

LITERATURE REFERENCES ON THE SUBJECT OF DEVELOPMENT OF RESISTANCE TO QUATERNARY AMMONIUM COMPOUNDS

1. **Bacterial resistance to disinfectants containing quaternary ammonium compounds**

Sundheim, G.; Langsrud, S.; Heir, E.; Holck, A. L.; Bessems, E.; Terpstra, P. M. J. (eds.)

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ABSTRACT

Quaternary ammonium compounds (QAC) are widely used as disinfectants in both medical and food environments. Microbial contaminants are, therefore, regularly exposed to their action and the isolation from clinical and food sources of resistant bacteria continues to be reported in many countries. Resistance to QAC in clinical strains of staphylococci is encoded by one of at least three resistance genes, designated *qacA*, *qacB* and *qacC*. Using hybridisation analysis, we have shown that these QAC resistance genes are also distributed among staphylococcal strains in the food industry. In addition, we have discovered two new resistance determinants in these food isolates, which are now being characterised and sequenced. Although the general level of resistance of pure cultures is low, the resistant strains have originally been isolated after exposure to the recommended user concentration of a commercial brand of QAC. We have also studied resistance to QAC in pseudomonads isolated from the food industry. Their level of resistance is much higher than that found in staphylococci. About 30% of the collected strains were able to grow in 200 $\mu\text{g ml}^{-1}$ benzalkonium chloride, the lowest recommended use concentration for this commonly used type of QAC.

2. **Investigation of bacterial resistance to hatchery disinfectants .**

Willinghan, E. M.; Sander, J. E.; Thayer, S. G.; Wilson, J. L.

Avian Diseases, 40, 3, 510-515 (1996)

ABSTRACT

Three commercial chicken hatcheries were sampled for environmental bacteria. Isolated bacteria were tested for resistance to commercial preparations of quaternary ammonia, phenolic, and glutaraldehyde liquid disinfectants. Bacterial isolates were exposed to several disinfectant dilutions bracketing the dilutions recommended by the manufacturer for 5-, 10-, and 15-min exposure periods before subculturing to broth medium. Approximately 8% of the isolates from two of three hatcheries were resistant to disinfectant concentrations at and above the

manufacturers recommended dilution and time of exposure. Resistant bacteria included *Serratia marcescens*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus badius*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas stutzeri*, and *Enterobacter agglomerans*.

3. **Does a bacterial resistance to disinfectants exist?**

Agolini, G.; Grassi, F. A.

Igiene Moderna , 105 , **NO. 1** , pp 1-27, (1996)

ABSTRACT

Positive results in hospital disinfection rely primarily on the knowledge of the antibacterial and antiviral spectra of different chemicals as well as on the choice of proper concentration and contact time between disinfectant solution and microorganism. However it is also important to know that "natural" or "intrinsic" resistance to some disinfectants may be a peculiar property of some bacteria and viruses. On the other hand, "acquired" bacterial resistance by mutation or by transmission through plasmids is much less frequent and trouble some than for antibiotics. This type of resistance has been demonstrated to occur with a low degree of frequency, severity and stability for formaldehyde, quats and ampholytes among disinfectants , and for clorexidine among antiseptics. The problem of "acquired" resistance is even less important for chlorine disinfectants and detergent polyphenolic mixtures. The biocidal activity of all chemical disinfectants may be greatly reduced by errors of choice, by improper preparation and storage of their diluted solutions, by physical or chemical incompatibilities, by the inactivating property of some organic fluids (such as blood, serum, and in general, proteinaceous material) or by other factors which can inactivate substances highly effective in vitro. Therefore it must be stressed that only by taking into account such interfering factors can chemical disinfectants be properly utilized, thereby contributing to the prevention of transmissible bacterial and viral infections in community and hospital settings.

4. **Resistance to quaternary ammonium compounds in *Staphylococcus* spp. isolated from the food industry and nucleotide sequence of the resistance plasmid pST827.**

Heir, E.; Sundheim, G.; Holck, A. L.

Journal of Applied Bacteriology, 79, 2, 149-156, (1995)

ABSTRACT

The complete nucleotide sequence of the 2.8 kb plasmid pST827 involved in resistance to the quaternary ammonium compound (QAC) benzalkonium chloride in

meat-associated staphylococci was determined. An open reading frame (ORF) similar to the QAC resistance genes *qacC*, *ebr* and *smr* previously reported from clinical staphylococcal strains was identified (*qacC'*). In addition an ORF coding for a protein (Rep827) showing extensive homology to reported replication proteins of Gram-positive organisms was found. The occurrence of known QAC resistance genes (*qacA-C*) among staphylococcal strains isolated from food processing plants was studied by hybridization analysis. Of 191 isolates, 25 were resistant to benzalkonium chloride. Five of these gave no hybridization signals to probes specific for *qacA-C*. Further hybridization analysis indicated that pST827 or closely related plasmids are widespread among QAC-resistant staphylococcal strains. The finding of resistant staphylococci in different areas of the food processing industry indicates that QAC resistance is a potential problem in the food processing industry.

