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GUEST COMMENTARY

Towards an Understanding of Chromosomally Mediated Penicillin Resistance in *Neisseria gonorrhoeae*: Evidence for a Porin-Efflux Pump Collaboration†

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In this issue of the *Journal of Bacteriology*, Olesky et al. (13) report a novel observation regarding the mechanism by which *Neisseria gonorrhoeae* developed clinically significant levels of resistance to penicillin. Although yet to be fully defined, their results link changes in the structure of a gonococcal porin (PorB), which was presumed to modulate permeation of penicillin due to precedents set by studies with porins from *Enterobacteriaceae* (1, 11), with overexpression of a multidrug efflux pump and the development of penicillin resistance in gonococci. The implications of their work for further advancing our knowledge regarding the structure-function relationships of the gram-negative cell envelope, differences between such bacteria in this respect, and the connection of efflux pumps with other cell envelope proteins in the development of antibiotic resistance are substantial. Moreover, the findings justify continued research on basic problems of antibiotic resistance even when the antibiotic in question is no longer used clinically to treat the disease in question.

Historical review of chromosomally mediated penicillin resistance in gonococci. The introduction of antibiotics in general and penicillin specifically as a means to treat bacterial infections is arguably one of the greatest advances in modern medicine. Unfortunately, soon after its introduction, certain pathogens (e.g., *Staphylococcus aureus*) were noted to have quickly developed resistance to penicillin due to their production of penicillinase. Other infectious diseases, such as gonorrhea, remained treatable with the relatively inexpensive penicillin G for several years. With respect to *N. gonorrhoeae*, strains expressing clinically significant levels of penicillin resistance emerged slowly. However, by the late 1960s and early 1970s, the peak of the gonorrhea epidemic in the United States, isolates were identified that displayed decreased susceptibility to penicillin. Studies in the 1970s (6, 7, 16) and 1980s (2, 3, 17) showed that these strains contained chromosomally borne mutations that could additively increase resistance of gonococci to penicillin to a level approaching or at clinical significance (e.g., treatment failures). It is important to stress that these strains did not produce detectable penicillinase, although other (comparatively rare) strains bearing a plasmid encoding a TEM-1-type beta-lactamase were identified in the mid-1970s (15).

With the report in 1985 (3) of a community-based outbreak of penicillin-resistant gonorrhea due to a strain not producing a beta-lactamase, the final blow to penicillin therapy for treatment of this sexually transmitted infection was, unfortunately, realized. The culprit strain (FA6140 [3]) from this outbreak contained (12) a number of chromosomal mutations (*penA*, *penB*, *ponA*, and *mtr*) that are known to alter cell envelope structure and/or function. In general terms, these mutations impact penicillin's accumulation in gonococci (*penB* and *mtr*) or affinity (*penA* and *ponA*) for penicillin-binding proteins; this commentary will be restricted to issues related to *penB* and *mtr*. The *penB* mutation was originally linked (7) to production of an altered major outer membrane protein (termed POMP or protein I) and was found to confer two- to fourfold increases in MIC levels of penicillin and tetracycline. Curiously, phenotypic expression of *penB* required the presence of the *mtr* mutation, which was found to confer single-step resistance to structurally diverse hydrophobic antimicrobial agents (10) and was presumed to decrease cell envelope permeability to such agents (6).

Present knowledge regarding *penB* and *mtr*. Considerable research over the past 30 years, of which space does not permit an adequate review, has shown that expression of *penB* results in amino acid replacements at position 120 alone (G120K) or positions 120 and 121 (G120D/A121D) of PorB, while *mtr* is related to a single-base-pair deletion in the promoter that drives transcription of the gene (*mtrR*) that encodes a repressor (14) of the *mtrCDE*-encoded efflux pump operon; this promoter mutation abrogates expression of *mtrR* and, as a consequence, enhances *mtrCDE* expression (8). The amino acid replacements in PorB associated with *penB* are within loop 3 of this porin and were previously suggested to impact penicillin and tetracycline entry into gonococci (5). The inference that permitted the development of this model had its root in reports of other studies (1, 11) that used porins from *Enterobacteriaceae*. Results from these studies associated similar amino acid replacements, also positioned within loop 3, with significant changes in

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† This commentary is dedicated to memory of the late Alice E. Shafer who, as a nurse in the United Kingdom during World War II, was one of the first health care providers to administer penicillin to patients with infected wounds and told W.M.S. countless stories regarding the miracle of penicillin.

