Effects of biocides and metals on antibiotic resistance: a genomic and metagenomic perspective

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal Arvid Carlsson, Medicinaregatan 3, Göteborg, tisdag den 31 Jan 2017, klockan 09:00

av
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The thesis is based on the following papers and manuscripts:

Paper I. Chandan Pal, Johan Bengtsson-Palme, Christopher Rensing, Erik Kristiansson, DG Joakim Larsson. (2014) **BacMet: antibacterial biocide and metal resistance genes database.** *Nucleic Acids Research*; 42:D737-43. DOI: 10.1093/nar/gkt1252

Paper II. Chandan Pal, Johan Bengtsson-Palme, Erik Kristiansson, DG Joakim Larsson. (2015) **Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential.** *BMC Genomics*; 16:864. DOI: 10.1186/s12864-015-2153-5

Paper III. Chandan Pal, Johan Bengtsson-Palme J, Erik Kristiansson E, DG Joakim Larsson. (2016) **The structure and diversity of human, animal and environmental resistomes.** *Microbiome*; 4:54. DOI: 10.1186/s40168-016-0199-5

Paper IV. Carl-Fredrik Flach, Chandan Pal, Carl-Johan Svensson, Erik Kristiansson, Marcus Östman, Johan Bengtsson-Palme, Mats Tysklind, DG Joakim Larsson. (2016) **Does anti-fouling boat paint select for antibiotic resistance?** *Submitted*

UNIVERSITY OF GOTHENBURG

PhD thesis – Abstract

Avdelningen för Infektionssjukdomar, Institutionen för Biomedicin, Sahlgrenska akademin,
Göteborgs universitet, Sverige, 2017.

Background and aim: There is a concern that biocides and metals can co-select for antibiotic resistance. The aim of this thesis is to enhance our understanding of the roles of antibacterial biocides (e.g. antiseptics, disinfectants) and metals (e.g. copper, zinc) in developing, promoting and maintaining antibiotic resistance in bacteria.

Methods: In **paper I**, published studies on resistance genes to antibacterial biocides and metals were compiled and used as the basis to develop a database (BacMet) of such genes. In **paper II**, 2522 completely sequenced bacterial genomes and 4582 plasmids were studied for resistance genes and their co-occurrences. In **paper III**, 864 metagenomes from human, animal and external environments were studied for resistance genes, taxonomic compositions and mobile genetic elements. In **paper IV**, marine microbial biofilms growing on surfaces painted with copper- and zinc-based anti-fouling paint were studied using phenotypic assay (i.e. culturing of bacteria on agar containing antimicrobials) and metagenomic sequencing.

Results and discussion: The BacMet database (**paper I**) was used to characterise biocide/metal resistance genes in genomes, plasmids and metagenomes in **papers II-IV**. In **paper II**, co-occurrences of resistance genes were studied to identify biocides and metals, such as mercury and quaternary ammonium compounds, with the potential to co-select for resistance to certain classes of antibiotics. Co-occurrences of resistance genes to both antibiotics and biocides/metals were highly prevalent in bacterial isolates from human and domestic animal sources, and in genera comprising many pathogens. In general, plasmids were predicted to provide limited opportunities for biocides and metals to promote horizontal transfer of antibiotic resistance through co-selection, whereas ample possibilities existed for indirect selection via chromosomal biocide and metal resistance genes. In **paper III**, air and environments subjected to pollution from pharmaceutical manufacturing were identified as under-investigated transmission routes for antibiotic resistance genes. The high genetic and taxonomic diversity of external environments suggests that they could serve as sources of unknown resistance genes with the potential to be transferred to pathogens in the future. In **paper IV**, it was found that antifouling paints enriches RND efflux systems conferring cross-resistance, as well as integron-associated integrases and ISCR transposases but not known mobile antibiotic resistance genes, thus providing limited support for selection of mobile antibiotic resistance.

Conclusions: Overall, this thesis provides tools to study co-selection of antibiotic resistance, and enhances our knowledge of risk scenarios and the underlying genetic basis. It identifies compounds, environments and taxa with identified opportunities for co-selection, thereby also provides a basis for risk-reducing actions. It also identifies point sources and reservoirs for resistance genes with a possibility to be transferred to human pathogens. Finally, the work in this thesis also highlights that copper and zinc-based antifouling paint has the potential to co-select for antibiotic resistance via cross-resistance mechanisms.

Keywords: Antibiotic resistance, biocide, metal, co-selection, metagenomics

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