From the Hospital effluent to the Municipal Wastewater Treatment Plant: bacterial communities and antibiotic resistance

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Scope and objectives

Previous studies showed that wastewater treatment reduces considerably the bacterial load of the effluents but may contribute for the increase of the prevalence of antimicrobial resistance in the treated wastewater[1].

This work aimed at assessing the impact of the discharge of a raw Hospital Effluent (HE) into a Municipal Waste Water Treatment Plant (MWWTP).

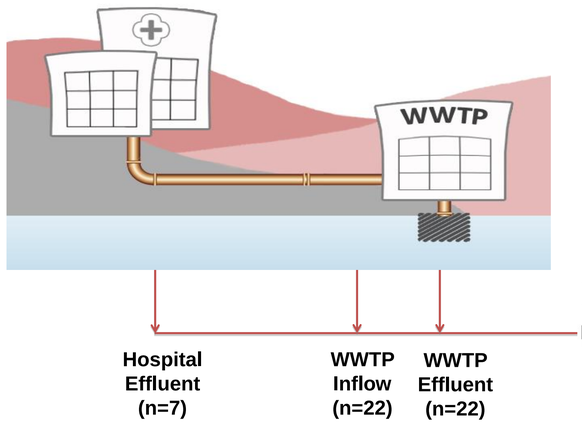
Specifically, it was intended to:

•compare the levels of antimicrobial resistance in the raw Hospital effluent with those in MWWTP inflow (IF) and the final MWWTP treated effluent (Ef);

• compare the fate of bacteria belonging to different groups, resistant to beta-lactams or quinolones.

•compare HE, If and Ef bacterial community structure based on 16S rRNA gene PCR-DGGE analysis.

Methodology



Hospital
•**Population served**: 3.7 million inhabitants; aprox. 1000 beds
•**Wastewater discharge**: into urban sewer system, untreated

WWTP
Population served: 200 000 inhabitants
Biological treatment: activated sludge
Daily treated outflow: 54 000 m³
Discharge: river mouth

Culture-dependant methods

Isolation		
Total heterotrophs	Aero/ Pseudomonas	Enterobacteria
PCA	GSP	mFC
PCA + 4 mg/L ciprofloxacin	GSP + 4 mg/L ciprofloxacin	mFC + 4 mg/L ciprofloxacin
PCA + 32 mg/L amoxicillin	GSP + + 32 mg/L amoxicillin	mFC + 32 mg/L amoxicillin

-CFU/mL
-Percentage of resistance → ANOVA
-Bacterial removal rates → Tukey test (SPSS 20)

Culture-independant methods → Total DNA extraction → 16S rRNA gene PCR with primers 338F+518R → DGGE analysis (Bionumerics + CANOCO)

Results

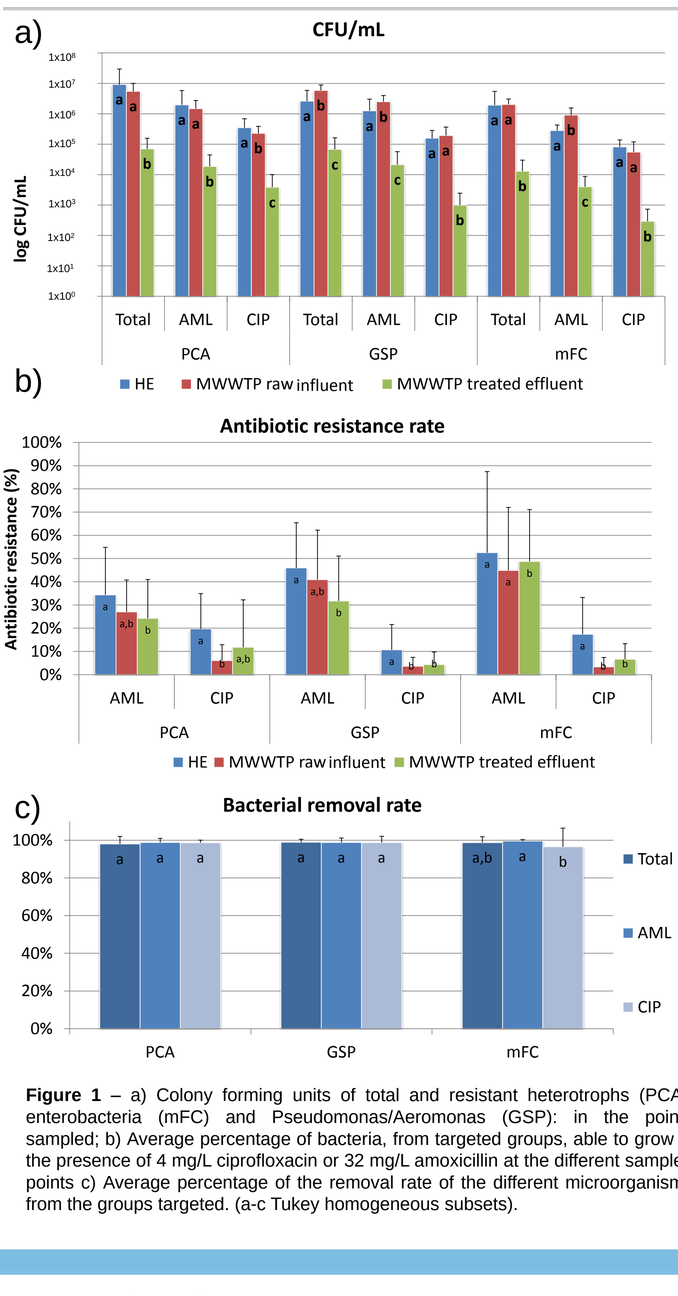


Figure 1 – a) Colony forming units of total and resistant heterotrophs (PCA), enterobacteria (mFC) and Pseudomonas/Aeromonas (GSP): in the points sampled; b) Average percentage of bacteria, from targeted groups, able to grow in the presence of 4 mg/L ciprofloxacin or 32 mg/L amoxicillin at the different sampled points c) Average percentage of the removal rate of the different microorganisms from the groups targeted. (a-c Tukey homogeneous subsets).

