Microscopic and macroscopic characterization of diet-induced adaptive antimicrobials resistance in gut microbiota swarmers

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Introduction

- one of the key strategies for bacteria to develop resistance/tolerance to antibiotics is to change their shape and physiology status in order to migrate collectively, a well-known phenomenon called **bacterial swarming**, one of the **adaptive antimicrobial resistance (AAR).**
- Bacterial swarming is characterazed by greater cell length, hyperflagellation, mutilplooidy. It is commonly observed in many human pathogens, including *Pseudomonas*, *Escherichia*. Intriguingly, **swarmers have an elevated resistance to antibiotics**. For example, *Salmonella* swarmers are more resistant to plolymyxins and aminoglycosides whereas *E. coli* and *P. aeruginosa* swarmers had higher resistance to tobramycin and ciprofloxacin.

Fig 1. Antibiotic resistance is associated with multicellular mobility (e.g. swarming) (INDEPTH Network website).

- However, the mechanisms underpinning swarming-enhanced antibiotic resistance are still under debate between two main hypotheses; One hypothesized that **changes of outer membrane permeability and modification of lipopolysaccharides (LPS)** are mainly responsible, whereas the other stated that **higher cell density and cell mobility (speed of swarming) are crucial** for protecting swarmers against antibiotics. Recent published work has largely concentrated on population-level response (i.e. treating swarming cells as a uniformed population) to antibiotics and neither of these hypotheses has been directly tested at subpopulation (micrometer scale resolution on swarming plates) or singe-cell level.

- In a recent paper that I co-authored, I demonstrated that **choline metabolism significantly increased swarming speed** of the model bacterium, *Proteus mirabilis* **(Fig 2)** (Jameson *et al.*, 2015) and **swarmer cells have an elevated resistance to antibiotics (Fig 3)** (Pearson *et al.*, 2010).

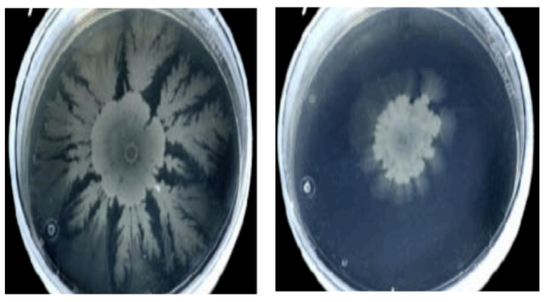

Fig 2. Choline promotes anaerobic swarming of *Proteus mirabilis*. Left, glycerol + choline; right, glycerol only. Photos were taken after 10 days incubation at 30°C.

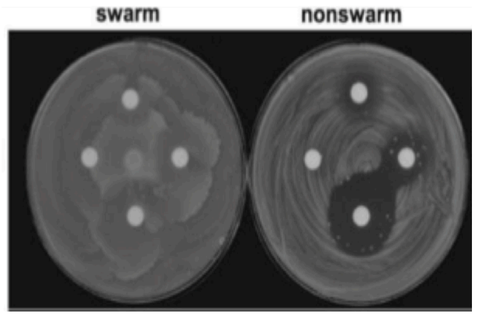

Fig. 3 Elevated antibiotic resistance to chloramphenicol (chl) in *P. mirabilis* swarming cells. On both plates, discs infused with (clockwise) 2, 10, 20, 50 µg/ml chl were placed on agar plates and zone of inhibition visible on non swarming plates.

Project aim:

To investigate the role of choline metabolism enhanced swarming on antimicrobial resistance, using *Proteus mirabilis* as the model, and test the hypothesis that choline enhances swarming speed and thus elevated resistance to antibiotics.

