Is Antibiotic Therapy Driving Selection for Gonococci that Fail to induce Symptomatic infection, L-C Wang, J Qiu, Q Yu, W Song, and DC Stein*, University of Maryland, College Park, MD USA

Abstract:

Background: *Neisseria gonorrhoeae* (GC) colonizes and infects the human genital tract as well as rectal and pharyngeal mucosal tissues in both men and women. Subclinical infections are becoming more common with asymptomatic rates as high as 56% in men and 80% in women. Meanwhile, antibiotic resistant GC has become an emerging threat worldwide. The relationship between increases in antibiotic resistance and asymptomatic infection has not been explored.

Methods: We used standard MIC testing and a bacter assay to measure the antibiotic susceptibility. Confocal microscopy was used to visualize and analyze the distribution of bacteria that survived ceftriaxone treatment and bacteria-bacteria interactions, as well as biofilm formation on abiotic and biotic surfaces. Lastly, we used electron microscopy to study bacteria-host cell interactions.

Results: We found a relationship between the ability of a strain to form aggregates and their antibiotic susceptibility. Aggregated GC have a higher survival rate than non-aggregated GC when treated with ceftriaxone. Viable bacteria locate in the core of the aggregates. The lack of opacity-associated protein (Opa) or pili, or expression of a truncated lipooligosaccharide, three surface molecules that mediate GC-GC interactions, reduce both GC aggregation and ceftriaxone survival. In addition, Opa expressing strains form aggregates that colonize more efficiently but are less able to penetrate into polarized human epithelial monolayers and the human cervical epithelial in a tissue explant model.

Conclusions: This study demonstrates that the aggregation of *N. gonorrhoeae* can reduce the susceptibility to antibiotics, and suggests that antibiotic utilization can select for surface molecules that promote aggregation, therefore driving pathogen evolution for better colonization but with reduced invasive capabilities.

Lessons Learned: Inhibiting aggregation may be a potential way of increasing the efficacy of antibiotic treatment, consequently reducing treatment failure.

Introduction:

Gonorrhea, caused by *Neisseria gonorrhoeae* (GC), is the second most commonly reported sexually transmitted infection in the United States. GC colonizes and infects the human genital tract but can also infect rectal and pharyngeal mucosal tissue in both men and women [2]. Symptoms include painful urination, genital pain, and abnormal discharge. Asymptomatic infections cause extended colonization without treatment. The emerging threat of cephalosporin resistance, in combination with subclinical gonorrhea, highlights the need for further understanding gonorrhea treatment failures.

Figure 1. Comparison of survival rate and distribution in GC aggregates that lack Opa or Pili or express truncated LOS under ceftriaxone treatment.

GC were allowed to aggregate for 6 h incubated with 1 μg/ml ceftriaxone (+CFX) stained with Live/Dead BacLight stain for visualizing live (Green) and dead (Red) GC, and analyzed using a confocal fluorescence microscope.

Figure 2. Survival and distribution of GC aggregates on cervical epithelial cells

GC were with ME180 cells for 6 h. (a) +1 μg/ml ceftriaxone (+CFX) or mock treated (-CFX) for 2 h, stained with Live/Dead BacLight stain for visualizing live (Green) and dead (Red) GC, and analyzed using a confocal fluorescence microscope.

Figure 3. Survival rate and distribution of pre-aggregated MS11Opa+Pil+ GC under ceftriaxone treatment.

GC were inoculated in a 96-well plate and allowed to aggregate. Various concentrations of ceftriaxone was added into different aggregation condition and incubated for 24 h. (a) Bacter assay (b) viable plate count or (c) microscopic analysis.

Working model

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