Identification of *Staphylococcus aureus*'s adaptation capacity to antiseptics by microplate-laser-nephelometry

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Introduction

Infection is the main cause of delayed healing in surgical, traumatic and burn wounds, and may lead to the formation of a chronic wound. Therefore, wound dressings with antiseptics are increasingly utilized in the treatment of critical colonized or infected chronic wounds. Antiseptics have a lower potency to induce bacterial resistance than antibiotics; however, concerns have been expressed regarding their overuse and the emergence of bacterial adaptation. *Staphylococcus aureus* is one of the most important pathogen of nosocomial infections and is common complication during the treatment of chronic wounds. Hence, we have used an experimental system employing microplate-laser-nephelometry to test the adaptation capacity of *Staphylococcus aureus* to continued treatment with common antiseptics.

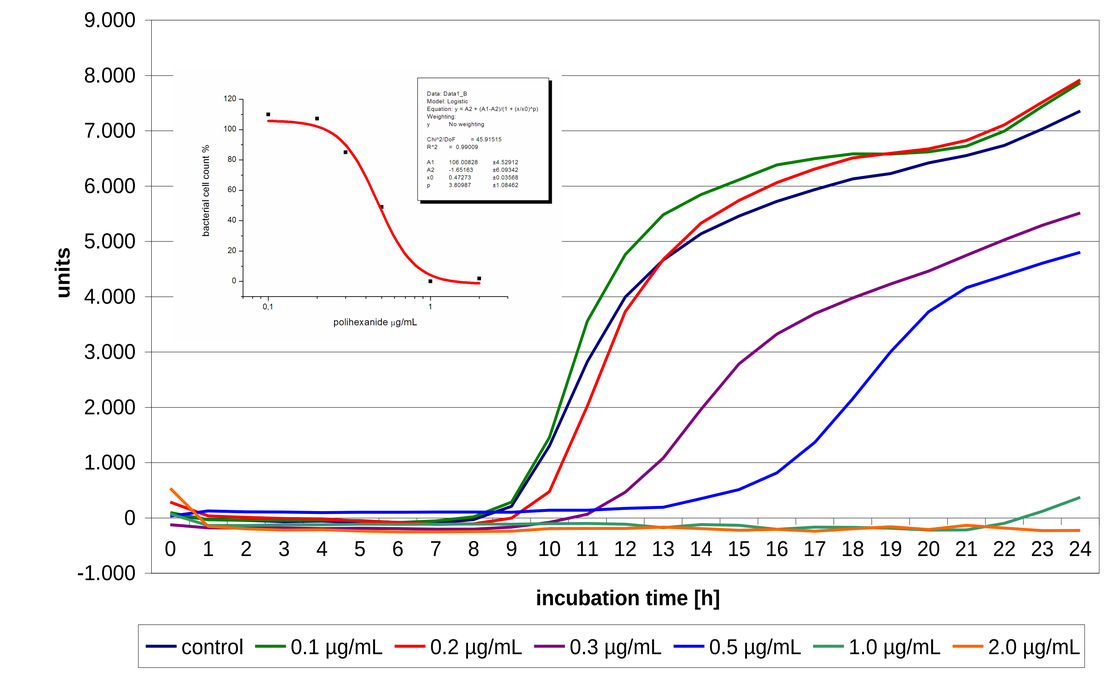

Fig. 1: Nephelometric measurement of the antibacterial activity of polihexanide against Staphylococcus aureus to determine the IC_{50}.

Material & Methods

Following antiseptics have been tested: polihexanide, polihexanide+macrogolum*, polihexanide+betaine**, polihexanide-containing wound dressing extract***, chlorhexidine, PVP-Iodine, silver nitrate, and octenidine. The antibiotic mupirocin was used as a reference. *Staphylococcus aureus* growth was investigated by lasernephelometry and the respective IC_{50} concentrations of the antiseptics tested were determined. Subsequently, the microorganisms were repeatedly incubated with these IC_{50} concentrations for 100 days. Influence of the continued treatment was determined by calculation of the current IC_{50}.