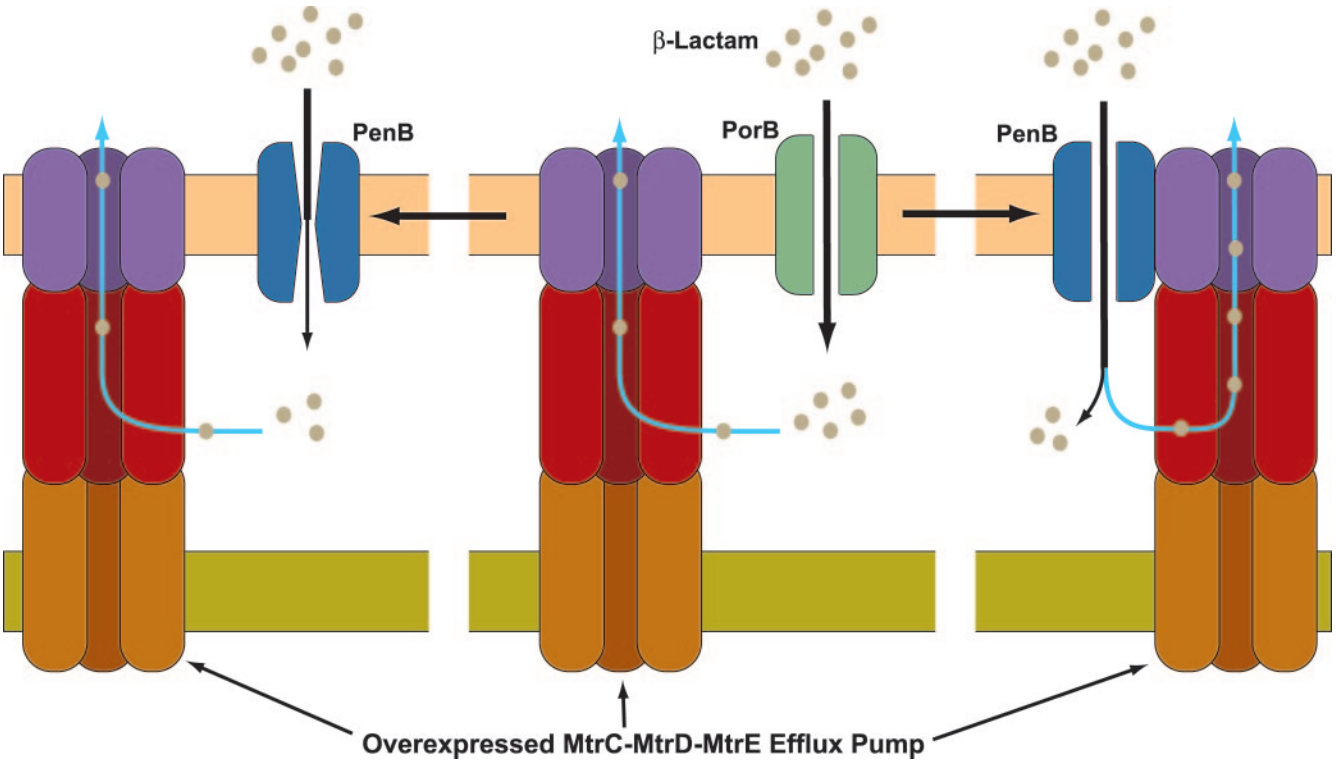

FIG. 1. Shown are possible mechanisms by which expression of *penB* in an *mtrR* mutant, which overexpresses the *mtrCDE*-encoded efflux pump, enhances resistance of gonococci to penicillin. In an *mtrR* mutant producing wild-type PorB (light green), permeation of penicillin is at wild-type levels. In this case, gonococcal susceptibility levels to penicillin, in the absence of other chromosomal mutations (e.g., *penA*) or the presence of beta-lactamase, are dictated by recognition of periplasmically located penicillin by the MtrC-MtrD-MtrE efflux pump (shown as a tripartite structure spanning the entire cell envelope). However, in the presence of *penB*, the altered PorB (PenB; shown in blue) can have a small impact on penicillin permeation (left and right sides of the figure). PenB and the MtrC-MtrD-MtrE pump may physically interact (right side of figure), and this close association may allow efficient penicillin efflux even though permeation rates are unaffected. Another possibility is that PenB and MtrC-MtrD-MtrE do not interact but that the permeation rates are slightly altered, and the pump is able to magnify this difference to decrease the levels of penicillin in the periplasm (left side of the figure). In either case, overexpression of the efflux pump (due to *mtrR* mutations) is required in order for *penB* to increase resistance.

