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## *Dear Readers*

We are very happy to present the most recent Electronic Supplement of the EWMA journal (click on the image) which includes reprints of four instructive scientific articles from two German wound healing journals.

Two of these papers were originally published in the journal WUNDMANAGEMENT. WUNDMANAGEMENT is the official publication of the following German-speaking societies:

- Initiative Chronische Wunden e. V. (ICW e. V.)
- Österreichische Gesellschaft für Wundbehandlung (AWA)
- Schweizer Gesellschaft für Wundbehandlung (SAfW)
- Wundnetz Kiel e. V.
- Wundverbund Südwest e. V.
- Wundzentrum Hamburg e. V.

WUNDMANAGEMENT serves as a platform for all those dealing with care, prevention and treatment of chronic wounds. It encourages the exchange of information and experiences relevant to very practical issues as well as the publication of scientific expertise and reports on wound management and health economics.

The readers will be informed of the latest developments in the care of acute and chronic wounds as well as of state-of-the-art scientific insights. In particular, the journal aims to promote the dialogue and fruitful cooperation among all those involved in wound care as well as pragmatic approaches for standardised measures.

The Editorial Board consists of a multidisciplinary team and ensures a high standard of the submitted scientific manuscripts through a peer-review process in accordance with the "Uniform Requirements" of biomedical journal editors.

Although it is primarily directed at the German-speaking readership, extended abstracts of the main articles are published in English to facilitate international contacts.

Despite the fact that approximately 90% of the German population are members of a statutory health insurance which includes access to wound care and bandaging materials, considerable differences in individual wound treatment can still be observed. From an economic viewpoint, the optimisation of wound care through prevention of wounds, early detection and specific treatment harbours a significant money saving potential. Thus, the recent and current activities of the Initiative Chronische Wunde e. V. concentrate on pooling clinical and scientific data, on education and on public relations.

The articles were carefully selected to reflect these activities and we are proud to have the opportunity to make them available to an international readership by publishing them in the EWMA journal.

We hope that these articles will provide valuable information to all those who are engaged in the care of patients suffering from chronic wounds. In particular, we hope that readers will be able to implement the insights gained from these articles in their daily routines for the benefit of their patients.

*Knut Kröger, Wolfgang Niebel*  
WUNDMANAGEMENT

## *Dear Readers*

We are very happy to make a contribution to the October issue of the EWMA Electronic Supplement. We are very grateful for the invitation and we would like to thank the EWMA for the good cooperation in 2011.

Two of the following articles were originally published in the “Zeitschrift für Wundheilung” (ZFW) (Journal for Wound Healing). The ZFW is an body of the “Deutsche Gesellschaft für Wundheilung und Wundbehandlung e.V.” (DGfW) (the German Society for Wound Healing and Wound Treatment).

The DGfW is a non-profit organisation with the aim of supporting the transfer of knowledge between science and practice in wound care. It was established in 1994 as a scientific society and has been a member of the “Arbeitsgemeinschaft der Wissenschaftlich Medizinischen Fachgesellschaften e.V.” (AWMF) (German Scientific Medical Society) since 2004.

The statutory mission of the DGfW is to gain and promote new knowledge of wound healing and wound treatment in practice, research and science in the fields of diagnosis, therapy and prevention. To that end the society supports targeted projects within the scope of working groups, an academy and a study centre.

The DGfW is financed through membership fees and donations. All funds are used exclusively to fulfil the statutory mission.

The society acts as editor of the ZFW. The journal is published quarterly by the publishing house “Congress Compact Verlag Berlin”. The content of the ZFW addresses different medical disciplines and other professions involved in wound care. Reports concerning possible active and preventive measures for improving the situation of people suffering from chronic wounds provide new impetus for everyday practice. Thus, an overview of useful and targeted treatment strategies is given to the reader. Hence, the ZFW pursues the objective of being a scientific journal with a focus on practical implementation.

The two articles presented are taken from two different issues of the ZFW published during the last five years.

*Zeitschrift für Wundheilung*

# Effector Molecules of Innate Immune Response: New Therapeutic Options for Diabetic Wounds?

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## Summary

Diabetes mellitus is one of the most frequent chronic diseases. Infected and chronic wounds present a major complication in patients with chronic diseases such as diabetes and are challenging for health care providers and the health care system. Antibiotic resistant infections are emerging and common therapeutic strategies are often insufficient. Furthermore, the insight into the process of diabetic impaired wound healing and immunological interactions is not elucidated sufficiently to date.

Host defense peptides are effector molecules of the innate immune system and possess antimicrobial and immunomodulatory properties. They are expressed in a number of human cell

types and tissue. Host defense peptides are considered to play a key role in immune response and interaction between innate and adaptive immunity. This review will focus on host defense peptides as effector molecules in immune response, particularly in the process of wound healing and soft tissue infection in diabetic patients. Furthermore we will discuss its future potential as a novel therapeutic option for the treatment of infected diabetic wounds.

## Key words

Host defense peptides, infection, innate immune system, wound infection, diabetes

## INTRODUCTION

Diabetes mellitus is one of the most frequent chronic diseases. It affects around 10% of the German population. According to the American Diabetes Association, more than 25% of currently 14 million diabetics in the US will eventually suffer from a wound healing disorder at some point during their illness. It is estimated that diabetes is the underlying disease responsible for more than 60% of all non-traumatic lower leg amputations. Impaired wound healing as a consequence of diabetes has a number of other effects such as vasculopathy, neuropathy or foot deformities<sup>(52,53)</sup>. On the molecular level, an increase in inflammatory immune cells, inadequate migration of neutrophil granulocytes and macrophages, reduced chemotaxis and reduced cell proliferation and migration in the epidermis have so far been identified as causes of wound healing disorders<sup>(25,29,93)</sup>. A common yet severe complication of diabetic wound healing is infection. It often results in delayed wound healing or osteomyelitis<sup>(40)</sup> and, by way of bacteremia, can even lead to sepsis<sup>(89)</sup>. The treatment of

infected diabetic wounds thus poses a great medical and health economic challenge<sup>(1,75)</sup>. *Staphylococcus aureus* (*S. aureus*) is one of the most common bacteria (76%) found in infected diabetic wounds. Already in 1949, some *S. aureus* bacteria were found to be penicillin-resistant. In the 1960s, the first methicillin-resistant bacterial strains (MRSA) were described<sup>(4)</sup> and in 2002, a first case of vancomycin-resistant *S. aureus* (VRSA) was discovered in a patient suffering from an infected diabetic foot ulcer<sup>(22)</sup>. This increasing limitation of treatment options and the fact that the understanding of pathophysiological and immunological mechanisms in diabetic wound infections remains incomplete highlight the urgency of finding new therapeutic approaches and options for treating infected diabetic wounds<sup>(7,76)</sup>. A therapeutic approach covering antimicrobial activity, immunomodulatory and anti-inflammatory properties could dramatically improve the treatment of chronically infected wounds.

