Development of bacterial resistance to biocides and antimicrobial agents as a consequence of biocide usage - DTU Orbit (09/12/2018)

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Biocides are chemical compounds with antimicrobial properties and they are widely used for disinfection, antiseptic and preservation purposes. Biocides have been applied for centuries due to early empirical approaches, such as cleansing of wounds with wine, vinegar and honey and salting of fish and meat. Today, large amounts of biocides are used for disinfection to achieve a satisfactory level of hygiene in various settings and use of biocides has become an integrated part of the industrialized world.

Despite the widespread use and application of biocides knowledge about their exact mechanisms of action, especially at sub-inhibitory concentrations, and the bacterial response to such exposure, is relatively limited. The increasing use of biocides has within recent years lead to concerns about development and emergence of biocide resistant microorganisms that might make the task of eradication of pathogenic bacteria more difficult. Furthermore, it has been suggested that use of biocides may contribute to the development of resistance in bacteria to antimicrobial agents used in human and animal therapy. So far, it is evident that cross- and co-resistance mechanisms to antimicrobials agents and biocides exist. However, much less is known about the potential effect of biocides on development of antimicrobial resistance in bacteria by promoting the horizontal transfer of resistance genes or by inducing the mutation rate. Even though biocides are commonly used at working concentrations way above the lethal bacterial dose, the efficacy of these compounds can be significantly reduced by incorrect use or the presence of residual concentrations hence, bacterial exposure to sub-inhibitory concentrations of biocides is likely to occur.

The overall objective of this study was to examine if natural bacterial isolates become less susceptible to biocides used in their environment and if this can lead to spread of antimicrobial resistant clones due to co-selection. Furthermore, the objective was to examine if exposure to subinhibitory concentrations of biocides induce development of resistance to antimicrobial agents.

So far, only few studies have investigated the susceptibility of livestock-associated isolates to biocides used in their environment. Pigs are increasingly recognised as a potential reservoir of community-acquired methicillin resistant Staphylococcus aureus (CA-MRSA), especially clones belonging to clonal complex (CC) 398. Recently, methicillin resistant S. aureus (MRSA) isolates belonging to CC30 was for the first time detected among Danish pigs. The susceptibility of 79 porcine S. aureus isolates belonging to CC398 or CC30 to commonly used biocides in pig farming was therefore determined (Manuscript III). The biocides comprised benzalkonium chloride (BC), hydrogen peroxide (HP), sodium hypochlorite (SH), formaldehyde (FH), and caustic soda (NaOH). S. aureus isolates did in general not show reduced susceptibility to the biocides tested. However, a quaternary ammonium compound (QAC) resistance gene, qacG, was detected in MRSA CC30 isolates. The presence of qacG in MRSA CC30 is worrying, since use of QACs may contribute to the selection and spread of these isolates. MRSA CC30 is often associated with MRSA types giving rise to clinical infections in Denmark and porcine MRSA CC30 may be prone to adapt to humans.

Residues or inaccurate use of biocides may lead to bacterial exposure to sub-inhibitory concentrations. The bacterial response to such exposure is however unclear. It has been suggested that the SOS response contribute to antimicrobial resistance development in bacteria by inducing mutagenesis. Therefore, the effect of sub-inhibitory concentrations of the five common biocides; BC, CHX, HP, PAA, and SH on the SOS response, indicated by the use of a recA-lacZ expression assay, and mutagenesis in S. aureus isolates was studied (Manuscript II). BC, CHX, and HP was found to induce the SOS response. In addition, HP and PAA were found to significantly (p = 0.05) increase the mutation rate by 5-15 and 3-8 fold, respectively. These results suggest that exposure to sub-inhibitory concentrations of HP and PAA may contribute to emergence of antimicrobial resistance in S. aureus. This may be of potential risk for human health, since these disinfectants are widely used at hospitals and in the food industry.

Mobile genetic elements such as conjugative transposons are important vectors in the dissemination of antibiotic resistance determinants. Tn916 including the tetracycline resistance gene tet(M) is a conjugative transposon and the prototype of a large family of related elements. They have an extremely broad host range and have been found in both pathogenic and commensal bacteria. In the study of Manuscript I, the effect of sub-inhibitory concentrations of ETOH, HP, CHX, and SH on the conjugal transposition of the mobile genetic element Tn916 was investigated. ETOH was found to significantly (p < 0.05) increase transfer of Tn916 by an average of 5-fold, whereas an increase of 4-fold on Tn916 conjugation frequency was observed (p = 0.12) when donors were exposed to hydrogen peroxide. These results suggest that exposure to sub-inhibitory concentrations of ETOH and HP may induce the spread of Tn916-like elements and their resistance genes, which is clinically important since these biocides are frequently used in hospitals.

In conclusion, no widespread selection for reduced susceptibility to commonly used disinfectants in pig farming was detected in porcine S. aureus isolates. However, a biocide resistance gene, qacG, was identified in several of the MRSA isolates, which has also been found in other animal related staphylococci. Surveillance of the occurrence and emergence of reduced susceptibility to biocides in bacteria are however, still encouraged, since this will provide important data to determine if decreased susceptibility to biocides happen over time. Importantly, the data from this thesis demonstrated a potential of certain biocides to contribute to antimicrobial resistance development and emergence in bacteria through increased mutagenesis and transfer of the antimicrobial resistance gene tet(M). On the short term these results emphasises that correct use of biocides are of outmost importance and should not be compromised. On the long term, more studies are needed to elucidate the actual risk of biocide use on generating antimicrobial resistant bacteria in practice.

**General information**

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