Evaluation of Ampicillin, Ciprofloxacin and Fosfomycin Sequential Antibiotic Treatment Regimen Against *Escherichia Coli* CFT073 in the Hollow Fiber Infection Model

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Abstract

Antibiotic resistance among gram-negative bacterial pathogens causes widespread morbidity and mortality worldwide and has resulted in limited therapeutic options. To address this concern, global research efforts are underway to develop new antibiotics, non-antibiotic molecules, as well as modifying or repurposing of existing antibiotics. Our previous work on Escherichia coli CFT073 in the hollow fiber infection model (HFIM) with single, double and triple simultaneous combination antibiotic treatment regimens with ampicillin, ciprofloxacin and fosfomycin antibiotics showed that double and triple simultaneous combination regimens significantly delayed resistance subpopulations compared to monotherapies (N. Garimella et al., Int J Antimicrob Agents, 2020, 55(4):105861*). To follow up this work, we explored a sequential antibiotic treatment strategy, in which the three antibiotics were administered individually in a sequence of ampicillin, ciprofloxacin and fosfomycin and continued this sequence for up to 10 days post-infection. We studied the effect on total and resistant subpopulations evolution at 3X, 10X and 30X minimum inhibitory concentration (MIC) levels in the HFIM. The sequential triple antibiotic's regimen resulted in the appearance of single, double and triple antibiotic resistant subpopulations as early as in the sequence of antibiotics administered. The sequential triple antibiotic's treatment regimen had less effect on delaying the emergence of resistant subpopulations compared to a simultaneous triple antibiotic combination treatment regimen.

Introduction

Antibiotics are used to treat bacterial diseases and their efficacy is endangered due to rapid emergence of resistant bacteria worldwide. This has made conventional antibiotics less effective in treating infections caused by gram-positive and gram-negative bacteria. Due to the growing need to combat this antibiotic resistance crisis, it is imperative to reconsider the monotherapy standard and actively explore combination therapies of existing antibiotics. To study the synergy with antibiotic combinations, recently the hollow fiber infection model (HFIM) has become an important tool in exploring the reasonable use of antibiotics and its relation to antibiotic resistance development. Our previous study with antibiotic combinations showed that simultaneous double or triple antibiotic combinations significantly delayed the emergence of resistant bacteria compared to single antibiotic treatments. In the present study, we explored a sequential antibiotic treatment strategy, in which ampicillin, ciprofloxacin and fosfomycin antibiotics were administered individually in a sequence and repeated this sequence for up to 10 days and studied its effect on total and resistant subpopulation evolution.