Tab. 1: Overview of the point of origin and the immunomodulatory activity of the Host Defense Peptides LL-37 and  $\beta$ -defensins

Host Defence Peptide	Expressed in the following cell and tissue types	Immunomodulatory function
hCAP18/LL 37	Neutrophil granulocytes, epithelial cells of the skin, the gastrointestinal tract, the genitourinary tract and the respiratory tract, mast cells, monocytes/macrophages, CD4 cells, myelocytes, wound fluid, seminal fluid, cervix, vagina, esophagus, mouth, tongue	Broad antimicrobial activity as well as antifungal and antiviral activity. Capable of binding endotoxin; modulation of the pro-inflammatory immune response through induction of chemokines and cytokines, chemotaxis, influence on cell proliferation and differentiation, enhances reepithelialization and angiogenesis, induction of gene expression, induction of the adaptive (innate) immune respons.
Human $\beta$ -defensin-1	T cells, dendritic cells, epithelial cells of the skin, the gastrointestinal tract, the genitourinary tract and the respiratory tract, astrocytes, microglial cells, uterus, pancreas, kidney, lung, prostate, placenta, thymus, testicles, vagina, gingiva, conjunctiva, cornea, mammary gland, salivary and lacrimal glands, tongue, joints	Broad antimicrobial activity as well as antifungal and antiviral activity; recruiting of immune cells, activation of the adaptive immune response through induction of chemokines and cytokines such as IL-8, -18, and -20, degranulation of mast cells, enhances phagocytosis, induction of dendritic cell maturation through TLR-4, LPS and LTA-binding properties, inhibition of TNF- $\alpha$ production, induction of matrix metalloproteinases (MMP) and inhibition of MMP inhibitors (TIMP)
Human $\beta$ -defensin-2	Mast cells, T cells, dendritic cells, epithelial cells of the skin, the gastrointestinal tract and the respiratory tract, conjunctiva, cornea, astrocytes	
Humanes $\beta$ -Defensin-3	Neutrophil granulocytes, monocytes, T cells, epithelial cells of the skin, the gastrointestinal tract, the genitourinary tract and the respiratory tract, uterus, placenta, testicles, esophagus, heart, muscle, tongue, kidney, liver, pharynx, tonsils, salivary glands	

## EFFECTOR MOLECULES IN THE INNATE IMMUNE SYSTEM

Host Defense Peptides (HDP) are part of the human innate immune system<sup>(97)</sup>. To date, more than 900 HDPs have been discovered in eukaryotes. These small, cationic peptides show a broad antimicrobial activity against gram-positive and gram-negative bacteria as well as against some fungi and viruses<sup>(33,41)</sup>. In addition, HDP have been found to have immunomodulatory properties. They are capable of binding bacterial toxins such as lipopolysaccharide acid (LPS) and lipoteichoic acid (LTA) directly and indirectly or inhibiting their pathways<sup>(24,55,84,87)</sup>. These bacterial toxins are of critical importance during sepsis and can be enhanced if a large number of bacteria are killed as a consequence of antibiotic treatment. An experimental therapeutic application of HDPs in a small animal model was found to significantly reduce mortality in sepsis<sup>(20,85)</sup> and to considerably reduce the bacterial contamination of wounds<sup>(88,89)</sup>. Further, it was shown that certain human HDPs actively contribute to wound healing (angiogenesis, keratinocyte migration, reepithelialization)<sup>(45,69,82,88)</sup> and interact with growth factors<sup>(82)</sup>. Current studies have shown that, within the framework of immune response, human HDPs do not only have a direct antibacterial effect but they also show a strong immunomodulatory activity supporting the fight against bacterial infections<sup>(66,94,95)</sup>.

The cathelicidin (hCAP18/LL37) and the defensin families ( $\alpha$ - and  $\beta$ -defensins) are among the best-researched human HDPs<sup>(68,69,80)</sup>. They can be found in various cell and tissue types in the human body where they are being synthesized as propeptides and either continuously expressed or released upon specific immunological triggers<sup>(17,47,55)</sup>.

## ANTIMICROBIAL ACTIVITY

The group of HDPs first became known when its direct antimicrobial activities were described for the first time<sup>(41)</sup>. They were and still are therefore referred to as antimicrobial peptides. Their activity targets bacteria, fungi, parasites and sometimes also viruses. This activity is achieved through direct effects at the membrane, such as penetration via hydrophobic and electrostatic interactions with lipid A or LPS of the cell wall and subsequent pore formation (development of transmembrane channels)<sup>(42,43)</sup> or effects inside the microbes through interaction with bacterial DNA and other intracellular targets eventually leading to cell destruction<sup>(11,26,35,41,90,96)</sup>. HDPs are particularly interesting with regard to a potential clinical usage against the background of the spread of antibiotic-resistant bacteria<sup>(59,86)</sup>.

## IMMUNOMODULATORY FUNCTION

HDPs also influence the immune reaction of the innate and adaptive immune systems in various ways. They can directly or indirectly neutralize bacterial toxins and they have both pro- and anti-inflammatory properties. HDPs also facilitate apoptosis, influence cellular differentiation, induce cytokine and chemokine release, stimulate mast cell degranulation and have chemotactic properties<sup>(3,9,23,30,46,66-68,78,81,94)</sup>. Toll-like receptors (TLR) are another key mechanism the innate immune system uses to recognize bacteria. These TLRs can be considered a bridge between the innate and the adaptive immune system<sup>(92)</sup>. Upon examining injuries in human skin, the authors of a recently published study were able to detect an increased expression of the CD14 receptor and the Toll-like receptor 2 (TLR-2) in keratinocytes in the wound environment. They also described that an increased expression of TLR-2 and CD14 induces an increased expression of LL37, a human HDP (79). Cathelicidins and defensins are able to attract T-lymphocytes, dendritic cells and monocytes. In addition, they were also found to stimulate the release of pro- and anti-inflammatory substances, such as interferons and interleukins, from T-lymphocytes. These results indicate that HDPs are not only effectors of the innate immune system but they also play a key role in activating the adaptive immune system<sup>(5,12,60,61,63,64)</sup>. Furthermore, the results also demonstrate that HDPs exert their antimicrobial activity also via their immunomodulatory function<sup>(6)</sup>.

## HOST DEFENSE PEPTIDES AND WOUND HEALING

As long as the protective skin layer is intact, HDPs make an important contribution to the skin's bacterial balance, just like RNase 7 or psoriasin<sup>(8,80)</sup>. In case of an injury or an infection of the skin, certain HDPs are increasingly synthesized by neutrophils and keratinocytes and then released. For instance, a continuous and inducible expression of LL37, a human cathelicidin, and the human  $\beta$ -defensins-2 and -3 in epidermal keratinocytes was described<sup>(26,31,44)</sup>. Another study has shown that the insulin-like growth factor-1 (IGF-1) and the transforming growth factor  $\beta$  (TGF- $\beta$ ) have a stimulating effect on the expression of LL37 in wound tissue<sup>(82)</sup>. The other way around, the porcine cathelicidin PR-39 stimulates dermal fibroblasts to synthesize a larger amount of extracellular matrix proteoglycans syndecan-1 and -2 which, in turn, are responsible for the activation of many growth factors. In case of a shortfall, wound healing can be delayed or might remain ineffective<sup>(14,27,34)</sup>. Heilborn et al, have shown a high LL37 concentration in wounds whereas much lower concentrations were found in chronic wounds<sup>(45)</sup>. Several other groups were able to demonstrate a protective

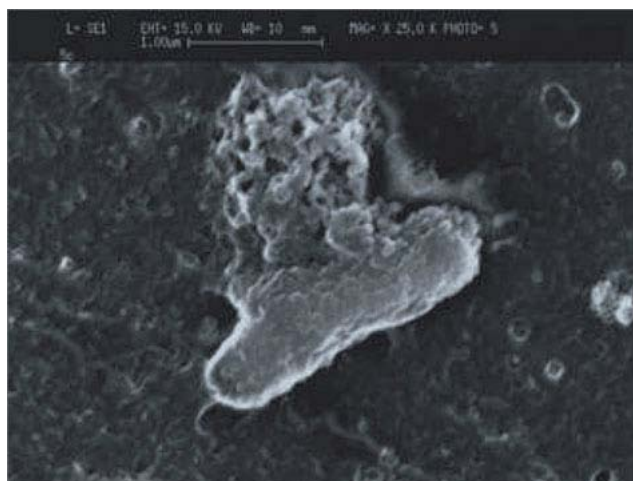


Figure 1: *Escherichia coli* bacterium destroyed by the host defense peptide protegrin-1. Electron microscopic image taken upon pore formation and destruction of the bacterial membrane.

effect of LL37 in bacterial skin infections, especially infections with *P. aeruginosa*, *S. aureus* and Group A *Streptococcus*<sup>(10,26,70,71)</sup>. The Ong lab described an improved immunocompetence with respect to *S. aureus* and an increased intralesional expression of LL37 in psoriasis patients. By contrast, patients suffering from atopic dermatitis showed a decreased LL37 expression<sup>(58,71)</sup>. These findings could serve as an explanation for the susceptibility of atopic dermatitis patients to infections, especially as patients suffering from psoriasis have shown effective defense mechanisms against bacterial infections<sup>(16)</sup>. Our group's results have shown that the application of the human HDP hCAP18/LL37 to experimental, infected burn wounds within the framework of gene therapy can lead to a highly significant reduction of *Pseudomonas aeruginosa*<sup>(50)</sup>. Upon conducting an animal experiment study, Koczulla et al. reported that application of LL37 resulted in a proangiogenic effect and increased revascularization<sup>(56)</sup>. Another study conducted by our group confirmed the angiogenic effect of LL37<sup>(88)</sup>. Various groups have been able to identify and localize those cell types in skin and in burned areas expressing the HDP LL37 and the  $\beta$ -defensins -1, -2 and -3 by using immunohistochemical methods and fluorescence deconvolution microscopy<sup>(73,74,91)</sup>. The authors concluded that the cells located in deeper dermal and subdermal layers of the burned skin increasingly produced HDPs in order to maintain a certain barrier against intruding organisms<sup>(73,74)</sup>.

