

# Presence of genetic determinants of antibiotic resistance in Lugano Lake and tributary rivers.

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## Introduction

Antibiotic resistant bacteria and antibiotic resistance genes are considered important environmental contaminants but little is known yet about their fate in the environment or about how they influence the natural microbial populations. In the environment, especially in water affected by anthropic activities (discharge of hospital, urban and industrial waste water treatment plant and agricultural run-off), antibiotic determinants may become part of the environmental gene pool, may spread horizontally, and may move back to humans and animals via contaminated food and drinking water.

## Aim

The aim of our work was to investigate and quantify the presence of genetic determinants for antibiotic resistance in Lugano Lake and some tributary rivers in order to observe if different anthropic activities could influence the bacterial populations naturally present in water environments and the water quality.

## Lake Lugano and sampling area

Lake Lugano is located at the border between Italy and Switzerland and is divided into three basins (fig.1) with very different geomorphologic characteristics. In the last years human activities have caused an increase of eutrophication of the lake, so the quality of water have to be monitored especially because of its many uses (irrigation, potable water uptake, industries). We have chosen different sampling site (fig.1), at different depths and seasons.

Sampling campaign was performed from January 2016 to December 2017

Lake water samples were taken seasonally (mar, jun, aug, nov).

Gandria, Ponte Tresa, and Melide, integrated samples 0-20 mt pelagic waters.

Lugano and Riva San Vitale, (40 and 33 mt) near potable water uptake plant.

Rivers water samples were taken monthly (jan-dec) from three different rivers all located in Lugano district.

Veduggio, downstream of waste water treatment plant (WWTP).

Cassarate, downstream of waste water treatment plant that has been closed in January 2017.

Magliasina, not directly influenced by waste water treatment plant but located nearby a fish farm.

## Results and discussion

We analysed water samples by PCR to investigate the presence or absence of integrase gene (*intI*) and of seven genes (*bla<sub>CTXM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>*, *teta*, *qnrS*, *sullI*, *ermB*) that confers resistance to the major classes of antibiotics used in clinical and veterinary ( $\beta$ -lactams, tetracycline, quinolones, sulphonamides and macrolides). After this qualitative screening, we quantified antibiotic resistance genes (ARG) using qPCR and comparing the amount of ARG with the 16S. We found at least five quantifiable genes *sullI*, *qnrS*, *teta*, *bla<sub>TEM</sub>*, *ermB* especially in rivers influenced by WWTP (tab.1) while the integrase gene (*intI*) was stably present both in rivers and lake. There was a global increase of resistance genes in 2017 (tab.1 and 2). The closure of the waste water treatment plant seems to have reduced rapidly the quantity of antibiotic resistance gene in Cassarate river (tab 1) and, because this river flows into the lake near the sampling point of Lugano, could be involved in the reduction of *sullI* gene in this area (fig.1 and tab.2). Low number of resistance genes in lake (tab.2) could also be explained by the distance from WWTP and dilution effect of water.