Materials and Methods

Microorganisms, antibiotics, media and agar

The clinical Escherichia coli strain CFT073-WAM4505 (ATCC BAA-2503) purchased from ATCC (Manassas, VA). Ampicillin sodium salt, ciprofloxacin hydrochloride and fosfomycin disodium salt were obtained from Sigma-Aldrich, St. Louis, MO. LB broth and agar were obtained from bioWORLD (Dublin, OH, USA). Polysulfone cartridges (C2011, FiberCell Systems Inc., Frederick, MD, USA) were used in the HFIM system.
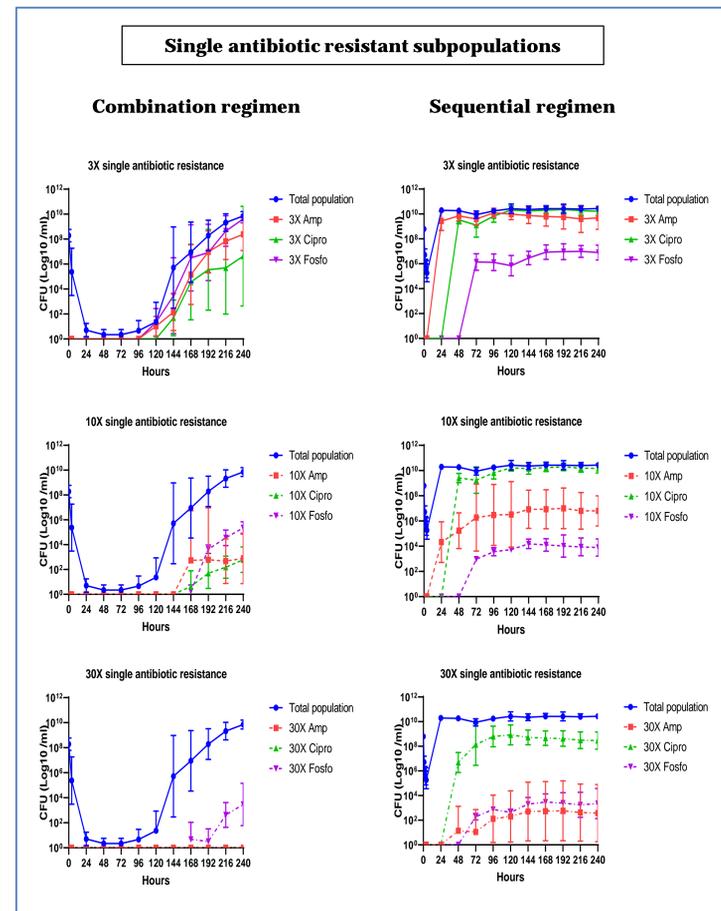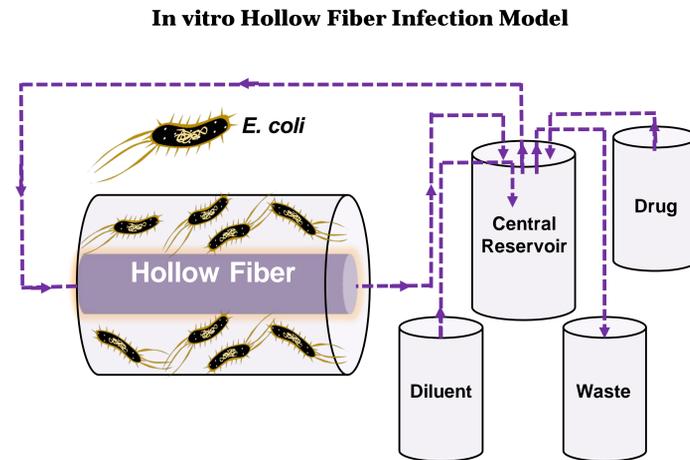
In vitro Hollow Fiber Infection Model

Single antibiotic resistant subpopulations

Figure 1. Single antibiotic resistant subpopulations at 3x, 10x and 30x MIC levels were shown as geometric mean ± geometric standard error from three independent hollow fiber experiments.

Double antibiotic resistant subpopulations

Figure 2. Double antibiotic resistant subpopulations at 3x, 10x and 30x MIC levels were shown as geometric mean ± geometric standard error from three independent hollow fiber experiments.

Results and Discussion

Simultaneous triple antibiotic combinations resulted in sustained killing of total bacterial population until 96 h post treatment. Analysis of antibiotic resistant subpopulations showed that triple combinations significantly delayed the emergence of resistant subpopulations and only single antibiotic resistant populations at all MIC levels and double antibiotic resistant populations at 3x MIC level were present. No other resistant subpopulations were observed for double and triple antibiotic combinations.

Sequential triple antibiotic treatment regimen had no effect on total bacterial population until 10 days post sequential treatment. Single antibiotic resistant subpopulations for all three antibiotics appeared rapidly at 3x, 10x and 30x MIC levels. Double antibiotic resistant subpopulations appeared after 24 h of second antibiotic treatment at 3x and 10x MIC level only. Triple antibiotic resistant subpopulations appeared on 96 h post treatment and only at the 3x MIC level.
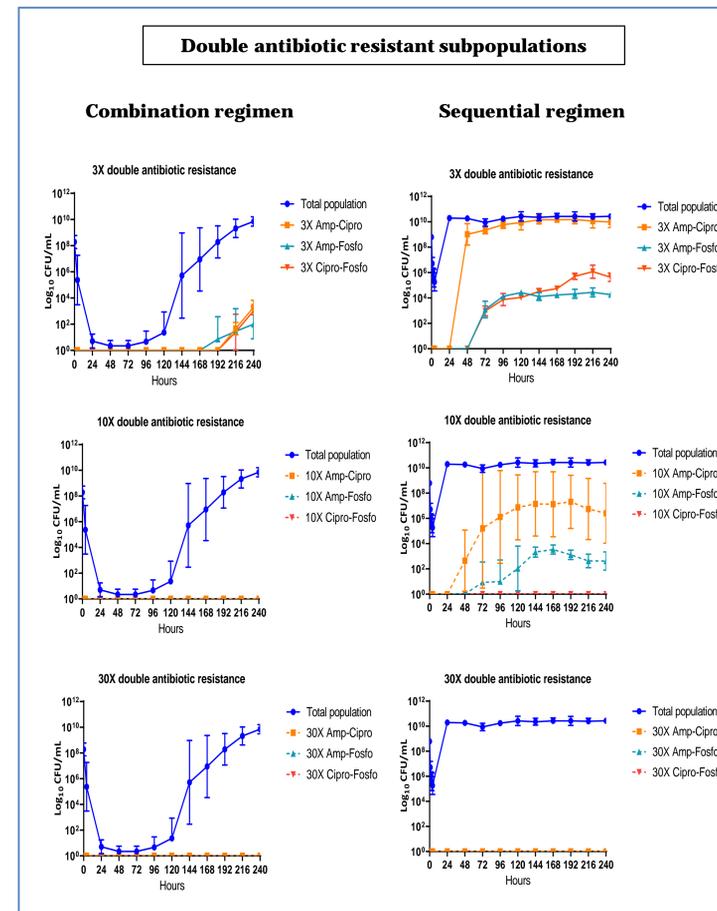
Triple antibiotic resistant subpopulations