The Niyonsaba lab found that  $\beta$ -defensins stimulate the migration and proliferation of epidermal keratinocytes and thus might make a contribution to the cutaneous wound healing process<sup>(69)</sup>. Furthermore, an increased concentration of human  $\beta$ -defensin-2 had been found in chronic and acute wounds<sup>(13)</sup>. It turned out that in skin infections of keratinocytes with *S. aureus*, human  $\beta$ -

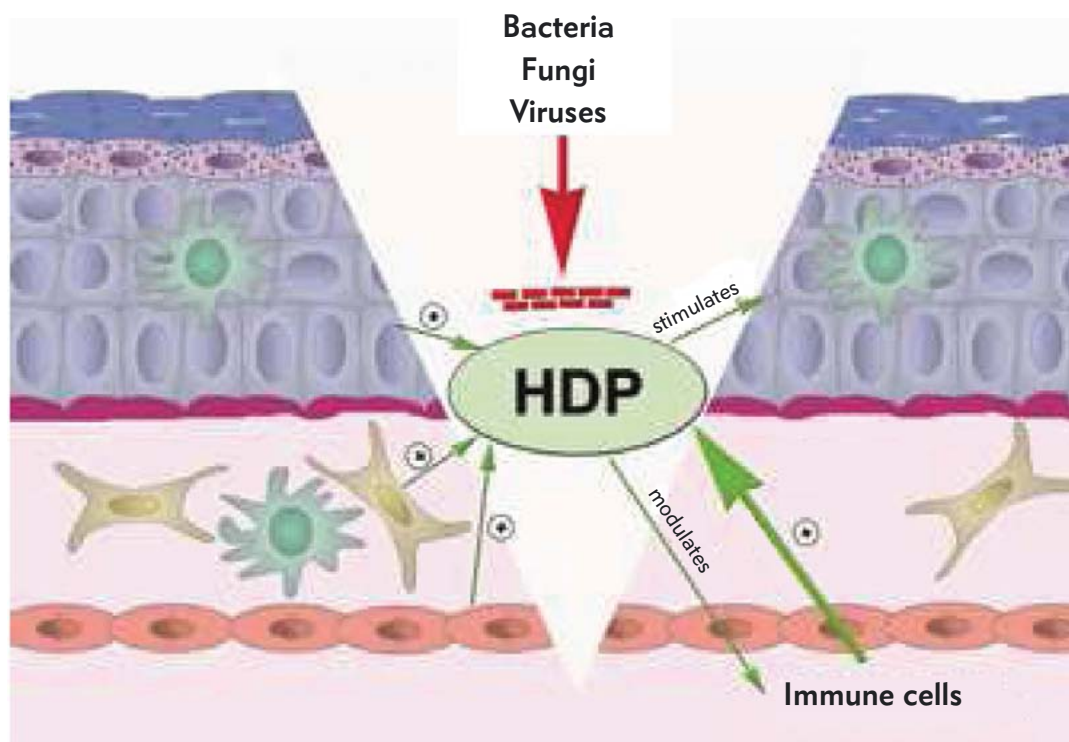


Figure 2: Diagram of the immunomodulatory function of host defense peptides.

defensin-3 is induced by TLR-2 and the epidermal growth factor receptor (EGFR)<sup>(62,83)</sup>. Kisich et al. showed that the ability of human keratinocytes to resist infections (*S. aureus*) depends on their  $\beta$ -defensin-3 expression<sup>(54)</sup>. We were able to prove in an experimental set-up that the designer peptide novispirin G-10 shows a high antimicrobial activity against *P. aeruginosa* in infected wounds and burn wounds. The fact that the synthetically produced novispirin G-10 had fewer cytotoxic properties than the natural original HDP ovispirin turned out to be beneficial<sup>(51,89)</sup>. Furthermore, our group found out in the animal model that the human HDP histone H1.2 is effective in infected burn wounds<sup>(49)</sup>. In summary, these studies show that application of human HDPs in experimental models delivers some promising results.

## OUTLOOK

Current publications suggest that an *in vitro* combination of human HDPs produces much better immunomodulatory results than the respective mono-applications: Niyonsaba et al. examined the expression of interleukin-18 (inducing a number of important immunomodulators such as IFN- $\beta$ , GM-CSF, IgE, IL-4, IL-5, IL-10 and IL-13) in keratinocytes upon incubation with the human HDPs  $\beta$ -defensin-1, -2, -3, -4 and LL37 alone and in combinations of two, three and four. It was shown that a combination of human  $\beta$ -defensin-2 and -3 with LL37 can cause an immunomodulatory effect up to 10 times higher than the respective mono-applications. A synergistic effect between human  $\beta$ -defensin-2 and LL37 was

also discovered with respect to antimicrobial activity against *S. aureus* and *E. coli*<sup>(15,65)</sup>. Further studies have shown synergistic effects between the HDPs lactoferrin and LL37<sup>(2,36)</sup>. The results at hand indicate a complex interaction between these effector molecules of the innate immune system in the immune response and give an interesting impetus towards developing new treatment options. Another important aspect in the use of HDPs is their combination with regular antibiotics. For instance, a combination of the HDP temporin L and a  $\beta$ -lactam antibiotic produced the highest survival rates in an experimental sepsis model<sup>(38)</sup>. The results for a cecropin-B- $\beta$ -lactam combination were similar<sup>(37)</sup>. A combination of vancomycin and the two HDPs cecropin A and magainin B proved equally successful<sup>(21)</sup>. Interestingly however, a negative effect was reported for the application of the bacteriostatic antibiotics erythromycin and chloramphenicol when combined with cathelicidins. If growth of *E. coli* or *S. aureus* is inhibited by these antibiotics, HDPs and the serum complement will also be less effective. The authors suspect that the negative interaction between bacteriostatic therapeutics is due to the fact that HDPs primarily target propagating bacteria<sup>(57)</sup>. A significant obstacle standing in the way of using HDPs in clinical practice seems to be the cytotoxic effect<sup>(18,72,77,85)</sup>. So-called "designer" HDPs, i.e. modifications of native HDPs, could be a possible solution to this challenge. Ideally, they have the same or an even more powerful antimicrobial activity while, at the same time, being less cytotoxic. The designer peptide novispirin-G10 for instance has a lower hemolytic and cytotoxic activity while *in vitro* showing a broad activ-

ity against gram-positive and gram-negative bacteria<sup>(89)</sup>. Ciornie et al. described a modified LL37 peptide fully capable of chemotaxis and binding LPS but causing a lot less hemolysis than the native peptide<sup>(19)</sup>. New forms of application such as gene therapy<sup>(50)</sup> or the development of HDPs targeting specific bacteria, the so-called STAMPs (specific targeted antimicrobial peptides)<sup>(28)</sup> shine a light on promising, new and alternative options for treating inflammations and infections.