Results

- Anaerobic swarming assay against different antibiotics

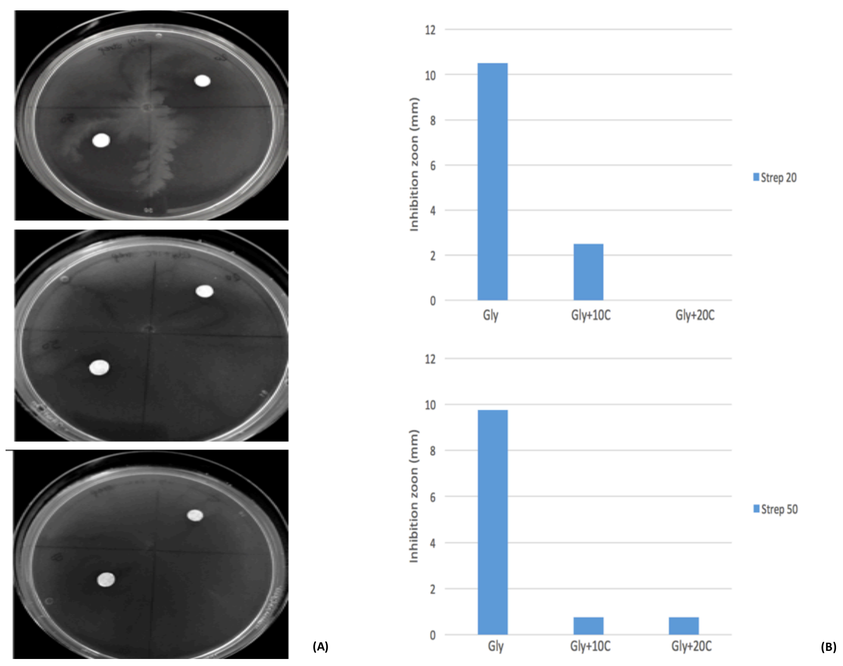

Figure 4. (A) Swarming assay with Streptomycin at concentrations of 20 µg ml^{-1} and 50 µg ml^{-1}, upper: 10 mM glycerol, middle: 10 mM glycerol+10 mM choline, lower: 10 mM glycerol+20 mM choline. **(B)** Bar charts shows decreased Streptomycin resistance of *P. mirabilis* with the increased amount of additional choline.

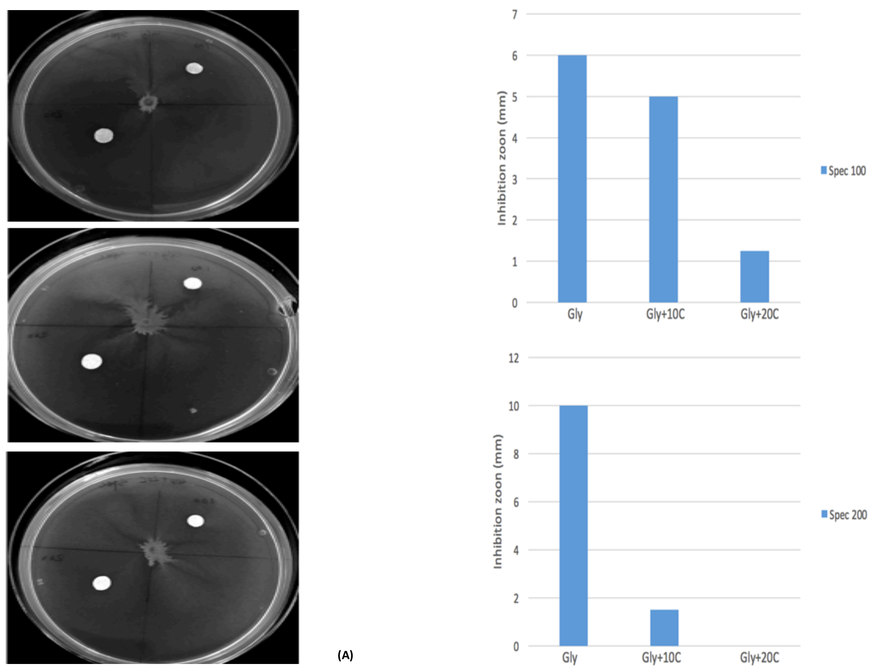

Figure 5. (A) Swarming assay with Spectinomycin at concentrations of 100 µg ml^{-1} and 200 µg ml^{-1}, upper: 10 mM glycerol, middle: 10 mM glycerol+10 mM choline, lower: 10 mM glycerol+20 mM choline. **(B)** Bar charts shows decreased Spectinomycin resistance of *P. mirabilis* with the increased amount of additional choline.

