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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 ¢9 (13) to yield strains ¢9ΔPorA and ¢9ΔPorB. 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 5×10^5 CFU/ml based on the optical density at 600 nm (OD_{600}). 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 ¢9ΔPorB mutant strain had reduced susceptibility to doxycycline. We noted decreased susceptibility to cephalothin for

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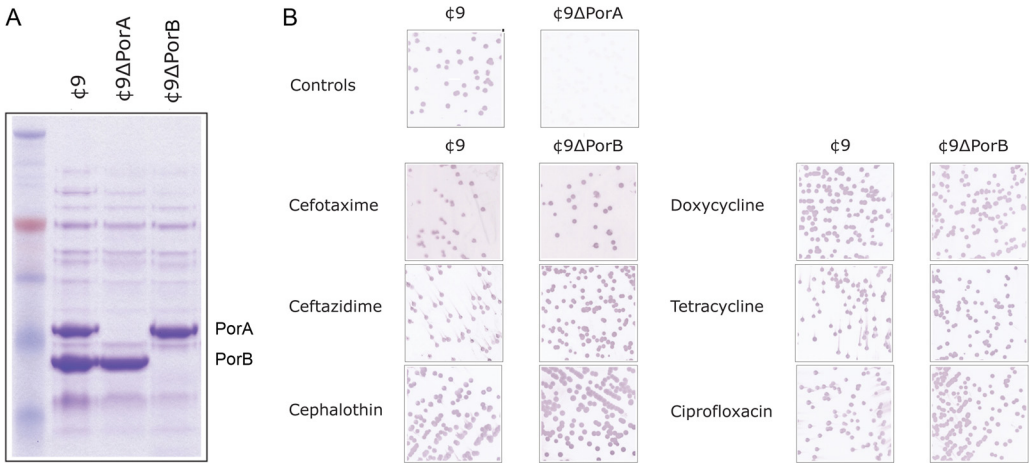

FIG 1 Analysis of porin expression during MIC analysis. (A) Membrane proteins were isolated by Sarkosyl extraction, and 10 μg was separated on 8 to 12% bis-Tris acrylamide gels prior to Coomassie staining. Lane 1, ¢9; lane 2, ¢9ΔPorA; lane 3, ¢9ΔPorB. (B) Samples from the last well showing turbidity were plated on BHI agar and immunoblotted with the PorA-specific MAb MN14C11.6.

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

Antibiotic	MIC (μg/ml)		
	¢9	¢9ΔPorA	¢9ΔPorB
Cefotaxime	0.003125	0.003125	**0.00625**
Ceftazidime	0.03125	0.03125	**0.0625**
Cephalothin	0.3125	0.3125	**0.625**
Ampicillin	0.0625	0.0625	0.0625
Carbenicillin	0.0625	0.0625	0.0625
Cloxacillin	1.25	1.25	1.25
Penicillin G	0.03125	0.03125	0.03125
Piperacillin	0.03125	0.03125	0.015625
Tetracycline	0.3125	0.3125	**0.625**
Doxycycline	0.1875	0.1875	**0.375**
Ciprofloxacin	0.003125	0.003125	**0.00625**
Nalidixic acid	1.25	1.25	1.25
Imipenem	0.0625	0.0625	0.0625
Rifampin	0.125	0.125	0.0625

[a] MICs are reported as the last well in which turbidity was observed. Bold indicates reduced susceptibility of ¢9ΔPorB.

¢9ΔPorB and also for the cephalosporins cefotaxime and ceftazidime. 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 *Neisseria* PorB modulates cephalosporin susceptibility raises the possibility that reduction in susceptibility to this class may arise clinically or be facilitated by mutations in PorB in either meningococci or gonococci.

Fluoroquinolone use is no longer recommended for gonococcal infection (18), and point mutations in PorB1b of *N. gonorrhoeae* contribute to decreased susceptibility of *N. gonorrhoeae* to ciprofloxacin. Expression changes in gonococcal porin alter ciprofloxacin resistance (19); we found that mutation of meningococcal PorB also results in reduced ciprofloxacin susceptibility. Conversely, this strain was more susceptible to rifampin, the other major choice for meningococcal prophylaxis, perhaps through altered membrane architecture, as has been suggested for altered rifamycin resistance of colistin-resistant *Acinetobacter baumannii* (20).

In contrast, we observed no changes in susceptibility of ¢9ΔPorA compared to the PorA$^+$ parent strain for any of the antibiotics tested (Table 1). This suggests that either PorA has no direct role in entry of antimicrobials into the cell or there is selection for reduced PorA expression via phase variation during the MIC assay, either in the parental strain or in ¢9ΔPorB, potentially masking PorA-mediated antibiotic entry. To assess this, we isolated bacteria from the final well in which turbidity was observed and assessed PorA expression by colony immunoblotting using the anti-P1.7 murine monoclonal antibody (MAb) MN14C11.6 (obtained from NIBSC, United Kingdom). As controls, reactivity was compared between the parental strain ¢9 and the PorA mutant strain ¢9ΔPorA. This showed that PorA expression was unaltered between control wells and after exposure to any of the tested antimicrobials (see Fig. 1B); thus, decreased susceptibility of ¢9ΔPorB is not due to altered PorA expression levels.

Our results are consistent with previous reports for a role for *N. meningitidis* PorB in cephalosporins and tetracycline resistance (9, 11, 15). The parallels with gonococcal PorB1b with respect to antibiotic resistance are striking. Knocking out PorB expression also decreased meningococcal susceptibility to doxycycline, one of the 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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