## CONCLUSIONS

HDPs show some interesting properties with respect to the treatment of infected wounds. If combined with conventional treatment methods, the usage of HDPs could significantly enrich the current spectrum of therapeutic options. However, at current scientific knowledge, side effects which have not yet been finally determined, promising developments which are still in the experimental phase (such as modified HDPs) and the significant barriers of clinical investigation and approval still stand in the way of a clinical application. So far, very little data is available on

the role HDPs could play with respect to diabetes mellitus: Froy et al. were able to show in a rat model that defensin expression in different organs of diabetic rats was very different from values measured in healthy rats<sup>(32)</sup>. Another study has shown decreased expression of rat  $\beta$ -defensin-1 (rBD1) mRNA in the kidneys of diabetic rats and mice<sup>(48)</sup>. The authors concluded that there might be a connection to urinary tract infections occurring frequently in diabetes patients. HDP expression and their interaction in diabetic wound healing disorders in other animal models or in humans has not been further investigated yet<sup>(32)</sup>. Therefore, the mechanisms of action and the interactions of HDPs in organisms with a diabetic metabolism and diabetic wound tissue have not yet been fully clarified. To allow for a better understanding of pathophysiological processes in a diabetic and infected wound environment, the role of HDPs in vivo and with respect to tissue regeneration, infection and immune response must be further investigated in the near future. The knowledge generated that way could provide important new stimulus for the understanding and treatment of diabetic wound infections.

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# Enhanced Resistance against Local Antiseptics in MRSA Bacterial Strains

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## Summary

**Introduction:** The efficacy of skin antiseptics, wound irrigants and antiseptic wound dressings is tested using a standardized lab test. To that end, a standardized bacterial lab strain is employed. However, it remains unclear whether the strain used for laboratory analysis is comparable to clinical (MRSA-) isolates.

**Material and Methods:** Susceptibility of three laboratory strains (ATCC) was compared to four MRSA-wildtype strains ("International Basic Set", Robert Koch Institute Wernigerode, Germany) using PHMB-based products (Prontosan, Lavanid and Wunddressing; 0.005% concentration). The quantitative suspension assay (EN 1040) was used for that.

**Results:** The standardized laboratory strain (*S. aureus* ATCC 6538) showed no significant difference compared to a second laboratory strain (*S. aureus* ATCC 29213). The MRSA laboratory strain ATCC 33592 showed 8-fold (0.9 log, highly significant) increased resistance towards PHMB-products compared to the standard strain (ATCC 6538). For each of the 4 MRSA wildtype strains, highly significant elevated resistance towards the standard laboratory strain was detected: MRSA wildtype strains on average showed a 160-fold (2.2 log)

increased resistance with a maximum of 400-fold (MRSA 93-0134: 2.6 log).

Even towards a standardized MRSA lab strain (ATCC 33592), wildtype MRSA (RKI 93-0134) showed 80-fold elevated resistance (1.9 log; highly significant).

**Conclusion:** This study shows that clinical isolates of MRSA possess highly significant elevated resistance towards local antiseptics compared to standardized strains used for standardized product testing. Products containing low concentrations of PHMB should be evaluated using MRSA wildtype strains to guarantee clinical efficacy and safety. The choice of the antiseptic product used in daily health-care routine should be critically reevaluated in view of the literature and available data.

**Key words:** Chronic wound, antiseptic, ATCC, *Staph aureus*, PHMB

## INTRODUCTION

Standardized bacterial strains are used for determining the microbicidal effect of products such as antiseptic solutions, irrigation solutions, moist and dry wound dressings. The procedures for assessing antiseptic products with the above-mentioned strains has been established and is standardized according to national and international (EN 1040, EN 13727) criteria<sup>(1,2)</sup>.

Laboratory strains are offered by international strain banks collecting, breeding, storing and shipping cultures of microorganisms to destinations all over the world such as the "American Type Culture Collection" (ATCC), Manassas/VA or the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig/Germany. Generally, the lab use of these strains for standardized test procedures forms the basis of comparable data. Assessing the efficiency of antiseptic products in different laboratories will lead to more objective results.

However, the basic prerequisite for any in vitro evaluation of an antiseptic product with using a lab strain is that the strain used represents the characteristics of the wildtype

strains occurring in clinical practice also with regard to their susceptibility. Against the background of health and safety regulations, most microbiology labs prefer to work with lab strains as they pose a containable risk and allow for a "targeted use" as defined in the Technical Rules for Biological Agents<sup>(3,4)</sup> published by the German Health and Safety Agency at a mostly low virulence. This helps to minimize the risk of infection for the staff involved.

Accordingly, the standard EN 1040<sup>(1)</sup> notes in its introduction that "there is no indication of a virulence of the strains used under this standard". Thus, according to the test standard, the following test strains can usually be used as a substitute for the infecting agent *Staphylococcus aureus*: ATCC 6538 and ATCC 29213. Furthermore, in order to test methicillin- or oxacillin-resistant *Staphylococcus aureus* (MRSA), the lab strain ATCC 33592 can be used. The susceptibility of these lab strains then allows for conclusions as to the similar susceptibility of wildtype strains occurring in clinical practice towards the microbicidal products tested.

On the other hand, clinically relevant MRSA wildtype strains e.g. occurring during (sometimes epidemic) infection outbreaks are also collected and characterized. The Robert Koch Institute's (Wernigerode) "International Basic Set" currently contains a total of 41 strains used as national and international MRSA reference strains. These not only include nosocomial MRSA wildtype strains isolated during hospital outbreaks but also so-called "community-acquired MRSA strains". These strains contain Panton-Valentine leukocidin, a special toxin, and started to spread among the US normal population in 1992<sup>(5,6,7)</sup>.

However, a few years ago, these CA-MRSA strains were first detected in Germany as well<sup>(8)</sup>. They can cause severe skin and soft tissue infections, in rare cases can lead to necrotizing pneumonia and, in isolated cases, can even result in the patient's death<sup>(9)</sup>. Currently, several different agents are being used in antibacterial solutions or wound dressings for antiseptic wound treatment including a moist wound environment. Due to its relatively low toxicity<sup>(10,11,12,13)</sup> PHMB has prevailed over the traditional PVPI preparations (Betaisodona, Braunol) and Octenisept<sup>(14)</sup>. The products available now range from PHMB-containing antiseptics with an active ingredient concentration of 0.02% (Lavanid, Lavasept, Lavasorb, Serasept) and irrigation solutions containing 0.01% of PHMB (Prontosan) to PHMB-impregnated wet dressings at 0.3% (Suprasorb-X + PHMB) and, since a few months ago, a dry wound dressing containing (according to the manufacturer's information) 0.5% PHMB.

When deciding on the active ingredient concentration – also with respect to PHMB products – it is important to take the respective objective (local infection treatment, infection prevention) and the type of application (wound dressing, irrigation) into consideration in order to ensure the desired killing or conservation effect. On the other hand, the toxic side effects on the three cell types relevant for wound healing (fibroblasts, keratinocytes and endothelial cells) need to be minimized to avoid negative effects on granulation and epithelization. Therefore, the PHMB concentration used on chronic yet clean wounds should be as low as possible.

The range of products offered by the industry with PHMB concentrations varying by a factor of 25 (!) (0.02 to 0.5%) despite very similar advertising indicates that a coherent treatment concept is yet to be developed. The objective of this study was to test the susceptibility of *Staphylococcus aureus* strains and MRSA laboratory strains to PHMB-containing antiseptics, wound irrigation solutions and wound dressings.

