Bacterial tolerance to antimicrobials - factors affecting treatment success in vitro and in vivo

During evolution bacteria has established a multitude of mechanisms to overcome environmental stressors. Working with beta-hemolytic streptococci (BHS) isolated from the endometrium of problem mares, we identified the presence of small colony variants (SCV). SCV typically only appears following normal incubation conditions (37 C) on blood agar plates for 72 to 96 hrs and will thus be missed during most routine procedures. We have also observed the phenotypic variant "persister" cells after antimicrobial exposure (Figure 1). As opposed to BHS, persister cells are characterized by a remarkable tolerance to antimicrobials including penicillins. When BHS enters the persister or dormant state tolerance changes dramatically. In our laboratory investigations, a small subset of the bacterial cells tolerated indeed very high concentrations of penicillin, approaching 10.000 times the minimal inhibitory concentration (MIC) of normal BHS. The persister BHS were found to be genetically identical to their fully susceptible relatives, ruling out a genetic cause of the antimicrobially tolerant phenotype.

Based on this we hypothesized if a similar phenomenon could take place in vivo. BHS from endometritis are practically always fully susceptible to penicillin, yet we have observed several cases where the antimicrobial treatment were unsuccessful. One explanation could be that some of the endometrial BHS were in a persister or dormant state and thus virtually untreatable.

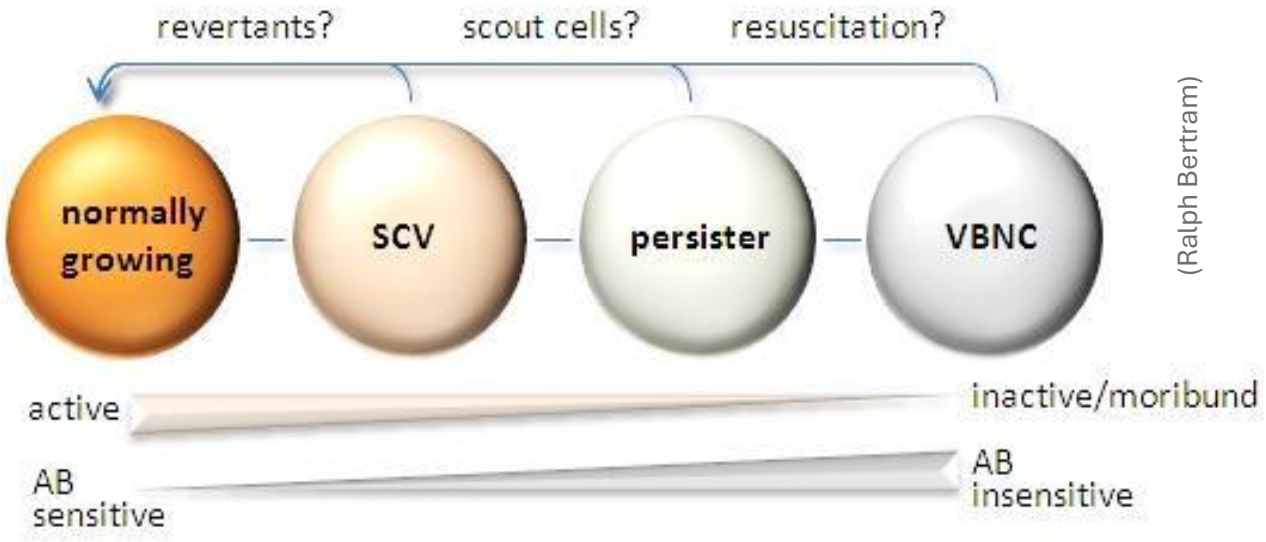

Figure 1, by Ralph Bertram, University of Tübingen, Germany.

To test that hypothesis, we performed a study on 21 client-owned problem-mares with a history of repeated uterine infections. All mares had been treated repeatedly in the past yet remained infected. Mares could only participate if they were found to be carry a latent infection with BHS following activation with bActivate. bActivate works as a resuscitation factor activating dormant bacterial cells in the uterine wall. Following activation, mares were treated for 3 days with intrauterine lavage and

subsequent installation of penicillin ($10x10^6$ IU) once daily. Uterine treatment was supported with 10 IU of Oxytocin if intrauterine fluid was demonstrated. Uterine treatment was supported with 10 IU of Oxytocin if intrauterine fluid was demonstrated. In parallel, systemic treatment was performed with penicillin was injected intramuscularly twice daily (25.000 IU/kg).

The following estrus an endometrial culture was recovered immediately prior to re-activation with bActivate in order to stimulate growth of remaining BHS. An endometrial sample was recovered 2 days later. Among the treated and re-activated mares, a single mare was positive to BHS could be found following activation illustrating a high efficiency of treatment. It was later found that this particular mare had been treated by intrauterine treatment only.

In other studies, using research mares, activation positive mares were only treated with intrauterine lavage and oxytocin, but no antimicrobials. In the following cycle the majority of these mares were initially culture negative, but following activation BHS could be identified.

When information from these studies is compiled, the same conclusions can be made. Endometrial BHS are very sensitive to antimicrobials, e.g. penicillin, when they are metabolically active, however, extremely tolerant when they are in dormant state.