

TITLE: DIVERSITY, BIOFILM FORMATION AND ANTIMICROBIAL SUSCEPTIBILITY OF AEROBIC HETEROTROPHIC BACTERIA ISOLATED FROM COOLING TOWERS

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ABSTRACT:

Cooling towers (CTw) technologies provide water refrigeration cycles to keep varied equipment working properly in industrial plants and other settings. The conditions of CTw water (such as temperature and organic matter) create a suitable environment for microbial growth and biofilm formation, what may not only impair the CTw efficiency due to biofouling but also can select and spread antimicrobial-resistant bacteria (ARB) due to the spraying of CTw water in the air or disposal of water on aquatic environments. Here we investigated the diversity of planktonic and biofilm-associated bacteria from two CTw of a Brazilian industrial plant using approach based on culture and metataxonomic (16S amplicon libraries sequencing on the Illumina Miseq platform). We also assessed the ability of biofilm formation *in vitro* of culturable aerobic heterotrophic bacteria and their susceptibility to clinically relevant antimicrobial drugs. We used culture approach and identified a total of 14 bacterial genera and 22 species. Most of them are associated to different diseases. Regardless of the sample origin (CTw1 that uses treated recycled water or CTw 2 that uses clarified and chlorinated water as makeup water) or age biofilm (7 or 14 days), *Bacillus* was the genus most frequently isolated followed by *Acinetobacter* and *Pseudomonas*. We observed elevated frequencies of poor susceptibility of the isolates to cephalexin, ampicillin, and nitrofurantoin, and the highest frequency of susceptibility for isolates exposed to meropenem. By the principal coordinate analysis (PCoA), close OTUs clustering was observed in function of CTw type and biofilm age. The EdgeR analysis showed significant variations of abundance on the OTUs on the Phyla, Family and Genera levels on the CTw 1 and Phyla on the CTw2. The most abundant genera on CTw1 were *Methyloversatilis*, *Sphingopyxis*, *Acinetobacter*, *Alicyclobacillus* and *Sphingomonas*, with increase of *Methyloversatilis* and *Sphingopyxis* in the 14-days biofilm, while *Acinetobacter*, *Alicyclobacillus* and *Sphingomonas* decreased in this time. In CTw2, the most abundant genera were *Cytophaga*, *Hyphomicrobium*, *Rhodobacter*, *Flavobacterium* and *Acinetobacter*, with *Cytophaga*, *Rhodobacter*, *Hyphomicrobium* and *Flavobacterium* increased at 14-days, and *Acinetobacter* decreased within 14-days. Our study opens doors for more investigations on reclaimed water quality in CTw, as well as on possible risks of infectious diseases and dissemination of ARB on the environment.

Keywords: Biofilm, antimicrobial susceptibility, bacteria, diversity, cooling tower

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