, mixed with 12 μ L of the PCR product and incubated on ice for 15 min. Next, 1.5 mL of tryptic soy broth was added to the tube and the cells were grown at 37°C with shaking for 2 h. An aliquot of the cells was plated on a blood agar plate containing 2 mg/L penicillin and allowed to grow overnight at 37°C with 5% (v/v) CO₂. Transformants were screened by PCR and confirmed by sequencing.

Results

Description of case, characteristics of isolate and susceptibility to antibiotics

A female patient in her mid-50s presented to a hospital emergency department with fever and chills and complaining of left shoulder pain that came on suddenly. She was diagnosed with left shoulder septic arthritis. A blood culture was taken that grew *N. meningitidis* within 12 h. After admission, the patient was started on intravenous ceftriaxone and ciprofloxacin. Surgery was performed on the left shoulder the next day with debridement. Irrigation of the shoulder and joint was done as well. The patient was continued on intravenous antibiotics and after 10 days was switched to oral agents. The patient's subsequent recovery was uneventful.

The *N. meningitidis* blood culture isolate (designated NMLY144) was typed and found to have an antigenic profile of Y: 14, 19: P1.– (non-serosubtypeable). PorA genotype and MLST profiling identified the isolate to have PorA VR1–3 types of 5–2, 10–2 and 36–2, and to be ST3587 (ST23 CC).

When compared with isolates of β -lactamase-positive *N. gonorrhoeae* and *H. influenzae*, NMLY144 was found to be weakly positive for β -lactamase. It was determined to be resistant to penicillin by both Etest and the agar dilution method with MIC values of 3–6 and 12 mg/L, respectively. Etest also showed that NMLY144 was susceptible to ceftriaxone, chloramphenicol, rifampicin and ciprofloxacin with MIC values of 0.002, 1.0, 0.047 and 0.004 mg/L, respectively. As a control, we included another serogroup Y isolate (NMLY048), also recovered from the province of Alberta in the same year as NMLY144. NMLY048 was β -lactamase negative when tested by nitrocefin, and was susceptible to penicillin, ceftriaxone, chloramphenicol, rifampicin and ciprofloxacin with MIC values of 0.094, 0.003, 2.0, 0.012 and 0.004 mg/L, respectively. NMLY048 was typed as Y: NT (non-typeable): P1.5, 2 with PorA genotypes of P1.5–1, 2–2, 36–2 and ST12163 (ST23 CC).

Additional antigen gene typing of NMLY144 was obtained from WGS analysis, and the isolate was found to have *porB* allele 1038 (which encoded a class 3 PorB), *porA* allele 148 (which encoded

PorA VR1–3 types 5–2, 10–2 and 36–2), *fetA* allele 223 (which encoded FetA VR type F4-1), *fHbp* allele 104 (which encoded peptide 104; Novartis variant 2 and Pfizer subfamily A) and *nhba* allele 13 (which encoded peptide 8), and lacked the *nadA* gene (hence no NadA peptide) and *penA* allele 9 (encoding PBP2 with significant mutations A510V, F504L, H541N, I515V and I566V) associated with reduced susceptibility to penicillin.

Detection of β -lactamase genes in isolate NMLY144 and transfer of a β -lactamase gene to a susceptible strain

Genomic DNA from NMLY144 was initially tested by PCR for the presence of *bla*_{TEM-1} and *bla*_{ROB-1} genes and only specific primers for the *bla*_{ROB-1} gene gave a positive PCR product. Extraction and detection of plasmids in NMLY144 yielded negative results, whereas *bla*_{ROB-1}-positive *H. influenzae* isolates were found to have plasmids (data not shown) and plasmid DNA gave positive PCR amplicons with the *bla*_{ROB-1}-specific primers (data not shown).

The full-length (918 bp) *bla*_{ROB-1} gene was searched for in the assembled draft genome of NMLY144 and its location was identified in a 45 069 bp contig (Figure 1). Besides the *bla*_{ROB-1} gene, the 1719 bp insert, with a G + C content of 41.7% versus the overall G + C content of 51.7% for the NMLY144 genome, also consisted of 801 bp of non-coding DNA. Interrogation of the insert against GenBank data revealed 99.77% (1715/1719 bp) identity of the insert with a DNA segment found in the plasmid pB1000' from an *H. influenzae* strain BB1050 (GenBank accession number GU080066) (Figure S1). This part of the *H. influenzae* plasmid pB1000' has been found to be consistently inserted in NMLY144 between two *Neisseria* genes *Neis0807* (encoding a putative periplasmic protein) and *Neis0803* (encoding a hypothetical protein) when isolate NMLY144 was resequenced in two additional runs on the MiSeq or the MinION WGS platforms. In contrast, NMLY048 did not have the insert with the β -lactamase gene *bla*_{ROB-1}.

