



A high resistome diversity in a metropolitan hospital waste water in Turkey revealed using shotgun metagenomics

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Introduction:

According to WHO, Turkey has the highest antibiotic usage among OECD countries. The state of antibiotics resistance in the country, therefore, is an alarming public health concern not only locally, but at the global scale considering the worldwide dissemination of the antimicrobial resistant determinants (ARDs). However, traditional microbiological approaches fall short when the task aims to profiling a large spectrum of ARDs, conducting this at community level, and include non-pathogenic and non-culturable bacterial species. Comprehensive profiling of ARDs contained in "hot spots" (i.e. wastewaters) can monitor the current state of antibiotic resistance within the related society, furthermore it is essential to understanding the mechanisms and dissemination of antibiotic resistance in a resistome ecosystem.



Method:

- We utilized **shotgun metagenomics** to provide a description of the resistome of a HWW, and identify factors that significantly impact this resistome
- We collected 3 fortnightly samples from a metropolitan hospital (1300 bed capacity) wastewater. Microbial dsDNA isolation were done
- Microbial dsDNA were subject to high-throughput sequencing using Illumina NextSeq 500 platform
- Over 60,147,132 sequencing reads (totalling 9.02 Gbp DNA) were produced across all samples
- The reads were assembled to larger contigs using IDBA genome assembler and the genes were predicted using Prodigal microbial gene finding program
- The predicted genes were homology-searched in ResFams ARDs database
- The abundance of each ARDs mechanism were determined by mapping the sequencing reads on classified genes using BWA short-read mapper

Results:

- 1,500,294 reads were aligned to 46,636 predicted resistance genes. These resistance genes were distributed to **118 out of 170 resistance-related protein families** contained in ResFams database, where 103 of those families were observed in all three samples.
- **20 resistance mechanisms** that confer resistance to 15 classes of antimicrobial drugs were determined.
- Out of 23,405, 30,981, and 30,104 resistance genes detected for the three samples respectively, **12,575** were found to be contained in all samples.
- **All the detected resistance genes could be aligned to MetaHIT human gut microbiome gene catalogue.**
- The most frequent resistance genes belonged to **aminoglycosides** and **β -lactamases**. The most abundant genes were *APH3* (mean=4148.3ppm), *VIM-type metallo-beta-lactamase* (mean=3666.7ppm), *CfxA* (mean=1478ppm), *AAC6-II* (mean=1055ppm).
- *AdeB* (mean=585.6ppm), which is responsible for efflux pump-associated resistance, and *phoQ* (mean=232ppm), which is responsible for colistin resistance in *A. baumannii* were detected.
- **NDM-type beta lactamases** and *ccrA-type* beta lactamases genes were also detected in rare amounts
- Several metagenomics reads were mapped to MCR-I/II colistin resistance genes.
- Custom PCR amplification via genome walking revealed that those regions might code for a **homologous hypothetical proteins of colistin resistance.**

Conclusions:

- ✓ In Turkey, for the first time, ARDs profiled in a HWW
- ✓ We used protein homology search on known resistance-related protein families to discover antibiotic resistomes in deeply sequenced metagenomes.
- ✓ Over 100 ARDs with high diversity of unique resistance genes discovered imply an alarming health concern
- ✓ **New *mcr-homologous* gene might be the culprit for the emerging colistin resistance in Turkey.**