*Lavasept, B.Braun; **Prontosan, B.Braun; ***Suprasorb® X + PHMB, Lohmann&Rauscher

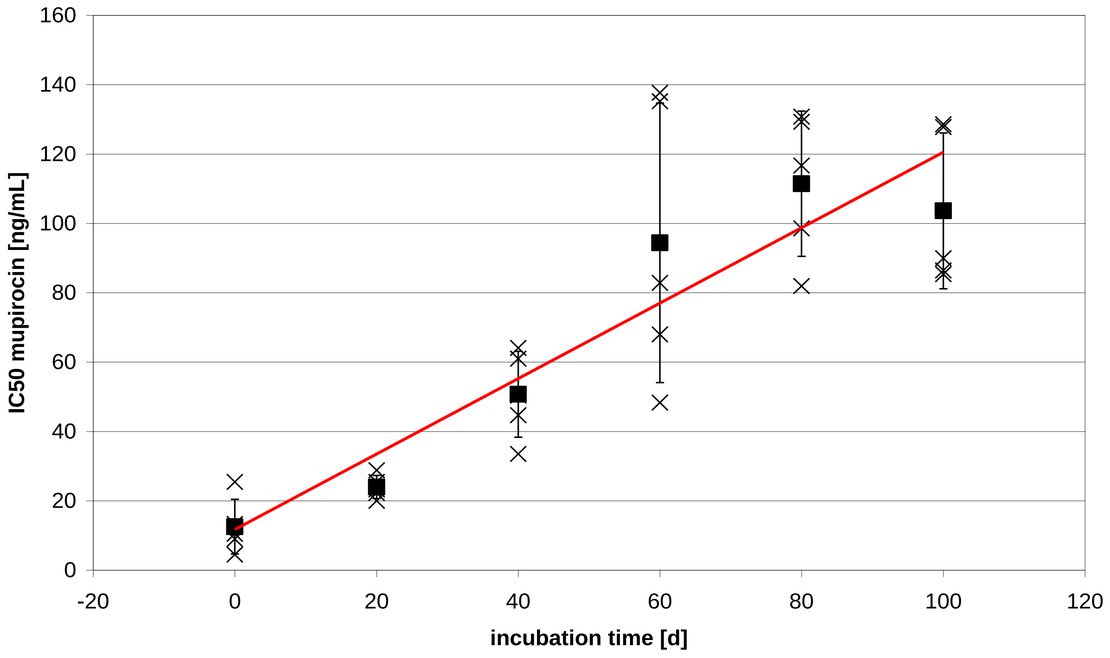

Fig. 2: Development of the IC_{50} under repeated incubation of Staphylococcus aureus with the antiseptic mupirocin for 100 days.

The study was supported with an educational grant of Lohmann & Rauscher GmbH, Rengsdorf, Germany

IX. Congresso Nazionale AIUC «L'Ulcera cutanea al centro del mediterraneo»
15.-18. Settembre 1010, Catania, Italia

substance	initial IC_{50}	IC_{50} after 100 days	p-value
mupirocin	12.5 ng/mL	103.6 ng/mL	<0.001
silver nitrate	5.3 µg/mL	10.8 µg/mL	<0.001
polihexanide	0.53 µg/mL	0.48 µg/mL	n.s.
polihexanide+macrogolum*	0.56 µg/mL	0.59 µg/mL	n.s.
polihexanide+betaine**	0.53 µg/mL	0.91 µg/mL	0.01
polihexanide-containing dressing extract***	10.4 %	9.6 %	n.s.
chlorhexidin	0.56 µg/mL	0.63 µg/mL	n.s.
octenidine	0.51 µg/mL	0.63 µg/mL	0.05
PVP-iodine	0.93 mg/mL	0.62 mg/mL	0.05

Fig. 3: Summary of the experimental results on the development of the IC_{50} during repeated incubation of Staphylococcus aureus for 100 days.