5. **Factors contributing to the survival of poultry associated *Pseudomonas* spp. exposed to a quaternary ammonium compound.**

Langsrud, S.; Sundheim, G.

Journal of Applied Microbiology, 82, 6, 705-712, 1997

ABSTRACT

30% of *Pseudomonas* spp. isolated from poultry carcasses were able to grow in 200 micro g/ml benzalkonium chloride (BC), indicating resistance. *Pseudomonas fluorescens* strains were generally less susceptible than strains of *P. lundensis* and *P. fragi*. An overnight incubation in medium containing 200 micro g/ml BC was sufficient to reduce the susceptibility of 2 *Pseudomonas* strains to the lethal effect of BC significantly. Adding EDTA enhanced the lethal effect of BC, but the effect was reduced after growing cells in medium containing BC and EDTA. Growth in medium with a quaternary ammonium compound (QAC) rendered the cells more susceptible to chlorine, phenolics and alkylaminoacetate. It is suggested that alternating use of QACs with these compounds can be used to avoid build-up of resistant strains. In addition, increased temperatures improved the lethal effect of BC and should be considered when planning disinfection protocols.

6. **Occurrence of and a possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*.**

Rorvik, Liv Marit; Aase, Brit; Langsrud, Solveig; Sundheim, Gunhild

International Journal of Food Microbiology, 62, 1-2, 57-63 (2000)

ABSTRACT

In a study of 200 *Listeria monocytogenes* isolates, 10% were determined to be resistant to benzalkonium chloride (BC). Serial subcultivation of initially BC sensitive

(BCS) and BC resistant (BCR) isolates in sublethal concentrations of BC resulted in enhanced and approximately equal resistance of all strains to the compound. Fifty per cent of the BCR isolates showed resistance to ethidium bromide (EB) as well. A proton motive force (pmf)-dependent efflux of EB was demonstrated in BCR isolates, and in originally sensitive strains adapted to grow in BC. This efflux was not found in BCS strains. The result indicate that BC can induce a broad resistance mechanism based on a pmf-driven efflux pump. There was no indication that this type of resistance was related to resistance to antibiotics.

7. **Specific variations of fatty acid composition of *Pseudomonas aeruginosa* ATCC 15442 induced by Quaternary Ammonium Compounds and relation with resistance to bactericidal activity..**

Guerin-Mechin, L.; Dubois-Brissonnet, F.; Heyd, B.; Leveau, J. Y..

Journal of Applied Microbiology, 87, 5, 735-742 (1999)

ABSTRACT

The role of membrane fatty acid composition in the resistance of *Pseudomonas aeruginosa* ATCC 15442 to the bactericidal activity of Quaternary Ammonium Compounds (QACs) was investigated. The strain was grown in a medium with increasing concentrations of a QAC, benzyldimethyltetradecylammonium chloride (C14) and two non-QACs, sodium dichloroisocyanurate and tri-sodium phosphate. In the presence of C14 only, the strain was able to grow in concentrations higher than the minimal inhibitory concentration. As the strain adapted to C14, resistance to bactericidal activity of the same biocide increased. For the non-QACs, no change was noted when cells were grown in the presence of biocides. The C14-adapted cells showed variations in membrane fatty acid composition. A hierarchical clustering analysis was used to compare all fatty acid compositions of cultures in the presence, or not, of the three biocides used here and another QAC studied previously. The clusters obtained underlined specific variations of membrane fatty acids in response to the presence of QACs. Furthermore, with a simple linear regression analysis, a relationship was shown between the membrane fatty acids and the resistance developed by the strain against the bactericidal activity of C14..

8. **Evaluation of bacterial contamination in disinfectants for domestic use**

Miyagi, F.; Timenetsky, J.; Alterthum, F.

Rev Saude Publica, 34, Oct 2000, 444-448

ABSTRACT

OBJECTIVE: To evaluate disinfectants for domestic use for the presence of bacteria, identify them, and determine their tolerance level to benzalkonium chloride.

METHODS: Fifty-two samples of commercially available disinfectants for domestic use were acquired at random in the metropolitan area of S .o Paulo, Brazil, and analyzed to detect the presence of bacterial contaminants. The isolated organisms were identified and their tolerance level to benzalkonium chloride was determined by broth macrodilution method. **RESULTS:** Sixteen (30.77%) of fifty-two disinfectants sampled were contaminated by Gram-negative bacteria, with counts varying between 10^4 and 10^6 UFC/ml. *Alcaligenes xylosoxidans*, *Burkholderia cepacia* and *Serratia marcescens* were the predominant organisms found. The minimum inhibitory concentration (MIC: mg/ml) of benzalkonium chloride for these bacteria were 2.48, 1.23 and 0.30 to *S. marcescens*, *A. xylosoxidans* and *B. cepacia*, respectively. **CONCLUSIONS:** The disinfectant formulation containing quaternary ammonium compounds (QACs) may be exposed to contamination by Gram-negative bacteria. The MICs of benzalkonium chloride against the isolated bacteria were low, indicating that the bacteria grown in culture media without QACs lost their tolerance to this biocide.