## MATERIAL AND METHODS

### Material

- Laboratory strains:
  - *Staphylococcus aureus* (SA) ATCC 6538, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig/Germany, Item No.: 799
  - *Staphylococcus aureus* (SA) ATCC 29213, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig/Germany, Item No.: 2569
  - *Staphylococcus aureus* (MRSA) ATCC 33592, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig/Germany, Item No.: 11729
- Wildtype strains of the Robert Koch Institute's "International Basic Set" of national and international MRSA reference strains:
  - MRSA 93-0134 → North German epidemic strain, MLST type 247
  - MRSA 98-0406 → North German epidemic strain, MLST type 247
  - MRSA 05-1825 → Community MRSA, MLST type 8
  - MRSA 05-2040 → Community MRSA, MLST type 8
- PHMB-containing solutions and wound dressings:
  - Prontosan wound irrigation solution, Mfr: B. Braun Melsungen, Melsungen/Germany, Item No.: 400403, Lot: 8163M14, expiration date: 03/2011
  - Lavanid 2, Serag-Wiessner KG, Naila/Germany, Lot: 220193, expiration date: 04/2011
  - Covidien Kendall™ AMD, Tyco Health Care Group LP, Mansfield/USA, Lot: 907710, expiration date: 03/2012
- Tryptone disinhibitor solution
  - 1.0 g tryptone, 8.5 g NaCl, 30 g Tween 80, 30 g saponin, 1.0 g histidine, 1.0 g cysteine, 3 g lecithin ad 1 l bidest water
  - Diluent (NaCl with tryptone: 1.0 g tryptone, 8.5 g NaCl ad 1 l bidest water)
  - Tryptone, Mfr: Oxoid, Hampshire/UK, Item No.: L42 (500g)
  - Tween 80 (Polysorbate 80), Mfr: Sigma Aldrich Chemie, Steinheim/Germany, Item No.: P1754-500 ML
  - Saponin, Mfr: Sigma Aldrich Chemie (Riedel-de-Haën®), Steinheim/Germany, Item No.: 16109
  - L-cysteine, Mfr: Merck, Darmstadt/Germany, Item No.: 1.02838
  - L-histidine, Mfr: Merck, Darmstadt/Germany, Item No.: 1.04351
  - Lecithin- $\alpha$ -phosphatidylcholine from egg yolk, Mfr: Fluka Chemie GmbH, Steinheim/Germany, Item No.: 61755

## Methods

Firstly, 20 ml of isotonic saline solution were applied to the (dry) wound dressing Covidien containing 0.5% of PHMB (according to the manufacturer's information),. Then, the dressing was put into an incubator at 37°C for 2 hours and afterwards was centrifuged (10 min./ 3000 rpm). Just like the irrigation solution Prontosan (concentrations tested: 0.005% and 0.08% PHMB) and the antiseptic Lavanid (solution, concentration tested: 0.005% PHMB), the dressing was tested in a quantitative suspension test for its "bactericidal effect" according to EN 1040 (basic test)<sup>(1)</sup> applying the dilution-neutralization method. Prontosan and Lavanid were applied to the test strains for 2 minutes at room temperature (22°C). The Covidien solution was applied on n=7 and Lavanid on n=6 different days for 200 minutes, both exclusively on Sa ATCC 6538 (concentration tested: 0.005 % PHMB). In addition, the Covidien solution with a PHMB concentration 10 times higher than the one previously tested (0.05%) was applied to the bacteria on n=5 different days for 200 minutes and 24 hours, respectively. For every laboratory and wildtype strain, Prontosan was tested on at least n=6 different days.

## Statistics

For statistical analysis, the unpaired t-test was used for assessing the significance of potentially differing means. The equality of variances of both samples was assessed using Levene's test.

## RESULTS

Table 1 and Figure 1 show the logarithmic reduction factors of the laboratory strains (*Staphylococcus aureus* ATCC 6538, 29213 and the lab-MRSA ATCC 33592) which reduced the original bacteria concentration by 3.8 and 3.1 log, respectively. Upon applying the same antiseptic concentration (0.005% PHMB/5% Prontosan) the 4 strains originating from clinical outbreaks which had been obtained from the "International Basic Set" of national and international MRSA reference strains of the RKI could only be reduced by an average of 1.7 log, the resistant wildtype strain could only be reduced by 1.2 log. Figure 1 shows the mean results and standard deviations; Table 2 depicts the significances; the higher resistance of all 4 clinically relevant MRSA to PHMB has been shown to be highly significant. Table 3 displays the bactericide effects of Lavanid and Covidien AMD on the lab strain *Staphylococcus aureus* ATCC 6538 in standardized PHMB concentrations (0.005%). Under similar test conditions, Lavanid led to a 3.9 log reduction after an application time of 2 minutes, while after being applied for 200 minutes, Covidien only achieved a 0.25 log reduction. Even if the Covidien concentration is increased 10-fold (0.05% PHMB), the initial amount of bacteria can only be reduced by 2.0 log within 200 minutes and 24 hours of application time are necessary at that same concentration in order to achieve a reduction of more than 5.6 log.

Table 1: Comparing the susceptibility of *Staphylococcus aureus* to and MRSA lab strains to that of MRSA wildtype strains to Prontosan with a PHMB concentration of 0.005% and 0.08%, AT: application time

Quantitative suspension array with 5% Prontosan										on	80% Protosan	
AT (min)	Temperature (°C)	Sa ATCC 6538		Sa ATCC 29213	MRSA ATCC 33592	MRSA 93-0134	MRSA 98-0406	MRSA 05-1825	MRSA 05-2040		Sa ATCC 6538	MRSA 93-0134
2	22	3,9	3,4	3,4	3,0	1,0	2,6	1,9	1,7		<7,58	<7,42
		4,1	3,6	4,1	3,0	1,2	2,2	1,3	1,2		<7,55	<7,42
		4,2	3,2	4,2	3,3	1,0	2,5	1,4	1,3		<7,59	<7,2
		4,2	4,1	3,8	3,2	1,6	2,0	2,3	1,8		<7,7	<7,09
		4,1	3,4	3,5	3,3	1,1	1,6	1,3	1,4		<7,72	<7,26
		4,0	3,8	3,9	3,1	1,4	1,7	1,8	1,7	1,9		<7,44
Mean		3,8	3,8	3,1	1,2	2,1	1,7	1,6		<7,72	<7,42	
Standard deviation		0,33	0,34	0,15	0,23	0,43	0,38	0,28		Mean		
Coefficient of variation		9%	9%	5%	19%	21%	23%	18%				
Differences												
Laboratory S.a. vs. laboratory MRSA										-0,7		
Laboratory MRSA vs. MRSA wildtype strain						-1,9	-1,1	-1,5	-1,6	-1,5		
Laboratoy S.a. vs. MRSA wildtype strain						-2,6	-1,7	-2,2	-2,3	-2,2		

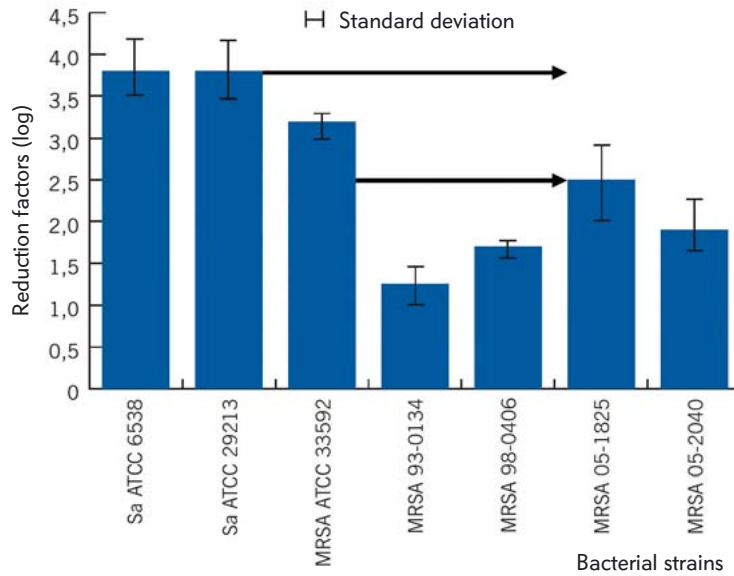


Figure 1: Quantitative suspension array testing the susceptibility of *Staphylococcus aureus* and MRSA lab strains as well as MRSA wildtype strains to Prontosan at a standardized PHMB concentration of 0.005%, star:  $p < 0.01$

Significance	Laboratory strains			MRSA laboratory strains			
	SA ATCC 6538	SA ATCC 29213	MRSA ATCC 33592	MRSA 93-0134	MRSA 98-0406	MRSA 05-1825	MRSA 05-2040
not significant	x	x	p=0,9232				
significant	x		x	p<0,0003			
significant	x	p<0,0001			x		
significant	x	p<0,0001		x			
significant	x	p<0,0001			x		
significant	x	p<0,0001				x	
significant	x	p<0,0001					x
significant		x	x	p<0,0008			
significant	p<0,0001		x		x		
significant	p<0,0001		x	x			
significant	p<0,0009		x		x		
significant	p<0,0001		x			x	
significant	p<0,0001		x				x

