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Enterococcus faecalis evolve biofilm development, antibiotic resistance, and quorum-sensing to nutrients in mesocosm experiments

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The emergence of multidrug-resistant bacteria has become a significant threat to public health. In aquatic environment the nutrients (cause nutrient pollution) that contributed to bacterial resistance and pathogenicity are poorly understood. Here, we conducted mesocosms experiments to evaluate the adaptability of *Enterococcus faecalis* by adding nitrogen and phosphorus periodically with different concentrations. *E. faecalis* were isolated from the experimental mesocosms on Days 0, 1, 6, 16, 22, 30, 45, 65 and 98 to evaluate antibiotic resistance, biofilm formation and quorum-sensing regulated genes expression. Broth micro-dilution method was used to assess the resistance profile of the following antibiotics: ampicillin, oxytetracycline, ciprofloxacin, cancomycin chloramphenicol erythromycin. Microtiter Dish Biofilm Formation Assay was used to assess the capability of *E. faecalis* biofilm development. Quantitative real-time PCR was used to compare mRNA levels of *E. faecalis* quorum-sensing related genes (*cylLS, cylLL, cylB, cylA, cylI, cylM, cylR1, cylR2 and gelE*) in Mueller-Hinton broth. Total of 411 *E. faecalis* isolates, 198 (48.2%), 29 (7.1%), 97 (23.6%) exhibited resistance to oxytetracycline, ciprofloxacin, and ampicillin, respectively. The biofilm development of *E. faecalis* isolates on day 65 and day 98 were significantly increased ($F_{11,24} = 42.36$, $p < 0.01$). The expression of quorum-sensing-related genes were significantly up-regulated, about 10-fold at the transcriptional level in nutrient enriched treatments than control (unenriched of nitrogen and phosphorus). The addition of nitrogen and phosphorus ions in water facilitates resistance in *E. faecalis* against commonly used antibiotics. In addition, the *E. faecalis* also contributed to biofilm development and regulation of virulence genes through quorum-sensing mechanism in aquatic environment.

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