Transfer of the gene insert including *bla*_{ROB-1} from NMLY144 into NMLW082 caused NMLW082 to produce β -lactamase and increased the penicillin MIC for this strain from 0.047 to 3 mg/L. This confirmed that the gene insert with *bla*_{ROB-1} was responsible for the penicillin resistance in NMLY144.

N. meningitidis of ST3587

Interrogation of the *Neisseria* MLST website for ST3587 (accessed on 26 February 2018) found four *N. meningitidis* isolates with this ST. Two were serogroup Y (from Italy and Canada), one was non-groupable (from the UK) and one was without serogroup information (from the USA). Other than the Canadian and the USA isolates, the other two isolates were not described as associated with invasive disease. WGS data were available for the Canadian, the UK and the Italian ST3587 isolates. The UK and the Canadian isolates were clustered together after cgMLST analysis, whereas the Italian ST3587 appeared to be different (Figure 2). Based on 1605 core loci, these three genomes exhibited allelic variations in 28–241 loci in pairwise comparisons. Maximum allelic differences ($n = 241$) were observed between Italian and Canadian isolates, whereas only a 28-loci difference was found between the Canadian and the UK isolates at the cgMLST level.

Comparison of the Canadian, the UK and the Italian ST3587 isolates showed that the UK isolate, but not the Italian isolate, was

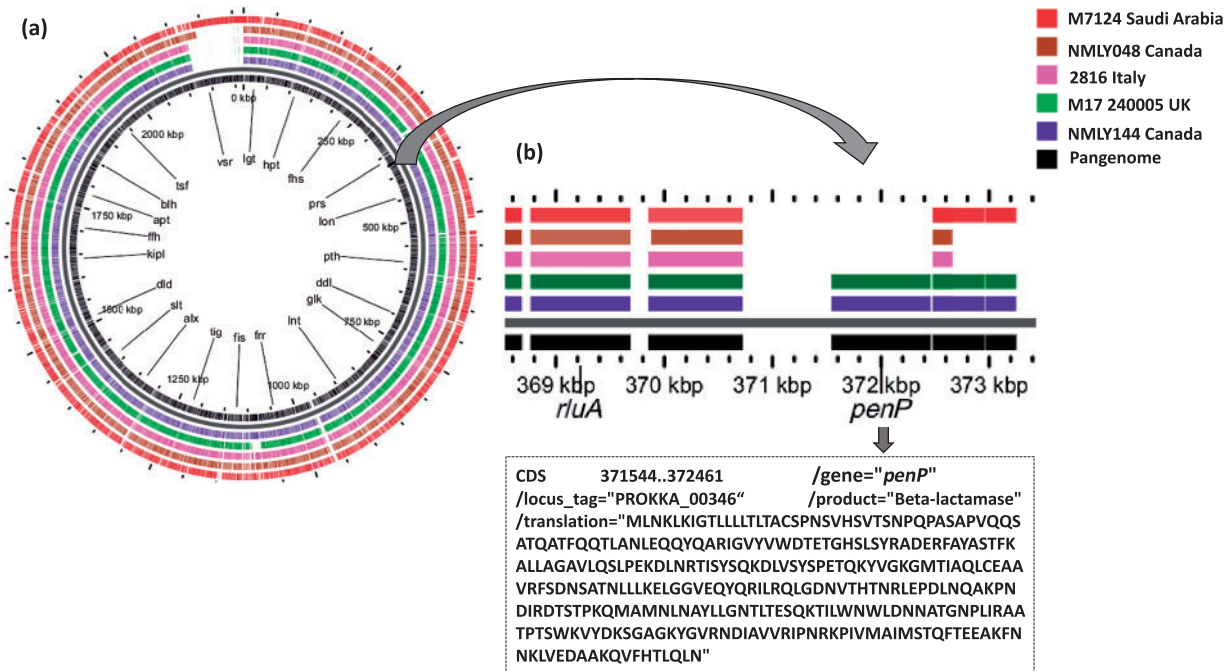


Figure 1. A pan-genome map of three ST3587 meningococcal strains (NMLY144 from Alberta, Canada; M17 240005 from the UK; and 2816 from Italy), a serogroup Y strain (NMLY048 from Alberta, Canada) and a serogroup W strain (M7124 from Saudi Arabia) generated using the GView server. A full-genome view (a) and a zoomed view (b) show an area centring around the β -lactamase (bla_{ROB-1}) gene, labelled as *penP*. The innermost slot (black) shows the constructed pan-genome using all uploaded genomes. A grey line separates the pan-genome from the other genomes, aligned to show sequence homologies and the absence of genes. Gaps denote non-coding sequences. Depicted in the box is the translated protein sequence of the ROB-1 β -lactamase annotated by PROKKA as *penP*. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

