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ePoster Viewing

Susceptibility testing methods

Reduced antibiotic susceptibility in wastewater *Planktothrix* sp.: evaluation of minimum inhibitory concentrations by a method designed for cyanobacteria – molecular identification of resistance genes

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Background: Cyanobacteria are ubiquitous in aquatic ecosystems, being *Planktothrix* spp. one of the most common bloom-forming species in freshwaters worldwide. In these environments, cyanobacteria are exposed to antibiotics and antibiotic resistant bacteria, but their role on water resistome was never investigated. One limitation of testing antibiotic resistance in cyanobacteria is the lack of standardized methods and interpretative guidelines. Based on the standard broth microdilution method, we developed a procedure for testing antibiotic susceptibility in cyanobacteria. This work aimed to test the applicability of that method in *Planktothrix mougeotti* from a wastewater treatment plant, in order to assess their susceptibility patterns and their putative contribution to the global pool of resistance determinants in freshwater.

Material/methods: We investigated 8 strains of *Planktothrix mougeotti* isolated from a wastewater treatment plant (WWTP) located in the north of Portugal (41°07'05.22"N, 8°34'015.40"W) and dimensioned for a population of 80,000 habitants. Antibiotic susceptibility was evaluated by a microdilution method previously adapted for cyanobacteria, against beta-lactams, aminoglycosides, quinolones, trimethoprim and tetracycline. Minimum inhibitory concentrations (MIC) were determined according to cyanobacterial cell density (optical density, 450nm) and microscopic examination of cultures integrity. All strains were subjected to the search of antibiotic resistance genes and class 1, 2 and 3 integrons by PCR/sequencing.

Results: The MIC values of *P. mougeotti* strains varied between 0.2-0.8 mg/L for ceftazidime, ceftriaxone, kanamycine and gentamicine. On the other hand, all of the tested strains were not susceptible to trimethoprim, quinolones (norfloxacin and nalidixic acid), tetracycline and amoxicillin, within the tested concentration range (0.0015-1.6 mg/L). None of the strains exhibited any gene conferring resistance to these antibiotics. On the contrary, a pair of *strA-strB*-type genes, a *sul1*-type gene and a class 1-type integron (*int1*) were detected in four, five and three strains, respectively.

Conclusions: The results suggests that the presence of antibiotic resistance genes and integrons in *Planktothrix mougeotti*, as well as their reduced susceptibility to some antibiotics, might be a result of their exposure to antibiotics and antibiotic resistance genes in the WWTP. This reinforces the hypothesis that cyanobacteria may play a role on freshwater resistome and point out the need to implement standard methodologies and guidelines for susceptibility testing in cyanobacteria.