

Spontaneous mutation accompanying decreased growth rate confers vancomycin resistance in the subpopulation of hetero-VRSA strain Mu3.

M. KAPI, F. TAKEUCHI, C. PIRIYAPORN, L.CUI, K. OKUMA, K. HIRAMATSU.

Juntendo Univ., Tokyo, JAPAN

Background: Mu3 is a clinical MRSA strain isolated in January 1996 (Hiramatsu et al, Lancet 1197; 350: 1668-1671), which shows a heterogeneous type of resistance to vancomycin. Although the MIC of Mu3 against vancomycin is 2 µg/ml, Mu3 generates VRSA within the population, which grew in the presence of 4 µg/ml to 7µg/ml vancomycin. In this study we focus on answering the following questions: (1) is spontaneous mutation responsible for the resistant subpopulation of Mu3? and (2) how big is the mutation rate?

Methods: About 1,000 CFU of Mu3 was inoculated into 100 independent tubes each containing 200 µl of BHI, and cultivated for 9 h. One hundred ml of each tube was spread on BHI agar containing 4 mg/L of vancomycin to enumerate the resistant colonies after 48 h incubation at 37C. Using the fluctuation test of Luria and Delbruck compensated for the reduced growth rate of mutants, we evaluated the degree of fluctuation and theoretical ratio of growth rate between the mutants and parent Mu3 strain.

Results: The experimental data did not conform to Poisson's distribution, which gave a strong evidence for spontaneous nature of the emergence of resistant subpopulation of Mu3. Smaller growth rate of the resistant subpopulations of Mu3 predicted by a mathematical model agreed well with that experimentally determined with selected resistant substrains.