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## Formation and stability of extracellular antibiotic resistance plasmid pool in seawater under existence of ciliate and heterotrophic nanoflagella

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[Background and aim] Extracellular DNA (exDNA) found in natural aquatic environment plays a role in horizontal transfer of antibiotic resistance genes (ARGs) among bacterial communities by probably natural transformation. exDNA is released from bacteria by various mechanisms. We aim to demonstrate the involvement of marine ciliates and heterotrophic nanoflagellates (HNFs) in the release/leakage of multi-drug resistance transferable plasmid pAQU1 from Photobacterium damselae subsp. damselae strain 04Ya311. [Results] Quantitative detection of pAQU1 was performed by qPCR targeted tet(M) coded on the pAQU1. In seawater microcosms containing 04Ya311 and ciliates, the marked increase of extracellular tet(M) was found at day-3 of incubation, while in microcosms containing 04Ya311 and HNFs, the highest copy number of extracellular tet(M) was reached at day-20. The addition of low concentration of oxytetracycline (OTC, 10  $\mu$  g/mL) was not effective to increase of extracellular tet(M). Under presence of ciliates, no significant loss of plasmid from bacterial cells was observed during the incubation with and without OTC. While under presence of HNFs, the addition of OTC accelerated loss of plasmid in cell fraction. In any cases, released/leaked tet(M) was stably present through the incubation period. [Conclusion] The results provide an insight into the effect of prey-predator interactions on plasmid behavior in microbial community, probably leading horizontal transfer of ARGs in marine environment. exDNA should be a pool of ARGs. This will be valuable evidence for risk assessment and management of marine environmental ARGs.