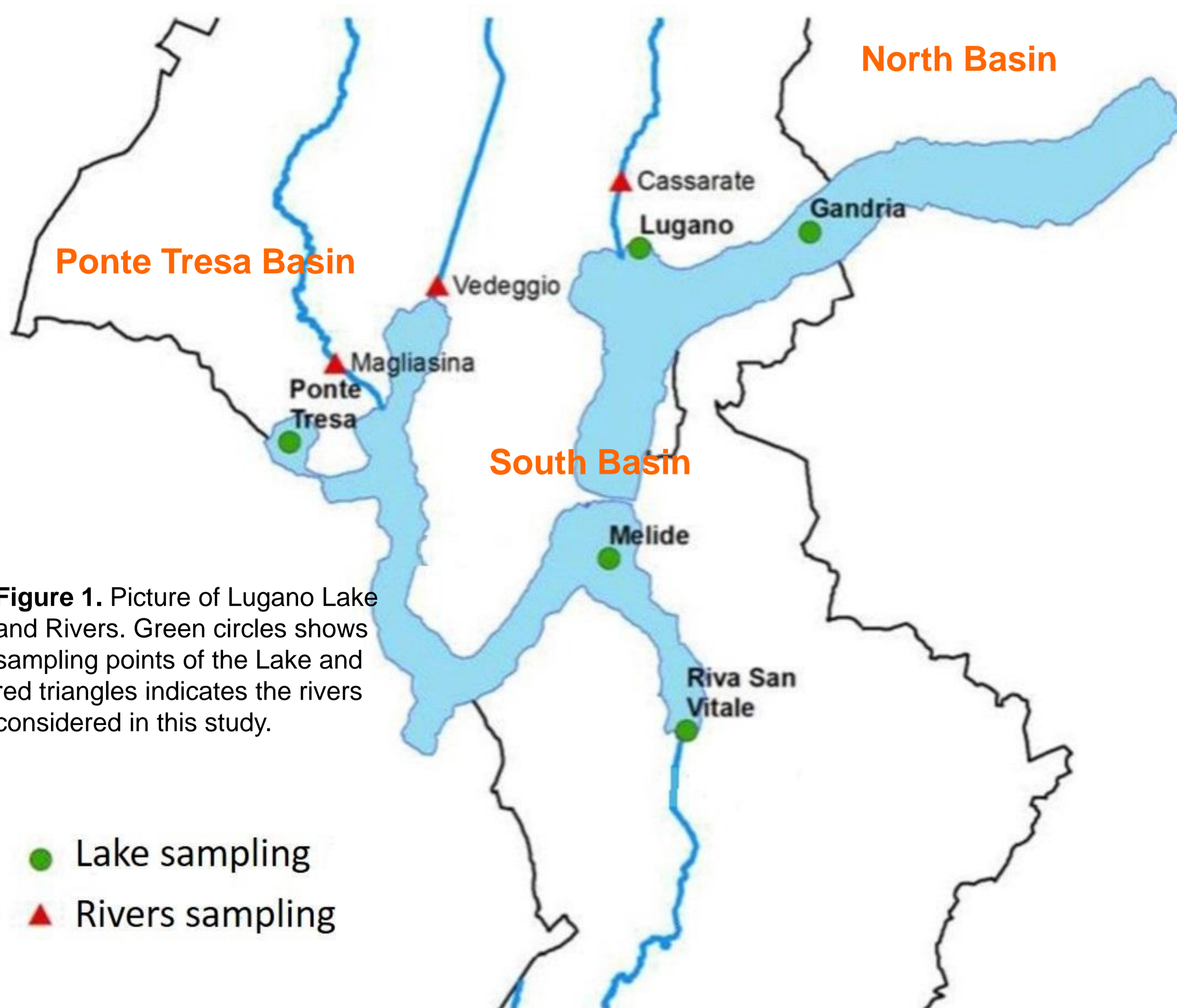


Figure 1. Picture of Lugano Lake and Rivers. Green circles shows sampling points of the Lake and red triangles indicates the rivers considered in this study.

Bla			
	2016	2017	Differences
Veduggio	0.00%	0.06%	0.06%
Cassarate	0.01%	0.04%	0.03%
Magliasina	0.00%	0.01%	0.01%

FQLs			
	2016	2017	Differences
Veduggio	0.05%	0.07%	0.02%
Cassarate	0.21%	0.10%	-0.11%
Magliasina	0.08%	0.02%	-0.06%

TETs			
	2016	2017	Differences
Veduggio	0.06%	0.06%	0.00%
Cassarate	0.61%	0.06%	-0.55%
Magliasina	0.87%	0.51%	-0.36%

Macr			
	2016	2017	Differences
Veduggio	0.08%	0.13%	0.05%
Cassarate	0.53%	0.32%	-0.21%
Magliasina	0.43%	0.12%	-0.31%

SULs			
	2016	2017	Differences
Veduggio	0.70%	1.02%	0.32%
Cassarate	2.33%	0.12%	-2.21%
Magliasina	3.46%	0.06%	-3.40%

Table 1. Percentage of antibiotic resistance genes in rivers in relation with the amount of 16S rRNA gene divided and compared by year (column "2016" and "2017" and column "differences" respectively). Bla,  $\beta$ -lactams resistant genes; Macr, macrolides resistant genes; FQLs, fluoroquinolones resistant genes; SULs, sulphonamides resistant genes; TETs, tetracycline resistant genes. Red, increase; green, decrease

FQLs			
	2016	2017	Differences
Ponte Tresa	0.06%	0.00%	-0.06%

Macr			
	2016	2017	Differences
Lugano	0.04%	0.01%	-0.03%

SULs			
	2016	2017	Differences
Lugano	0.80%	0.02%	-0.78%
Ponte Tresa	0.00%	0.02%	0.02%
Riva S.Vitale	0.00%	0.01%	0.01%
Gandria	0.00%	0.02%	0.02%
Melide	0.00%	0.02%	0.02%

Table 2. Percentage of antibiotic resistance genes in lake in relation with the amount of 16S rRNA gene divided and compared by year (column "2016" and "2017" and column "differences" respectively). Macr, macrolides resistant genes; FQLs, fluoroquinolones resistant genes; SULs, sulphonamides resistant genes; Red, increase; green, decrease

## Conclusion

Our results support the hypothesis that anthropic activities, in particular the waste water treatment plant, influence the bacterial population present in water environment. The resistance genes present in Magliasina river (not directly influenced by WWTP), could be part of natural resistome or could derive from other discharge not considered in this study (e.g. fish farm). The low level of antibiotic resistance genes in lake Lugano was not expected compared to other lake with similar characteristics, nevertheless the presence of resistance genes near potable water uptake plants and the increase of genes that confer resistance to  $\beta$ -lactams are worrying results.