Table 2: Significance of differences in susceptibility to Prontosan between *Staphylococcus aureus* and MRSA lab strains as compared to MRSA wildtype strains at a standardized PHMB concentration of 0.005%

Quantitative suspension array with the laboratory strain ATCC 6538								
Temperature	Lavanid 0,005% PHMB		Covidien 0,005% PHMB		Covidien 0,05% PHMB			
	AP (min)	Rf (log)	AP (min)	Rf (log)	AP (min)	Rf (log)	AP (min)	Rf (log)
RT	2	3,6	200	0,29	200	2,36	1 day	6,99
		3,8		0,34		1,38		4,45
		3,7		0,30		3,36		>7,68
		3,8		0,42		1,91		5,39
		3,9		0,08		1,02		3,48
		4,2		0,21				
Mean		3,9		0,25		2,01		>5,6
Standard deviation		0,23		0,13		0,91		1,49
Coefficient of variation		6%		53%		45%		27%

Table 3: Quantitative suspension array testing the susceptibility of the lab strain *Staphylococcus aureus* ATCC 6538 to Lavanid (solution) and Covidien (NaCl solution of dry wound dressing) at PHMB concentrations of 0.005% and 0.05%, AT: application time of 2 minutes, 200 minutes and 24 hours

## DISCUSSION

The test standard defines the bactericidal effect of a product as its potential to reduce the number of live vegetative bacteria cells of *Staphylococcus aureus* ATCC 6538 at least by a factor of 105 ( $\rightarrow$  5 logarithmic steps)<sup>(1)</sup>. An annotation to the standard further specifies that “the bactericidal effect of the undiluted product cannot be determined as adding inoculum culture always leads to a certain dilution”. Therefore, the effectiveness of the products we tested could only be assessed for concentrations of up to 80%.

The experiment showed that even Prontosan diluted down to 80% achieved a reduction of bacteria by 7 logarithmic steps within 2 minutes. This equals a more than 10 million-fold ( $>10^7$ ) reduction of the original number of bacteria; that means that this product sold by the manufacturer as “irrigation solution” (more than) meets the requirements for antiseptics in the laboratory experiment. In clinical practice however, the infection agents are normally protected by a number of different effects of the wound environment such as the dilution with serum (proteins), blood, sanies detritus, fibrin, etc.

As a consequence, the antiseptic agent cannot reach the bacteria at all or only in a low concentration. On the other hand, the normal test procedure for products as effective as this one does not allow a further quantification of effectiveness so that the result cannot be more specific than  $>10^7$ . These aspects may serve as a reason why the parameters “concentration” and/or “application time” are changed under laboratory conditions to allow for an exact quantification.

The objective of this study was to compare the bactericidal effects of PHMB products available on the market; for that reason we chose to start with an absolute concentration of 0.005% and an application time of 2 minutes. In some cases, we had to prolong the application period or increase the PHMB concentration in order to be able to represent the bactericidal effect. As a result, this comparative study of lab and wildtype strains of *Staphylococcus aureus* has shown that the laboratory strain (ATCC 6538) to be used according to the test standard<sup>(1)</sup> does not significantly differ from the alternative strain Sa ATCC 29213 when in contact with PHMB.

The MRSA laboratory strain ATCC 33592 showed resistance increased by 0.9 log (8-fold, highly significant) compared to the lab strain (ATCC 6538). However, even the MRSA lab strain (ATCC 33592) showed a 1.9 log (80-fold), highly significant, resistance to PHMB as compared to the most resistant MRSA wildtype strain (93-0134). Both the individual analysis of each of the 4 MRSA wildtype strains from the RKI’s “International Basic Set” and the statistic analysis of groups of strains has shown that their lower susceptibility towards a (Prontosan) PHMB concentration of 0.005% was highly significant

as compared to the laboratory strain chosen according to the test standard.

MRSA wildtype strains on average were 160 times (2.2 log) and at a maximum 400 times (MRSA strain 93-0134: 2.6 log) more resistant. As the test standard defines effectiveness as achieving a reduction by 5 logarithmic steps, a difference in efficiency of 2.6 logarithmic steps must be considered substantial. It seems to be sensible to test further wildtype strains taken from the RKI pool for their susceptibility as compared to the test standard laboratory strain. Furthermore, the effectiveness of the other antiseptic products (PVPI, Octenisept) should be tested as well. Against this background, especially those manufacturers selling wound treatment products with low or very low active PHMB concentrations should immediately reassess these products. Only that way can critical situations be avoided if, for example, there is no or not a sufficient antiseptic or conservative effect even though the user of the product (wrongfully) assumes that the product’s bactericidal effect is sufficient. This requirement is all the more urgent as more and more antiseptic applications containing a wide range of PHMB concentrations and designed for a multitude of purposes are offered on the market.

For our study we used Prontosan for instance which is advertised as being a wound irrigation solution keeping the wound moist and clean but at the same time contains 0.1% of PHMB: From a legal perspective, Prontosan should never be used for treating infections, i.e. when clinical symptoms like redness, swelling, pain or hyperthermia are apparent. For treatment of infectins, the same manufacturer offers the product Lavasept and other products such as Lavasorb or Lavanid whose PHMB concentration is only 1/5 as high (0.02 or 0.04%) as that of Prontosan. This may confuse the users of these products and thus urgently needs to be clarified. In addition to these PHMB-containing solutions advertised as being “antiseptic” (0.02-0.04% PHMB) or for “irrigation/cleansing” (0.1% PHMB), the manufacturer also sells a wet (Suprasorb-X-plus-PHMB) and a dry wound dressing (Covidien-Kendall™-AMD). According to the manufacturer’s information, these dressings have a much higher PHMB concentration than the solutions (Suprasorb: 0.3%; Covidien: 0.5%).

Based on the concentration of active ingredient as declared by the manufacturer, one might think that a product containing “0.5% PHMB” would have a particularly strong antiseptic effect and would thus be particularly suitable for treating severe local wound infections.

In line with this, the corresponding enterprise decided to advertise the product as follows: “*Kendall™ AMD antimicrobial foam dressings were designed to help you manage exudate and bacteria. This dressing is an open-cell polyurethane foam dressing impregnated with 0.5% polyhexamethylene biguanide (PHMB) - a powerful yet safe antiseptic.*”

[...] *Effective against gram+ and gram- bacteria, including: MRSA, VRE, Pseudomonas and acinetobacter baumannii. [...] The foam absorbs wound fluid; PHMB retains the fluid in the foam and kills the bacteria at initial contact. [...] is proven effective against cross-contamination for 7 days. [...] is cheaper than most antimicrobial treatment methods and silver dressings*<sup>(15)</sup>. As opposed to this advertising, the package insert of the product lists the following indication: „*Kendall™ AMD dressings are indicated for use in management of post-surgical incisions, pressure sores, venous stasis ulcers, diabetic ulcers, donor sites, abrasions, lacerations, 1st and 2nd degree burns, dermatologic disorders, other wounds inflicted by trauma and, as a secondary dressing or cover dressing for packed wounds. It is an ideal dressing in the local management of exudate that may occur at surgically induced body exit sites such as tracheostomy, G-tube, J-tube, Penrose drain, chest tube, nephrotomy tube, sump drain, IV catheters, central venous lines, arterial catheters, dialysis catheters, peripherally inserted coronary catheters, mid-line catheters, externally placed orthopedic pins and epidural catheters.*“<sup>(16)</sup>.

Following this advertising, especially the indication of the concentration of PHMB (0.5%), the user can not only expect a major bactericidal effect but consecutively also a significantly higher toxicity than in classic antiseptic solutions with a PHMB concentration of 0.02%. However, as a result of our comparative study with the test strain ATCC 6538 and using a standardized PHMB concentration (0.005%) we can claim that Lavanid solution achieved a reduction of 3.9 logarithmic steps within 2 minutes. This matches the result achieved with the 0.005% PHMB Prontosan solution (reduction: 3.8 log<sub>10</sub>-factor 8,000).