found to contain the *bla_{ROB-1}* gene and an identical 801 bp non-coding sequence inserted at the same position as the Canadian isolate NMLY144 (Figure 1).

Discussion

In this report we have described the first isolate, to our knowledge, of a penicillin-resistant serogroup Y *N. meningitidis* identified since 2000 through our routine IMD laboratory surveillance programme. The penicillin MIC for this isolate was found to be 3–6 mg/L by Etest and 12 mg/L by the agar dilution method, which was 6- to 24-fold above the CLSI MIC breakpoint for penicillin resistance (0.5 mg/L). Our routine surveillance over the past five years (2013–2017) on all invasive meningococcal isolates collected at the National Microbiology Laboratory submitted from across Canada did not detect any other resistant strains. Random testing of invasive isolates collected earlier in our laboratory surveillance programme from 2000 to 2012 also did not identify any resistant strains (R. S. W. Tsang, unpublished data). A previous survey in Ontario, Canada, examining 93.3% (363 isolates) of all their IMD case isolates collected in the years 2000–2006 also did not identify any penicillin-resistant or β -lactamase-positive *N. meningitidis* strain.²³

The last time a penicillin-resistant *N. meningitidis* was reported in Canada was in 1983.¹⁵ The isolate, designated M1-221, was described as β -lactamase positive, with a penicillin MIC of 256 mg/L. This *N. meningitidis* isolate contained two plasmids of 4.5 and 24.5 MDa in size, similar to the sizes of plasmids found in penicillinase-

producing *N. gonorrhoeae*. In contrast, the current isolate, NMLY144, was only weakly positive for β -lactamase when tested by the nitrocefin method and did not contain a plasmid. The β -lactamase gene *bla_{ROB-1}* was chromosomally located in NMLY144. The lower penicillin MIC in the presence of a β -lactamase gene may suggest the gene was poorly expressed and hence reflected in the weakly positive phenotypic test for β -lactamase.

The ROB-1 β -lactamase was first reported in 1981 in an *H. influenzae* serotype b strain causing meningitis that failed to respond to ampicillin treatment.³⁶ Since then, it has also been identified in ampicillin-resistant *Actinobacillus pleuropneumoniae* (a porcine respiratory pathogen) and *Pasteurella multocida*.^{37–39} Ampicillin resistance in *H. influenzae* is mostly due to TEM-1 β -lactamase, with the ROB-1 enzyme responsible for <10%.^{40,41} In contrast, 88% of the ampicillin resistance in *A. pleuropneumoniae* is due to the presence of ROB-1 β -lactamase.³⁹ Because the *bla_{ROB-1}* genes in both the human pathogen *H. influenzae* and the animal pathogens *A. pleuropneumoniae* and *P. multocida* are primarily carried on plasmids that are transmissible, ampicillin-resistant animal pathogens may serve as a reservoir for the *bla_{ROB-1}* genes for potential transfer into *H. influenzae*.³⁷

In the NMLY144 isolate, the *bla_{ROB-1}* gene was not carried on a plasmid and was integrated into the bacterial chromosome. We believe the *bla_{ROB-1}* gene was likely acquired from another organism because: (i) the insert carrying the *bla_{ROB-1}* gene has a distinct G + C content (41.7%) different from the G + C content (51.7%) of *N. meningitidis* genomes;^{42,43} (ii) the *bla_{ROB-1}* gene insert matched