Figure 3. Triple antibiotic resistant subpopulations at 3x, 10x and 30x MIC levels were shown as geometric mean ± geometric standard error from three independent hollow fiber experiments.
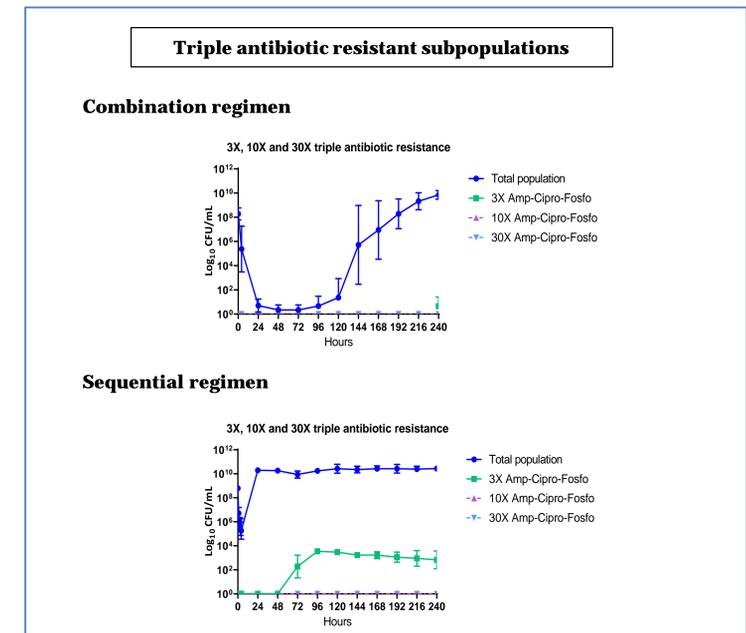
Conclusion

➢ Our findings between simultaneous and sequential treatment regimens show that administration of antibiotic combinations proves to be superior to sequential antibiotic administration, in reducing the development of resistance.

➢ The difference observed between these two treatment regimens may be due to several factors, such as different mechanisms of action of each drug administered, the sequence of drugs administered, time interval between each antibiotic administration, and the phenotypic and genotypic status of bacterial cells at the time of each antibiotic administration.

➢ The resistance pattern observed with the sequential therapy (ampicillin-ciprofloxacin-fosfomycin) in this study may be different if other sequences of sequential antibiotic treatment regimens of the same antibiotics were followed.

➢ Whole genome sequencing of the resistant subpopulations might provide a clearer picture of the genetic and phenotypic changes which led to the development of multi-drug resistance in combination as well as sequential drug regimens.

* Narayana Garimella, Tesfalem Zere, Neil Hartman, Adarsh Gandhi, Aschalew Bekele, Xianbin Li, Heather Stone, Leonard Sacks and James L Weaver. Effect of drug combinations on the kinetics of antibiotic resistance emergence in Escherichia coli CFT073 using an in vitro hollow-fiber infection model. Int J Antimicrob Agents, 2020 Apr;55(4):105861.