Prevention of the dermal colonization of MRSA by the application of algae microparticles

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ABSTRACT SUMMARY
The aim of this study was to investigate the prevention of the dermal colonization of methicillin-resistant Staphylococcus aureus strains (MRSA) by the application of microparticles called Maresome®. Maresome® were prepared from selected microalgae by a novel emulsion technique. They contain lipids and all other components of the microalgae in an encapsulated form. It could be shown that Maresome® prepared from a cyanobacterial strain of the order Nostocales were able to inhibit the dermal colonization of different MRSA strains (North German Epidemic Strain, Col, N315) and even of the vancomycin resistant strain MU50 in the models “mouse ear” and “cow udder teat”. Pre-treatment of the skin with Maresome® reduced the number of attached MRSA by 3 to 4 log units in comparison to the control. We assume that a prophylactic skin care with Maresome® could complete the multibarrier anti-infectious strategy of hospital hygiene.

INTRODUCTION
The occurrence of methicillin-resistant Staphylococcus aureus strains (MRSA) is a world-wide problem [1-3]. In Germany, presently more than 20 % of nosocomial Staphylococcus aureus isolates are MRSA. In other countries, e.g. Japan, France or the United States, the proportion of MRSA exceeds 50 % with increasing infections [4]. Colonized persons are the main reservoir for MRSA and hand-to-hand contact is the main vector for transmission.

In this presentation a new possibility to prevent the dermal colonization of MRSA by use of microalgae preparations for skin care is presented. Microalgae possess a high variety of active compounds like antimicrobial lipids, polynsaturated fatty acids, proteins, vitamins, and minerals and have a high capacity for water retention. The microencapsulation of the biomass of selected microalgae leads to microparticles with the trade name of Maresome®.

EXPERIMENTAL METHODS
The microalgal strain Bio 33 used was isolated from the Baltic Sea near Rügen Island (Germany) and cultivated in BG 11 medium + 0.5 % NaCl. Additionally, commercially available strains of Chlorella, Spirulina and Nostoc (IGV Potsdam, Germany) have been used.

The preparation of microparticles was done using a special emulsion technique according to PCT/DE 03/00747 [5, 6]. A mixture of biomass and ethanol was produced (1:10 v/v). The organic solvent was evaporated. A pre-suspension of the biomass and a surfactant-water mixture was produced by a stirring machine. This pre-suspension was homogenized by a high pressure homogenizer. The particle size distribution was determined with a laser diffractometer (Malvern Mastersizer X, Malvern, UK).

One milliliter of the microparticle suspension was mixed into 1 g w/o-emulsion (Heitland & Petre International GmbH, Celle, Germany). In the same manner, ointments based on biomasses of Chlorella, Spirulina, Nostoc and Oscillatoria were prepared.

TREATMENT WITH MARESOMES®
The microparticles were tested on two animal models: mouse ear and cow udder teats. For each test the skin was treated with Maresome® containing ointment. Maresome®- free ointment was used in the control. The mouse ears and cow udder teats were streaked on Mueller-Hinton II-agar plates and incubated for 90 min at 30 °C. The plates were incubated for 48 h at 30 °C. Thereafter the colonies were checked for MRSA and enumerated. The MRSA test strains used were “The North German Epidemic Strain” ST 247 (NES) Col, Mu 50 and N315. The MRSA strain Col exhibits high methicillin resistance as well as resistance against tetracycline. The strain MU 50 was isolated in 1996 from the pus of a Japanese male baby with a surgical wound infection that was resistant to vancomycin. The MRSA N315 was isolated in a Japanese hospital from pharyngeal smear in 1982. The strains Col, Mu 50 and N 315 are completely sequenced and frequently used in studies [7-9].

RESULTS AND DISCUSSION

Mouse ears

Fig. 1 Influence of Maresome® prepared from biomass of microalgae Bio 33 and other algae Spirulina, Chlorella, Nostoc and Oscillatoria redikei on the colonization (colony forming units) of MRSA NES in the skin model „Mouse ear“ (n=6)
At the direct site of contamination of the mouse ear, we found 675 ± 270 cfu (colony forming units) of MRSA (n=50) at the controls. Bio33-Maresome® completely inhibited the growth of MRSA on the mouse ear (Fig. 1). The other formulations with algae Spirulina, Chlorella, Nostoc and Oscillatoria redkiel had no inhibition effect on the colonization (colony forming units) of MRSA.

After simulation of the skin to skin contact, the mean number of colonies at the control-pre-treatment with Maresome® free ointment) was 601 ± 241 CFU. The pre-treatment of the acceptor with Bio 33-Maresome® led to a strong decline of contacted MRSA NES at the pre-treated acceptor site (significant, p = 0.001). In contrast to MRSA, the normal microbial flora grew with only a small inhibition.

Cow udder teat model: The prevention of MRSA colonization was also detected in the cow udder teats model (Fig. 2). Just as in the mouse ear model, only Maresome® completely inhibited the growth of the NES.

The prevention of MRSA colonization by Bio 33 Maresome® was not only detected for NES but also for the three other multi-resistant MRSA strains (Fig. 2). The inhibition of growth of the strains with a very broad resistance spectrum, including resistance to Vancomycin-resistant MRSA-strain (Mu 50), was remarkable. Just as in the model “mouse ear”, the pre-treatment of the acceptor with Bio 33-Maresome® inhibited completely the colonization of attached MRSA.

CONCLUSION

Maresome® ointment could play an important role in hospital hygiene in the prevention of nosocomial infections. The implementation of a skin care (bio) product as basic hygiene measure in the context of the worldwide accepted barrier system is a completely new concept. Additionally the Maresome® ointment avoids elaborated high tech support or dangerous chemicals. The overall suitability in preventive medicine is evaluated in clinical studies at the moment including settings with high risk prevalence of dangerous pathogens. A specific application could be the combination of skin care with preventive aspects in the management of patients suffering from chronic wounds, which are typically highly contaminated by nosocomial pathogens.

REFERENCES


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