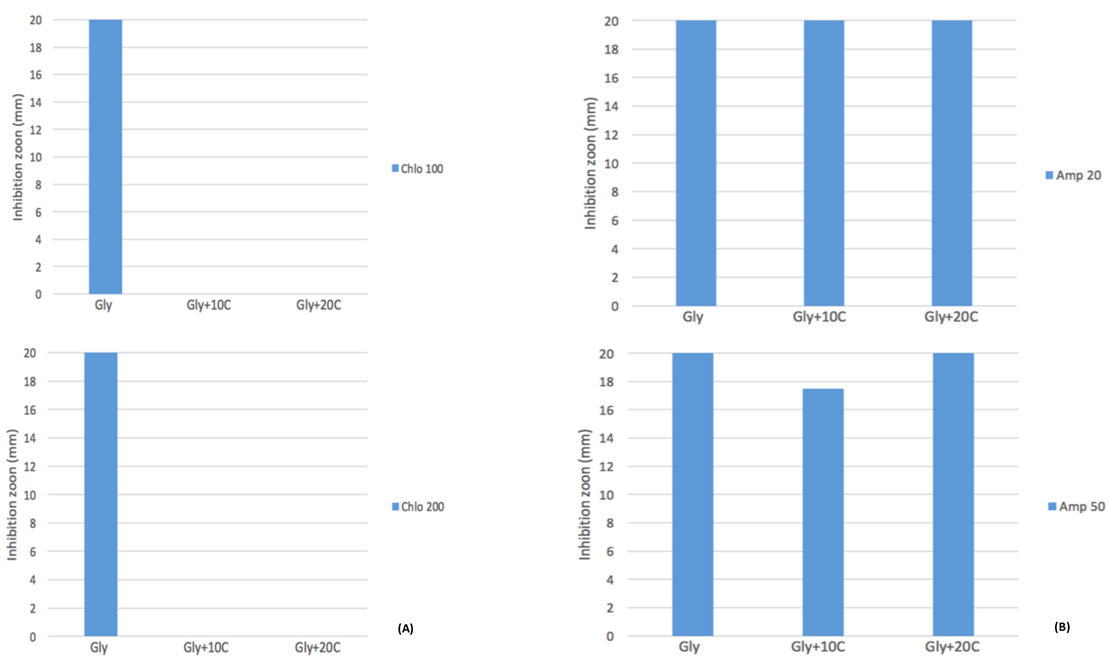

Figure 6. (A) Swarming assay with Chloramphenicol at concentrations of 100 µg ml^{-1} and 200 µg ml^{-1}. The two high concentrations of Chloramphenicol prevent *P. mirabilis* swarming on plates with glycerol alone, but there is no inhibition on choline addition plates **(B)** Swarming assay with Ampicillin at concentrations of 20 µg ml^{-1} and 50 µg ml^{-1}. *P. mirabilis* swarming cells didn't grow on ampicillin plates, even supplemented with additional choline.

Discussions

As can be seen from **Fig 4&5**, swarming plates supplemented with additional choline enhanced the swarming-related colony expansion of *P. mirabilis*, and swarming cells shows enhanced antibiotic resistance in response to increased additional choline.

It has been reported that Chloramphenicol interferes with protein synthesis by binding to the 50S subunit of the ribosome. In the presence of Chloramphenicol and in the absence of protein synthesis, *P. mirabilis* is be able to swarm more rapidly (Pearson *et al.* 2010). Supplying with high concentrations of Chloramphenicol inhibits swarming expansion on glycerol alone plates, but additional choline enhances the swarming ability to against it **(Fig6A)**. Ampicillin shows significant inhibition on *P. mirabilis* swarming with or without choline **(Fig6B)**.

Further work

- To investigate if choline enhanced swarming is associated with antibiotic resistance. Swarming assay will be repeated. The shape of swarming front and swarming velocity filed will be quantified against antibiotics (distribution of velocities and correlations in space and time).
- To investigate the macroscopic response of swarming cells on agar plated with/without choline. The population dynamics of swarming will be obtained by filming of fresh-inoculated plate for a few days using DSLR camera.
- To investigate the microscopic response of swarming cells on agar plated with/without choline. The swarming speed of individual cells and direction in response to antibiotics by phase-contrast microscopy .

Reference:

- INDEPTH Network website: http://www.indepth-network.org/groups/working-groups.
- Jameson, E., Fu, T., Brown, I.R., Paszkiewicz, K., Purdy, K.J., Frank, S., and Chen, Y. (2015) Anaerobic choline metabolism in microcompartments promotes growth and swarming of *Proteus mirabilis*. *Environ Microbiol* **18:** 2886-2898.
- Pearson, M.M., Rasko, D.A., Smith, S.N. and Mobley, H.L.T. (2010) Transcriptome of swarming *Proteus mirabilis*. *Infect Immun.* **78:** 2834-2845.