Based on the manufacturer's information for Covidien however, the 0.005% PHMB product only reached an efficiency of 0.25 logarithmic steps (~ factor 1.7), and even that could only be achieved upon prolonging the application period 100-fold. In the PHMB dry wound dressing, only a PHMB concentration 10 times higher (0.05%) than the original concentration led to a decrease by 2.0 logarithmic steps within 200 minutes. One might speculate whether the product might have been impregnated by the manufacturer with a 0.5% PHMB concentration and whether a reaction with the product's foam structure might have led to the decrease subsequently. For example, the PHMB molecules could have bonded to the supporting material, or the active ingredient could have been partly inactivated.

Regardless of the causes, the PHMB concentration applied to the wound requiring antiseptic treatment and the corresponding bactericidal effect is much lower than the declared 0.5%. Even if the “missing PHMB” were still to be found in the foam structure of the product, it would not have a bactericidal effect on the wound surface. In contrast to the advertising describing a “*powerful yet safe antiseptic*”, the user will surprisingly discover in the manufacturer's information (only after having bought the product) that it is not even indicated for infections! Assuming that the concentration of vegetative bacteria doubles every 20 minutes, the number of bacteria will increase 8-fold within an hour. Even without taking dilution and protective effects or protein binding of bactericidal agents<sup>(17,18)</sup> into account, roughly a 10-fold reduction (1.0 logarithmic steps) of bacteria per hour would be necessary just to have a conservative bactericidal effect maintaining a “steady state” situation.

As a precaution against effect-reducing wound effects (a further 10), we would therefore like to propose a minimum reduction of > 2 logarithmic steps per hour for containment. This in turn would require that the test strains used have the same susceptibility as the (MRSA-) wildtype strains. Products and preparations not fulfilling these minimum requirements should never be referred to as antibacterial, microbicidal, antiseptic, etc. Taking into account the potentially significant limitation of the susceptibility of MRSA strains as compared to laboratory test strains, the wound effects potentially further limiting the effectiveness of PHMB (such as proteins) and the lack of data on effectiveness and toxicity of antiseptic wound dressings, it seems to be sensible to only use those antiseptic solutions with a proven bactericidal effect and concentrations ranging from 0.02 to 0.04% PHMB for the time being. Furthermore, we urgently recommend to strictly stick to the indications listed by the manufacturer.

#### Conflict of Interest

The authors hereby declare that there is no conflict of interest as defined by the rules of the International Committee of Medical Journal Editors.

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# Cost-Benefit Analysis in the Treatment of Chronic Wounds

## – Cluster Analysis of Duration of Treatment and Cost Typology

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### Summary

*Introduction:* The annual incidence of chronic ulcerations is about 8 patients per 1000 population. For Germany this would mean that about 650.000 people develop a new chronic wound annually. In order to further develop efficient health care provision in chronic wound care, an integrated health care provision system including structured health care pathways has been introduced. Within the scope of a cost-benefit-analysis, the effect of the implementation of integrated health care pathways has been

evaluated.

*Methods:* A retrospective longitudinal study was conducted. As data source, routine data derived from patient documentation was used. Additionally, data was taken from our national wound register. Finally, secondary data analysis took place. All patients, being treated due to a chronic wound within the scope of an integrated health care contract were included into the study (N = 291). Analysis took place for mobile patients with complete wound healing (N = 240). A clustering by the degree of severity with the treatment duration as grouping criterion was undertaken. The time period under investigation was 2007-2010.

*Results:* Our analyses showed that about 50% of all patients had a treatment duration of less than 58 days (1. +2. quartile), while about 25% of all patients showed a treatment duration of more than 101 days (4. quartile). The treatment costs stay in line with the results and differences of the treatment duration. The ratio between costs and bene-

fits turned out to be better for modern wound care compared to conventional care. The costs of non-usage of the more favorable alternative (opportunity costs) in chronic wound care are striking for the whole population (see incidence rate) and amount up to a 1 billion EUR. *Discussion:* All in all a population-based implementation of integrated health care concepts, that are similar to the one that was evaluated within the scope of this study, can be recommended in chronic wound care due to the better cost-benefit- ratio compared to conventional care. However, even within the modern wound health care setting longer treatment durations have to be accounted for and need to be investigated more in-depth, in order to evaluate and finally assure the sustainability of integrated health care concepts.

### Keywords

Integrated care, Cluster analysis, costbenefit analysis, treatment duration, opportunity costs

## INTRODUCTION

We can almost certainly assume that both the absolute and the relative incidence of chronic wounds and thus also the total number of patients will continue to increase; not least due to a rising life expectancy and demographic change. Currently, the annual incidence of chronic ulcerations is 8 patients per 1,000 inhabitants<sup>(1)</sup>. For Germany, that equals an annual 650,000 new patients.

Research on the costs and benefits of medical treatment in general and the treatment of chronic wounds in particular is not very common in Germany.

Isolated studies have also shown shortcomings with regards to study design and definitions so that only a small number of valid findings on the functional connection between costs and benefits of chronic wound treatment are available to date.

At the same time, the wound healing process depends on many different factors and may be different in each individual patient. A lack of patient compliance and existing comorbidities can have a significant influence on the wound healing process. Any research on chronic wound treatment therefore has to be based on nuanced analyses.

International studies have shown that the treatment of chronic wounds is associated with high hidden costs. Important cost drivers are: a long treatment period, a lack of compliance, incorrect use of material, insufficient diagnostics, in-patient treatment and wound infections<sup>(2,3,4)</sup>.

Measures improving the efficiency of chronic wound treatment are therefore indispensable. International studies have shown that modern wound care with optimized treatment procedures will improve the cost-benefit ratio.

In the course of the present study, a comparative assessment of costs and benefits in wound treatment was conducted, with the incurred total costs on the cost side and the duration of treatment until healing on the benefit side. For the first time in Germany, the data of almost 300 patients who completed their treatment (wounds healed) were thus collected and analyzed during a multi-year observation period for this study. The data were taken from a national wound registry on modern wound care kept at the Institute of Health and Nursing Economics (Institut für Gesundheits- und Pflegeökonomie) in Bremen. The analyses discussed here will be continuously updated with an increasing number of patients.

## METHODS

The study conducted was a retrospective longitudinal study. Table 1 provides a brief overview of the study design.

The present study is based on an analysis of mobile patients with complete wound healing (n=240) observed in the period between 2007 and 2010. Within the framework of the cost-benefit assessment, the total treatment costs per patient and the duration of treatment for patients in the integrated medical care scheme are compared to the data collected in conventional wound treatment. Immobile patients are often affected by significant comorbidities requiring an increased amount of care. This group accounting for 51 patients of the total population needs to be analyzed separately. It is not uncommon for individual immobile wound patients to have extremely high treatment costs<sup>(6)</sup> which, due to a relatively small number of cases, lead to results which are hardly statistically valid and analyses which may be biased.

Chronic wound patients were treated by medical specialists under an integrated medical care contract. Medical specialists with expertise in chronic wound treatment conclude a medical care contract including fixed payment regulations with a management company. This arrangement is subject to the patient's approval. Nursing services are included in the concept. Patients are treated according to the standards of modern wound management. The material used is chosen based on the state of the wound. The medical care contract defines the services provided for the patient. In addition, the treatment plan includes a wound management schedule with defined quality assurance measures. This approach makes the concept discussed a structured treatment or case management option for the treatment of chronic wounds.

