Modulation of conjugation in pathogenic *Escherichia coli*

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Abstract — **As resistance outpaces new antibiotics, evolutionary mechanisms like horizontal gene transfer (HGT) become targets for resistance prevention and reversal therapies. Discrete modulation of HGT is difficult to quantify, however, because unequal fitness across mixed populations confounds HGT with selection dynamics. To address this, we present a high-throughput assay that uses time to optical density threshold for conjugation quantification. We apply this method to study antibiotic modulation in pathogenic *E. coli* strains collected from patient cultures. In contrast with previous literature, we find that sublethal antibiotics exert little to no effect on conjugation, although exceptions with biologically significant modulation may exist.**

Keywords — **Horizontal gene transfer, Conjugation, Antibiotic, Resistance**

I. Introduction

Horizontal gene transfer (HGT) is a fundamental process by which bacteria adapt to their environment. It is especially pertinent in recent decades, with sublethal antibiotic gradients permeating throughout diverse environments and selecting for antibiotic resistance genes (ARGs) [1]. Conjugation, the direct transfer of genetic material between a donor and recipient, has drawn particular attention for its ability to spread ARGs due to broad host range, high rates of transfer, and close association of ARGs with plasmids [2]. In certain cases, environmental stimuli appear to influence HGT [4]. Antibiotics may have a similar effect—accelerating their own demise by promoting the spread of ARGs.

Most antibiotics are natural in origin, multifaceted in purpose, and bacteria have been exposed to them for millions of years prior to human use [4]. Past studies have generally concluded that antibiotics can promote horizontal gene transfer [1]. However, selection dynamics may have overshadowed any true modulation of conjugation [5]. Moving forward, a framework for accurate and high-throughput quantification is needed to understand modulation of conjugation across a wide variety of conditions.

II. Results

In conjugation, three cell populations are at play: plasmid donors (D), recipients (R), and transconjugants (T). We define the conjugation rate (η_c) as:

$$\eta_C = \frac{T}{DR\Delta t}$$

with transconjugants being the critical measurement. To decouple selection from conjugation dynamics, we prevent growth during a limited conjugation period. This restricts conjugation, reduces transconjugants, and makes cell quantification in mixed culture more difficult.

We resolve these issues by taking advantage of exponential bacterial growth:

$$T_C = T_0 e^{\mu t}$$

where the time (t) it takes to reach an optical density threshold (T_c) is proportional the initial inoculum (T_0). A small initial population of transconjugants can therefore be amplified and easily quantified with corrections for further conjugation.

As a clinically relevant model, we characterized 219 pathogenic *E. coli* strains isolated from bloodstream infections. Of these, 65% displayed extended spectrum beta-lactamase resistance and 24% were able to transfer it to recipient *E. coli*. Using beta-lactamase as a marker for conjugation, we then tested for antibiotic modulation of conjugation with five classes of antibiotics at three sublethal concentrations. By and large, antibiotics exerted no significant effect on conjugation rates, although exceptions may exist. We follow-up on significant effects with whole-genome long-read sequencing capable of capturing difficult mobile elements and shedding light on potential mechanisms for modulation.

III. Conclusion

Identifying and understanding factors that modulate conjugation is key for addressing issues of bacterial evolution, notably antibiotic resistance. To this end, the time to threshold method enables high-throughput, selection-decoupled conjugation quantification in natural populations.

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