Figure 2. Neighbour-net phylogenetic network comparison of four *N. meningitidis* ST23 CC isolates with the meningococcal serogroup W reference strain M7124 as an outlier by means of the BIGSdb genome comparator tool using the *N. meningitidis* cgMLST version 1.0 scheme. The scale bar denotes the number of allelic differences. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

almost 100% with the corresponding segment on an *H. influenzae* plasmid pB1000',⁴⁴ and (iii) both *N. meningitidis* and *H. influenzae* are carried in the human oropharynx, and genetic exchange between these two organisms has been reported, suggesting a mechanism for the acquisition.⁴⁵ Although the origin of the *bla*_{ROB-1} gene in isolate NMLY144 is likely to be the *H. influenzae* plasmid pB1000', the exact steps taken by this gene when transferring to the chromosome of NMLY144 are not clear. Transposons and transposases were not found in the immediate vicinity of up to 1 kb from both ends of the insert in the NMLY144 genome. However, located on the 3'-end of the insert is a 6 bp DNA sequence (TGAAAA), which is found in both pB1000' and NMLY144 as well as in a number of other meningococcal chromosomes with (e.g. the UK M17 240005) or without (e.g. NMLY048 and the Italian strain 2816) the insertion of the β -lactamase gene. Whether this TGAAAA sequence was involved in the recombination process of the insertion of the β -lactamase gene is not known, and a similar sequence on the 5'-end of the insert could not be identified. Another interesting fact about the *bla*_{ROB-1} gene was its chromosomal location in an animal strain of *Pasteurella*.⁴⁶ In all other strains described in the literature, *bla*_{ROB-1} was uniformly found to be plasmid associated.

Genotyping identified NMLY144 as belonging to the ST23 CC, which is a common genotype among MenY.⁴⁷ Within the ST23 CC, the most commonly encountered ST is ST23. Because it is a triple-locus variant of ST23, ST3587 had not been identified among IMD isolates at the NML prior to 2016. On the public *Neisseria* MLST website (<https://pubmlst.org/neisseria/>), besides NMLY144, only three other entries of ST3587 were found (data accessed on 26 February 2018). Three of the isolates were identified in 2016 or 2017. Because of the rarity of this ST and the unique feature of our isolate, we compared NMLY144 with two other ST3587 isolates (strains 2816 and M17 240005) that were found to have WGS data on the *Neisseria* MLST website. When compared by cgMLST, the Canadian isolate NMLY144 and the UK isolate M17 240005 were

most similar, sharing 1577/1605 identical gene alleles. In addition, NMLY144 and M17 240005 both have the same 1719 bp insert carrying the *bla*_{ROB-1} gene found exactly in the same location in both genomes (Figure 1). However, M17 240005 is non-groupable and may lack some of the essential capsular genes for serogroup identification. In contrast, the Italian isolate 2816 was found to lack the insert, and hence lacked the *bla*_{ROB-1} gene, and was also found to have 241 and 238 gene allele differences from NMLY144 and M17 240005, respectively. During revision of our manuscript, a serogroup Y invasive *N. meningitidis* strain resistant to penicillin due to acquisition of the *bla*_{ROB-1} gene has been reported in France.⁴⁸ The origin of the *bla*_{ROB-1} gene in this strain was also suggested to be the *H. influenzae* plasmid pB1000 because 100% identity was found between the *bla*_{ROB-1} genes in these two organisms.

Currently there are now two MenB vaccines (Bexsero[®] and Trumenba[®]) licensed in Canada, which may have the potential to offer cross-protection to meningococci of other serogroups such as MenY.^{49,50} In this regard, it is interesting to note that NMLY144 lacks the *nadA* gene and, therefore, does not encode any NadA protein, which is one of the components present in Bexsero[®]. NMLY144 also does not have the PorA antigen of P1.4, which is another vaccine component in Bexsero[®]. It is also noteworthy that the *fHbp* and *nhba* genes of NMLY144 do not appear to encode fHbp and NHBA, which match specifically with the corresponding vaccine components in Bexsero[®] and Trumenba[®]. Nevertheless, protection by these vaccines may still be possible owing to cross-reactivity of fHbp of the same sub-family and the cross-reactive nature of NHBA antigen as well as minor antigens found in the outer-membrane vesicles of the Bexsero[®] vaccine.

In conclusion, this study highlighted the importance of antibiotic susceptibility testing and molecular characterization of strains in IMD surveillance by identifying an IMD isolate with a *bla*_{ROB-1} gene responsible for causing penicillin resistance. Comparison of WGS data identified another very similar isolate of the same ST in the

UK that also has a chromosomal *bla_{ROB-1}* gene. This type of molecular analysis is particularly relevant for surveillance in order to monitor for the emergence of strains with altered penicillin-binding protein genes that may affect penicillin susceptibility. This report also illustrates the usefulness of WGS analysis for the global surveillance of IMD and antibiotic resistance.

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Transparency declarations

None to declare.

Supplementary data

Table S1 and Figure S1 are available as [Supplementary data](#) at JAC Online.

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