Results

A fast and dramatic increase in the IC_{50} of mupirocin was observed (Fig. 2) while the antiseptics showed a much lower potency to induce adaptation in *Staphylococcus aureus* (Fig. 3). Only the use of silver nitrate provoked a distinct increase of the IC_{50} over time. A slight rise of the IC_{50} was also observed for polihexanide+betain, chlorhexidine, and octenidine. Furthermore, results for PVP-iodine showed a minor decrease of the IC_{50}. Development of the IC_{50} was displayed as slope of the regression line (Fig. 4).

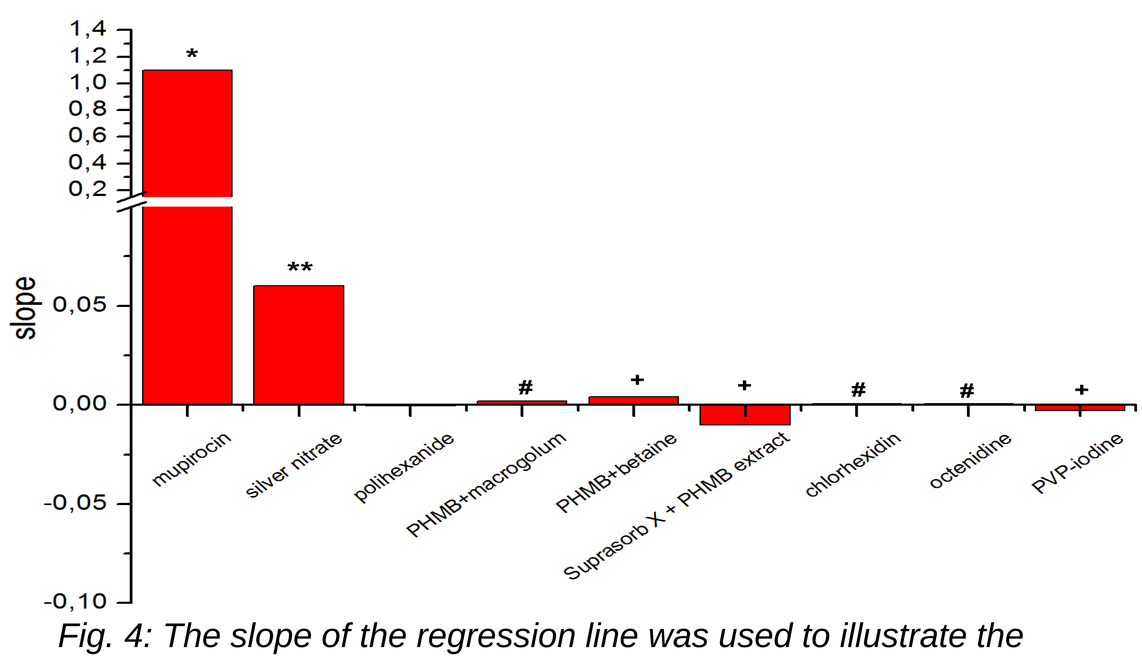

*Fig. 4: The slope of the regression line was used to illustrate the development of the IC_{50} of different antiseptics compared to mupirocin during repeated incubation of Staphylococcus aureus. * significant difference to antiseptics p < 0.001; ** significant difference to other antiseptics p < 0.001, # no significant difference to polihexanide (pure); + significant difference to polihexanide (pure) p < 0.05*

Conclusions

Staphylococcus aureus quickly adapts to high concentrations of the antibiotic mupirocin during repeated treatment. Rising use of antiseptics may result in bacteria that are less susceptible. As wound dressings with antiseptics are increasingly utilized in the treatment of critical colonized or infected chronic wounds it is of interest to determine the risk of triggering formation of resistant microbes. Employing microplate-laser-nephelometry it could be shown that commonly used antiseptics have a low potency to induce adaptation in *Staphylococcus aureus*. Only the IC_{50} for silver nitrate was found to increase distinctly with repeated treatment. Polihexanide, chlorhexidine, octenidine, and PVP-iodine on the other hand showed a low potency to induce adaptation in *S.aureus.* Especially polihexanide seems to be a valid option for an antimicrobial substance in wound dressings for treating chronic wounds as it possess a low risk to induce adaptation and shows a high biocompatibility