pore size, ion selectivity, and/or antibiotic entry, all of which would impact levels of bacterial resistance to beta-lactams. A simple explanation, yes, but the findings of Olesky et al. (13) strongly suggest that it is not applicable to explain how amino acid replacements in loop 3 of gonococcal PorB increase levels of penicillin resistance in gonococci.

Using purified native and recombinant wild-type or mutant PorB preparations in planar lipid bilayer experiments to measure electrophysiological properties of the different PorB proteins, Olesky et al. (13) discovered that the mutant porins, unlike wild-type PorB, were largely in the subconductance state. However, this could not be translated to changes in ion selectivity, pore size, or antibiotic permeation. In whole bacteria, a single amino acid replacement at position 120 (G120K) in PorB impacted levels of beta-lactam accumulation only in the presence of a coresident *mtrR* mutation.

One model replaces another. Since PorB, and its allelic form PorA, is essential for gonococcal viability, it is not possible to construct null mutants to directly test the functional consequences of *penB* mutations. However, the model that the effect of *penB* requires not only the presence of functional MtrC-MtrD-MtrE but also its overexpression due to a coresident *mtrR* mutation is supported by the work of Veal et al. (18). In the present study, a single point mutation (D405N) in the gene encoding the cytoplasmic membrane transporter (MtrD) of the efflux pump was found to significantly increase the susceptibility of gonococci to penicillin despite the presence of *penB* and *mtrR* mutations. This finding and the observation that wild-type levels of MtrC-MtrD-MtrE do not confer increased resistance of gonococci to penicillin even in the presence of *penB* suggest that a collaboration exists between the consequences of *mtrR* and *penB* mutations and that this collaboration is essential for chromosomally mediated resistance.

What might this collaboration be? One scenario (Fig. 1) is that the altered form of PorB (PenB) and MtrC-MtrD-MtrE physically interact, and that even though antibiotic permeation is not affected, the close association of the two proteins allows the efflux pump to efficiently remove penicillin entering through PenB from the periplasm. A second possibility is that the mutant porin has a small change in antibiotic permeation relative to the wild type and that this small decrease is amplified by the increased levels of the efflux pump (Fig. 1). An additional hypothesis, not advanced by Olesky et al., (13) is that MtrR regulates other genes involved in determining levels of penicillin resistance, independent of or dependent on a change in PorB functional status.

The first issue that needs to be resolved is whether PorB and MtrC-MtrD-MtrE physically interact, and immuno-colocalization studies might help in this determination. If this is the case, a genetic approach that seeks to identify mutations that impact this interaction may help to correlate physical association with phenotype. As is emphasized by the authors, a three-dimensional structural model for PorB is needed for understanding how loop 3 mutations change PorB function, and knowing the location of residues 120 and 121 is essential. Since missense mutations at 120 and/or 121 do not alter a number of PorB properties (see above), they may not line the channel. Rather, as suggested by Olesky et al. (13), residues 120 and 121 may face the outer wall of the pore or even face outside. Such information, along with results from additional biophysical studies, should help to determine if the gating action of PorB can be modified by amino acid replacements in loop 3. With respect to their model, it will also be important to know whether enhanced levels of MtrC-MtrD-MtrE modify PorB gating properties, and a system that permits transient manipulation of efflux pump levels may help in this determination.

It is now clear that MtrR can regulate genes other than *mtrCDE* (4, 9), and a complete understanding of the *mtrR* regulon should help in testing the model described above. This is particularly true if MtrR-regulated genes are important in determining levels of antibiotic resistance through a *penB*-dependent process; there is no evidence, however, that expression of *mtrR* influences levels of PorB (or the allelic PorA). In conclusion, it is important to stress that continued research on antibiotic resistance has significance for advancing not only our knowledge regarding how microbes, like gonococci, developed ways to subvert the action of antimicrobials but also, perhaps more importantly, this line of research can provide novel approaches to furthering our understanding on basic properties of bacteria. The study reported by Olesky et al. (13) provides us with reason to think more deeply about these issues.

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