S1 Text: To study antibiotic resistance, we have built customized bioreactors that are able to maintain a continuous culture at its fastest growth rate (a type of turbidostat) and favor the formation of long-term biofilm communities. While technically very challenging, this approach can provide advantages over using more traditional techniques, such as batch cultures (for example serial transfers or chemostats). Unlike batch cultures, the bioreactor maintains a continuous culture at a controlled concentration of antibiotic so that the ratio of cells to drug is controlled. TGC is also hydrolyzed more rapidly than many antibiotics further diminishing the utility of batch culture or serial transfer approaches. In addition, the population does not enter stationary phase. Unlike a chemostat, which maintains growth rates by limiting an essential nutrient, the population in the bioreactor is kept at its fastest growth rate by adjusting the in-flow of media in response to changes in cell density and respiration. Therefore, the bioreactor removes the selective pressure of nutrient limitation so that the population adapts to the stress of the antibiotic. Also, with a chemostat the addition of media is continuous, which could potentially bottleneck the population when growth is slowed by the stress of antibiotic. Since the in-flow of media into our bioreactor is dynamically changed in response to the growth rate of the culture, the possibly of bottlenecking the population is greatly reduced. Furthermore, our bioreactor allows for the persistence of very long-term biofilms, a trait shown to play an essential role in the pathogenicity of enterococci. By allowing biofilms to be established on the interior walls of the bioreactor during adaptation we can recapitulate some aspects of the clinical ecologies associated with the development of endocarditis or catheter colonization [15]. Overall, this means that the population has a more consistent, controllable and clinically relevant ecology. Also, previous studies we have conducted found that clinically relevant mechanisms of resistance can be identified using this setup [13]. Finally, while the technical challenges of this setup limit replication, there are several advantages that allow us to make robust predictions. Firstly, we are able to maintain genetically polymorphic populations (**S1-S5 Tables**), which indicate that our setup allows for multiple adaptive trajectories to be achieved in an individual

population. In addition, we observed similar trajectories across the two replicate populations indicating that the observed mutations can easily occur.