00451 Bacterial Persistence Facilitates Development of Antibiotic Resistance.

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Aims: To study role of residual "persister" bacteria that continue to survive in the presence of elevated antibiotic concentrations in development of antibiotic "resistance".

Methodology: Pseudomonas aeruginosa (PAO1) late exponential phase cultures were treated with therapeutically achievable concentrations of different antibiotics for 24 h at 370C. After incubation, culture suspensions were centrifuged, washed and suspended in phosphate buffered saline. Viable bacteria were determined for pre- and post-treatment cultures using standard methods and kinetic reads were captured at 600nm.

For invasion assay, persisters collected after besifloxacin (5 µg ml^{-1}) and amikacin (100 µg ml^{-1}) treatment were used for infecting human conjunctiva cells (IOBA-NHC), for 2 h. Invading bacteria were enumerated by culture methods and visualized using confocal microscopy.

Evolution of resistant bacteria from persisters was studied under controlled experimental conditions. Persister cells were maintained in presence of clinically achievable concentrations of antibiotics for 5 days. On different days, culture aliquots were subjected to selection on solid media. Minimum inhibitory concentrations (MICs) were also compared to parent strain.

Result: Bi-phasic kill curves were obtained against all antibiotics except polymyxin B. MICs for persister colonies were similar to parent strain. Extended lag phase was observed for residual cells obtained after polymyxin B and besifloxacin treatment with a delay of 4.98 and 5.12 h respectively. The invading percentage for PAO1 and persister cells harvested after exposure to besifloxacin and amikacin was 0.45 ± 0.15, 0.16 ± 0.07 and 0.14± 0.06 respectively. The antibiotic resistance for selected colonies was class specific, as colonies selected against besifloxacin showed up to 16-fold higher MIC values against all fluoroquinolones Similarly, colonies selected against polymyxin B showed up to 8-fold higher MICs against polymyxin B and colistin.

Conclusion: Our data suggest that persisters, in addition to survival advantage, continue to invade mammalian cells and also lead to the emergence of antibiotic resistance.