Hospital effluent *vs.* raw municipal wastewater

- **In general, the hospital effluent had bacterial densities (CFU/ml) similar to those reaching the MWWTP, i.e., the raw wastewater;**
- **For amoxicillin, the resistance rates in the Hospital effluent were not significantly different from those in the raw wastewater, irrespective of the bacterial group;**
- **For ciprofloxacin, resistance rates were significantly higher (at least three times higher) in the Hospital effluent than in the raw wastewater of the MWWTP, irrespective of the bacterial group.**

Antibiotic resistant bacteria in the MWWTP

- **Wastewater treatment led to a reduction of cell densities of about 100-1000 times, for both total and antibiotic resistant bacteria;**
- **In general, amoxicillin and ciprofloxacin resistance rates were not significantly altered due to wastewater treatment;**
- **The final treated effluent had lower resistance rates than the hospital effluent.**

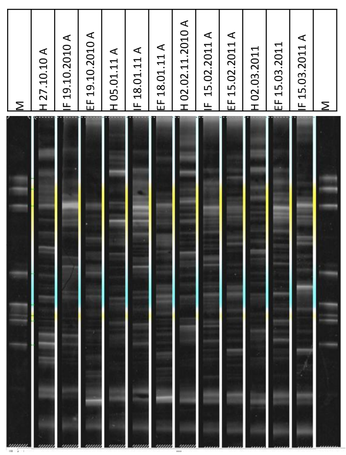


Figure 2 – DGGE band profile of 16S rRNA (180 bp) of total genomic DNA extracted from raw Hospital effluent, raw (inflow) and treated (Effluent) samples, collected from October 2010 to March 2011.

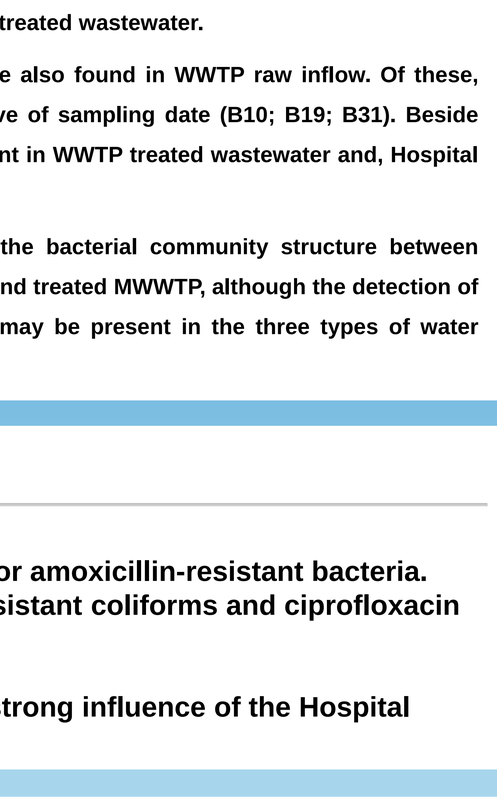


Figure 3 - Detrended Correspondence analysis (DCA) ordinal analysis of samples recovered. Axis 1 and axis 2 explain **39,1%** of the cumulative variation shown by DCA. Δ-DGGE bands, ●- Hospital, ●- Inflow ●- Effluent

DGGE profiling

- **The 16S rRNA-DGGE profiling yielded a total of 32 bands. Although variations over sampling periods were observed, in average each type of sample yielded 16-25 bands, suggesting identical richness of bacterial lineages in Hospital effluent and Municipal raw and treated wastewater.**
- **Eight bands always present in the Hospital effluent were also found in WWTP raw inflow. Of these, three persisted in the WWTP treated wastewater, irrespective of sampling date (B10; B19; B31). Beside these 3 bands, one (B20) and two (B11 and B30) were present in WWTP treated wastewater and, Hospital effluent and WWTP raw inflow, respectively.**
- **An exploratory DCA suggests a stronger variation of the bacterial community structure between Hospital and WWTP than the one found between raw inflow and treated MWWTP, although the detection of common bands suggests that some community members may be present in the three types of water analyzed.**

Conclusions

•Untreated hospital effluents are confirmed as a potential source of antibiotic-resistant bacteria reaching the MWWTP.;
•The number of ciprofloxacin-resistant bacteria decreases from the Hospital to the entrance of the WWTP; the same is not observed for amoxicillin-resistant bacteria.
•Wastewater treatment reduces bacterial cell densities (CFU/ml) in about 100-1000 but causes an increase in the rate of amoxicillin resistant coliforms and ciprofloxacin resistance bacteria of all groups targeted.
•DGGE profiling revealed similar bacterial richness in all the points sampled.
•The similarity of the bacterial community structure of the Hospital effluent with that of the Municipal Waste Water inflow suggests a strong influence of the Hospital discharges, which will be further studied.

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1. Novo & Manaia (2010), Factors influencing antibiotic resistance burden in municipal wastewater treatment plants, Appl Microbiol Biotechnol, 87:1157-1166.