Role of Neisseria meningitidis PorA and PorB Expression in Antimicrobial Susceptibility

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Neisseria meningitidis can cause potentially fatal systemic disease. Early diagnosis and prompt antimicrobial intervention are critical for favorable clinical outcomes. Antibiotic resistance has been reported for penicillins (1), tetracycline (2), and sulfonamides (3), as well as quinolones (4) and rifampin (5).

N. meningitidis expresses two major porins, PorA and PorB, which are antigenically variable between strains and within a strain, and PorA is phase variable (random on/off switching) (6). Neisseria gonorrhoeae expresses a single porin, PorB. Changes in porin expression or variant porins mediate antibiotic resistance in several Gram-negative bacteria, including N. gonorrhoeae (7–10).

In N. meningitidis, the absence of PorB increases resistance to tetracycline and cefsulodin in vitro (11). The role of PorA in antimicrobial resistance has not been reported for the meningococcus. In addition to its proposed role in immune evasion, we hypothesized that phase-variable PorA expression may provide an obvious mechanism for the meningococcus to evade antimicrobials if PorA mediates antibiotic uptake or exclusion. We generated strains lacking PorA or PorB and conducted MIC assays. We also tested whether altered PorA expression is selected by antimicrobial exposure during the course of the MIC assay.

The porA and porB genes with flanking sequences were amplified from N. meningitidis strain MC58 and cloned into pGEM T-easy. Inverse PCR followed by self-ligation yielded plasmids with internal deletions and introduced restriction sites. The LacZ/ kanamycin cassette (12) was cloned into the introduced SmaI site of the deleted porA allele, yielding plasmid pPorALacZKan. A chloramphenicol acetyltransferase gene was amplified and cloned into the introduced BglII site of the deleted porB allele, in plasmid pPorB:CAT. The porA lacZ kan or porB:cat constructs were transformed into N. meningitidis strain Ø9 (13) to yield strains Ø9ΔPorA and Ø9ΔPorB. Allelic replacement of wild-type porA or porB alleles with the mutant allele was confirmed by PCR and sequencing, as well as SDS-PAGE of Sarkosyl-extracted outer membrane proteins (Fig. 1A).

MIC were assessed by broth microdilution method in 96-well plates (14) using bacteria grown overnight on supplemented BHI agar at 37°C and subcultured shaking for approximately 4 h in BHI broth at 37°C before adjusting to approximately 5 x 10^8 CFU/ml based on the optical density at 600 nm (OD600). After addition of 50 µl to serially diluted antibiotics, MICs were recorded after overnight growth at 37°C (Table 1) as the concentrations at which no turbidity was observed. Each assay was done three times, each time in triplicate. For each treatment, MICs were identical within and between assays.

Our results confirmed that loss of meningococcal PorB expression increases resistance to tetracycline (11). Mutations in PorB also contribute to resistance to tetracycline in N. gonorrhoeae (9, 15). Recommended combination therapy for multiply resistant N. gonorrhoeae includes injectable ceftriaxone and oral doxycycline or azithromycin (16). In this context, it is notable that the meningococcal Ø9ΔPorB mutant strain had reduced susceptibility to doxycycline. We noted decreased susceptibility to cephalexin for

FIG 1 Analysis of porin expression during MIC analysis. (A) Membrane proteins were isolated by Sarkosyl extraction, and 10 µg was separated on 8 to 12% bis-Tris acrylamide gels prior to Coomassie staining. Lane 1, Ø9; lane 2, Ø9ΔPorA; lane 3, Ø9ΔPorB. (B) Samples from the last well showing turbidity were plated on BHI agar and immunoblotted with the PorA-specific MAb MN14C11.6.

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LETTER TO THE EDITOR
PorB and also for the cephalosporins cefotaxime and cefazidime. A previous report linked N. meningitidis PorB mutation with increased cefsulodin resistance (11). In N. gonorrhoeae, PorB loop 3 variants also contribute to enhanced cephalosporin resistance (17). Our confirmation that ciprofloxacin. Expression changes in gonococcal porin alter cip-rhoeae contribute to decreased susceptibility of N. gonorrhoeae to ciprofloxacin. Expression changes in antimicrobial resistance of invasive Neisseria meningitidis isolates in Belgium from 2000 to 2010: increasing prevalence of penicillin nonsusceptibility, Antimicrob. Agents Chemother. 56:2628–2672.

$\Delta$PorA and $\Delta$PorB are not recommended therapies for multiply resistant N. gonorrhoeae. Troublingly, this suggests that selective pressure may lead to emergence of gonococcal PorB variants with reduced doxycycline susceptibility. Although we recorded only 2-fold differences that are unlikely in isolation to lead to treatment failures, the synergy of PorB variation with other mutations has the potential to expand the meningococcal and gonococcal resistance spectrum. We found no evidence of a role for PorA in antimicrobial transit across the outer membrane.

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REFERENCES


TABLE 1 MICs for the wild-type and mutant N. meningitidis strains*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>$\Delta$PorA</th>
<th>$\Delta$PorA</th>
<th>$\Delta$PorB</th>
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</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td>0.003125</td>
<td>0.003125</td>
<td>0.00625</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.03125</td>
<td>0.03125</td>
<td>0.0625</td>
</tr>
<tr>
<td>Cephalothin</td>
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<td>0.3125</td>
<td>0.625</td>
</tr>
<tr>
<td>Ampicillin</td>
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<td>0.0625</td>
<td>0.0625</td>
</tr>
<tr>
<td>Carbenicillin</td>
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<td>0.0625</td>
<td>0.0625</td>
</tr>
<tr>
<td>Cloxacin</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Penicillin G</td>
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<td>0.03125</td>
<td>0.03125</td>
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<tr>
<td>Piperacillin</td>
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<td>0.03125</td>
<td>0.015625</td>
</tr>
<tr>
<td>Tetracycline</td>
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<td>0.3125</td>
<td>0.625</td>
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<tr>
<td>Doxycycline</td>
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<td>0.1875</td>
<td>0.375</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>0.003125</td>
<td>0.00625</td>
</tr>
<tr>
<td>Nalidixic acid</td>
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<td>1.25</td>
<td>1.25</td>
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<td>Imipenem</td>
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<tr>
<td>Rifampin</td>
<td>0.125</td>
<td>0.125</td>
<td>0.0625</td>
</tr>
</tbody>
</table>

* MICs are reported as the last well in which turbidity was observed. Bold indicates reduced susceptibility of $\Delta$PorB.

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