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Role of residual antibiotics in aquatic environments on selection and diffusion of bacterial resistances (RASDI)

Objectives Evaluation of the effects of antibiotic residues in surface waters on pathogenic and potential pathogenic bacteria belonging to the genera *Aeromonas*, *Acinetobacter* and *Legionella*. These bacteria come into contact with low level amounts of antibiotics in their natural habitats.

Conclusions Antibiotic compounds that reach the aquatic environments exert a selective pressure on the bacterial populations of *Aeromonas*, *Acinetobacter* and *Legionella*, and low level antibiotic contamination of waters can induce the onset of resistances in *Aeromonas*. Therefore, low level antibiotic contamination of waters represents a risk for at least some bacterial populations, since it has an impact on the appearance, the persistence and the spread of the antibiotic resistance.

Main results and findings

Resistance profiles and correlations to the strain origin From the water samples collected aseptically from hospital wastewaters (Ticino and Zurich), two wastewater treatment plants, two rivers and a mountain stream, the lakes Ceresio and Cadagno and tap waters in Ticino, a total of 812 *Aeromonas,* 463 *Acinetobacter* and 47 *Legionella* strains were isolated. The results can be summarised as follows:

– For *Aeromonas* (812 strains) and *Acinetobacter* (463 strains), it was shown that:
  - resistant or intermediate strains were more often isolated from hospital wastewaters and wastewater treatment plants than from rivers, lakes and, for *Aeromonas,* clinical samples.
  - for hospital wastewater strains, susceptibility was correlated to the concentration of antibiotics (the higher the antibiotic concentration, the smaller the diameter of the inhibition zone, indicating thus a decrease in susceptibility of the strain). In particular for *Aeromonas* strains, the susceptibility was highly correlated to the concentration of ciprofloxacin.
  - in *Aeromonas,* resistance to fluoroquinolones (ciprofloxacin and norfloxacin) was found in about 45% of strains from hospital wastewaters, in less than 5% of the strains from the activated sludge (wastewater treatment plant) and in none of the strains isolated in other locations.
  - *Acinetobacter* strains from the aquatic environment were found to be more susceptible to the investigated antibiotics (in particular fluoroquinolones) than *Aeromonas*.
– For *Legionella* (47 strains), since only few strains could be isolated from wastewater treatment plants, the experiments were carried out using *L. pneumophila* serogroup 1 strains (provided by the National Reference Centre for *Legionella*) isolated from patients, tap water and cooling towers from different regions of Switzerland. A clear statistical correlation was found between MIC (Minimal Inhibitory Concentration) values for erythromycin and *Legionella SBT* (Sequence Based Typing) type 1,4,3, regardless of the origin of the strains.

***In vitro* induction of resistances** Some *Aeromonas* and *Acinetobacter* isolates were grown *in vitro* (24h in Tryptcase Soya broth and 24h on Mueller-Hinton agar) in the presence of a mixture of antibiotics (ceftriaxone, ciprofloxacin and erythromycin) at concentrations like those measured in hospital wastewaters. This experiment showed the following results:

– For one *Aeromonas* clinical isolate, growth and resistance pattern were strongly affected by the lowest environmental concentrations of antibiotics.
– In some *Aeromonas* strains, the susceptibility profiles could be changed by growth in media containing low antibiotic concentrations. The induced profiles (slight reduction of susceptibility) were stable after 10 passages on Mueller Hinton agar without antibiotic.

– In *Legionella,* resistance to erythromycin could not be induced at the concentration found in the environment. However, resistant mutants could be obtained upon a single passage on BCYE agar containing high amounts of erythromycin, only for *Legionella* SBT type 1,4,3 (inoculum 0.5 McFarland). In strain ATCC 33152 Philadelphia (SBT type 3,4,1) resistance to macrolides could be induced only by plating inoculum concentrations higher than >1 McFarland. The induced strains were characterised by a decrease in susceptibility against different macrolides (erythromycin, clarithromycin and azithromycin).

***In situ* induction of resistances** A laboratory scale model of an activated sludge phase (from the wastewater treatment plants of Airolo and Bioggio) was used to verify if environmental concentrations of antibiotics could play a role in selecting *Aeromonas* and *Acinetobacter* resistant strains. The strains were isolated from the native sludge (without adding antibiotics) and 12h after addition of the antibiotic mixtures (ceftriaxone, ciprofloxacin and erythromycin) at concentrations normally found in this environment. The results can be summarised as follows:
– In general, no differences in resistance profile could be detected in the bacterial populations of *Aeromonas* and *Acinetobacter* after in situ induction of resistance. However, variations in bacterial counts (obtained by in situ whole cell hybridisation) could be observed.
– Chemical quantifications revealed that the native sludge from Bioggio (collecting wastewater from Lugano including some hospitals) already contained residual antibiotics. This was not the case for the native sludge from Airolo (collecting wastewaters from the alpine region without hospitals).

Resistance genetic determinants Integrons seem to play a major role in the dissemination of resistance in Gram-negative bacteria. In the *Aeromonas* and *Acinetobacter* strains isolated from environmental waters the following results were found:
– Class 1 integrons were present in 48% of the analysed strains, whereas class 2 integrons were never detected.
– For *Aeromonas,* the number of the strains harbouring integrons was high in the wastewater from both the Bioggio and Airolo treatment plants, as well as in the sludge of Ticino hospitals. This suggests that in sludge, *Aeromonas* can acquire resistance genes from other bacteria or develop resistance to antibiotics.
– For *Acinetobacter,* 91% and 40% of the strains isolated respectively from the Ticino and Zurich hospital wastewaters and 23% of the strains isolated from environmental waters were positive for class 1 integron.
– 60.9% of *Aeromonas* isolates and 64.4% of *Acinetobacter* isolates from the sludge from Bioggio carried class 1 integrons. The presence of integrons in these strains could be linked with the constant antibiotic pressure present in this environment and to which the strains are exposed.
– For *Legionella,* since spontaneous point mutation in the 23S rRNA seems particularly frequent in slow growing bacteria having 1 or 2 copies of *rrn* genes (*as Legionella*), DNA sequences of the macrolide binding site on the 23S rRNA molecule were investigated. Three different types of resistance to macrolide were identified and characterised at molecular level. In particular, high level erythromycin resistance (MIC>256µg/ml) was found due to a C→T replacement in position 2611 (*E. coli* numbering). In order to be able to screen rapidly for this mutation, a real-time assay for the detection of the C2611 mutation was developed.

Publications of the NRP 49 project

Corvaglia AR.
Rôle des résidus d'antibiotiques dans l'environment hydrique sur la sélection et la diffusion de bactéries résistantes des genres *Aeromonas*, *Acinetobacter* et *Legionella*
[diploma thesis]. Geneva: University of Geneva; 2006.

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