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Elucidating the balance between essential transpeptidase activity and antibiotic resistance in N. gonorrhoeae Penicillin-Binding Protein 2

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Background

H041 is a multidrug-resistant N. gonorrhoeae strain that was the first isolate fully resistant to ceftriaxone, the only remaining recommended antibiotic for gonorrhea. In N. gonorrhoeae, ceftriaxone targets Penicillin-Binding Protein 2 (PBP2), an essential peptidoglycan transpeptidase (TPase) that functions in cell division. PBP2 from H041 has undergone extensive recombination with non-pathogenic Neisseria species and has more than 60 mutations compared to wild-type PBP2, but it is still able to maintain its essential function. We have identified eight mutations that, when introduced into wild-type PBP2, confer ~80% of the resistance of

PBP2(H041) to ceftriaxone, but there is minimal data on how these mutations impact the native TPase activity of PBP2. PBP2 is a class B PBP and requires a partner transglycosylase (TGase) for full activity. From work in Pseudomonas aeruginosa, FtsW from N. gonorrhoeae was identified as the TGase that functions with gonococcal PBP2 to synthesize peptidoglycan. We predict that mutations in PBP2 that significantly increase resistance to ceftriaxone will proportionally hinder TPase activity, although some mutations may be more deleterious than others.

Aim/Methods

We are currently investigating the effects of these eight H041 mutations, individually and in groups, on PBP2 through an in vitro TPase assay. We are also assessing the influence of these individual and grouped mutations on N. gonorrhoeae strain MIC, growth, and fitness in both FA19 (antibiotic-susceptible) and H041 (ceftriaxone-resistant) strains.

Results

For assessing PBP2 TPase activity in vitro, purified full-length, membrane-associated PBP2 and the integral membrane protein FtsW are required. Purifying FtsW independently resulted in degraded, aggregated protein; however, upon coexpression and copurification of both PBP2 and FtsW, an active complex formed. For future TPase activity comparisons, there should a one-to-one ratio of PBP2 to FtsW. Thus, a fusion of FtsW-PBP2 as one peptide chain was expressed and purified for use in future assays. We also have recently purified large amounts of the peptidoglycan precursor lipid II from E. coli.

Conclusions

With purified PBP2, FtsW, and lipid II in hand, we are poised to complete in vitro TPase assays and assess the effects of resistance-conferring mutations on TPase activity of PBP2.