**Table 1: Summary of the Study Design**

Study Design	
Type of study	Retrospective longitudinal study
Observation period	2007-2010
Data source	Routine data for patient documentation, database: National wound registry Secondary analyses
Inclusion criterion	All patients treated for a chronic wound under an integrated medical care contract ("Integrierter Versorgungsvertrag") Mobile patients with complete wound healing
Data basis	N = 291
Analyzing procedure	Analysis for mobile patients with complete wound healing (N=240) Mobile wound patients: Clustering by degree of severity with the duration of treatment being the clustering method
Evidence level	Level 2+ according to the Scottish Intercollegiate Guidelines Network (SIGN) <sup>1</sup> (2008) <sup>(5)</sup>

<sup>1</sup> The advantage of these guidelines as compared to other schemes such as the one proposed by the Oxford Center for Clinical Evidence is that the SIGN approach includes aspects such as study quality or a potential bias of results. Therefore, we chose to use this scheme.

Due to a relatively large study population, the results of this study have a high level of evidence.

Cases in which complete wound healing had not yet been achieved were excluded from the analysis as the future wound healing process and thus the treatment required could not be foreseen. The data and the results will be continuously updated in the wound registry.

It became evident that there is a wide spectrum of different treatment options in the medical and nursing care of chronic wounds. In the majority of chronic wound cases, there are no complications and when treated according to modern wound management standards, the duration of treatment until healing will be relatively short. Nevertheless, a significant share of these wounds is rather longterm and complex in terms of the treatment required<sup>(6)</sup>.

Cluster analysis can be used to methodically group this practical evidence by duration and costs of treatment. In chronic wound treatment, we can distinguish between four different clusters.

Cluster 1 = Minor cases

Cluster 2 = Moderate cases

Cluster 3 = Severe cases

Cluster 4 = Very severe cases

The present cost-benefit analysis will, for the first time, provide a cluster analysis of chronic wound management grouped by the different treatment requirements. Figure 1 shows the treatment requirement clusters. Quartile analy-

sis based on the duration of treatment was chosen as a case-specific clustering method.

Wounds can be defined as an internal or external area of low tissue density combined with a loss of tissue cohesion. The wound will pass through different stadia before healing. A wound can generally be considered “chronic” if wound healing is impaired and complete healing cannot be achieved within a period of approximately six weeks<sup>(7)</sup>. From then on, the wound will be referred to as a “chronic wound”.

**Figure 1: Clustering by Type of Treatment**

Cluster 1 (n=60): “Minor” cases (1st quarter)	Cluster 2 (n=60): “Moderate” cases (2nd quarter)
Cluster 3 (n=59): “Severe” cases (3rd quarter)	Cluster 4 (n=61): “Very severe” cases (4th quarter)

Total N=240

A cluster-based typing provides evidence for practical treatment.

Cluster 1 contains chronic wounds which can then be closed and healed within 6 weeks. These are normally relatively “small” wounds healing without any complications.

Cluster 2 contains chronic wounds with a healing time of around 6 to 8 weeks. Also in these cases, treatment can be assumed to remain without complications.

Cluster 3 contains relatively severe types of wounds with a treatment duration of more than 8 weeks. The chronic wounds grouped in this cluster a “larger” and treatment takes longer and is more complex. In this group, there is normally no problem with patient compliance.

And finally, Cluster 4 contains the very severe cases with a duration of treatment which can sometimes be very well in excess of 3 months. This group includes patients with non-healing chronic wounds, a high risk of complications and, in some cases, a lack of patient compliance.

**RESULTS**

In this study, the average age of patients with complete wound healing is 65 years. Table 2 provides an overview of the study population’s basic data.

**Table 2: Basic Data of the Study Population**

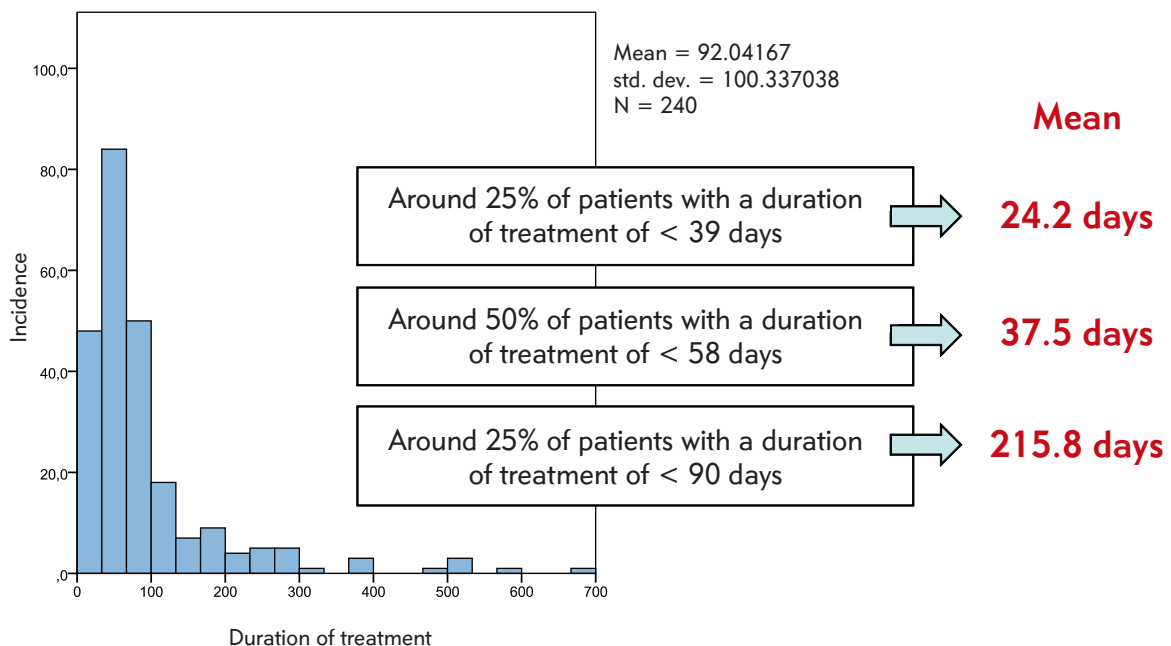
Basic Data	
Number of patients with complete wound healing	240
Average age in years	64.7
Share of male patients (in %)	54.2
Share of retired persons (in %)	53.6
Share of patients treated with compression therapy (in %)	28.3

The share of retired persons is 54%; so is the share of male patients. 28% of patients had been treated with compression therapy.

**Substantial Differences in the Duration of Treatment**

When analyzing the duration of treatment (see Fig. 2 and Table 3), substantial variations between the different clusters become apparent. While patients in Cluster 1 (minor cases treated according to modern wound management) experience complete healing after an average of about three weeks, the duration of treatment until complete wound

**Figure 2: Overall Analysis of the Duration of Treatment**



**Table 3: General Overview of Duration of Treatment and Treatment Costs by Cluster**

Assessing the Duration of Treatment (in Days) by Cluster					
Variables	Cluster				Total
	Cluster 1 = "Minor" cases	Cluster 2 = "Moderate" cases	Cluster 3 = "Severe" cases	Cluster 4 = "Very severe" cases	
Arithmetic mean	24.2	50.7	75.2	215.8	92.0
Median	24.0	53.0	77.0	172.0	57.5
Minimum	7	39	58	91	7
Maximum	38	57	90	671	671
Number of cases	60	60	59	61	240
Assessing Treatment Costs (in €) by Cluster					
Variables	Cluster				Total
	Cluster 1 = "Minor" cases	Cluster 2 = "Moderate" cases	Cluster 3 = "Severe" cases	Cluster 4 = "Very severe" cases	
Arithmetic mean	885.30	1,850.98	2,746.13	7,882.04	3,362.51
Median	876.78	1,936.22	2,813.00	6,283.59	2,100.62
Minimum	256	1,425	2,119	3,325	256
Maximum	1,388	2,082	3,288	24,513	24,513
Number of cases	60	60	59	61	240

closure is, at an average of more than 30 weeks, much longer in Cluster 4 (very severe cases of chronic wounds). This indicates that there is roughly a factor of 10 between the different types of chronic wounds with regards to the duration of treatment required. Half of the study population (Clusters 1 and 2 with a total of 120 out of 240 patients) had a mean duration of treatment of an approximated 38 days; that is a duration of treatment of around five weeks for chronic wounds treated with modern wound management.

### Shorter Treatment Periods with Modern Wound Management

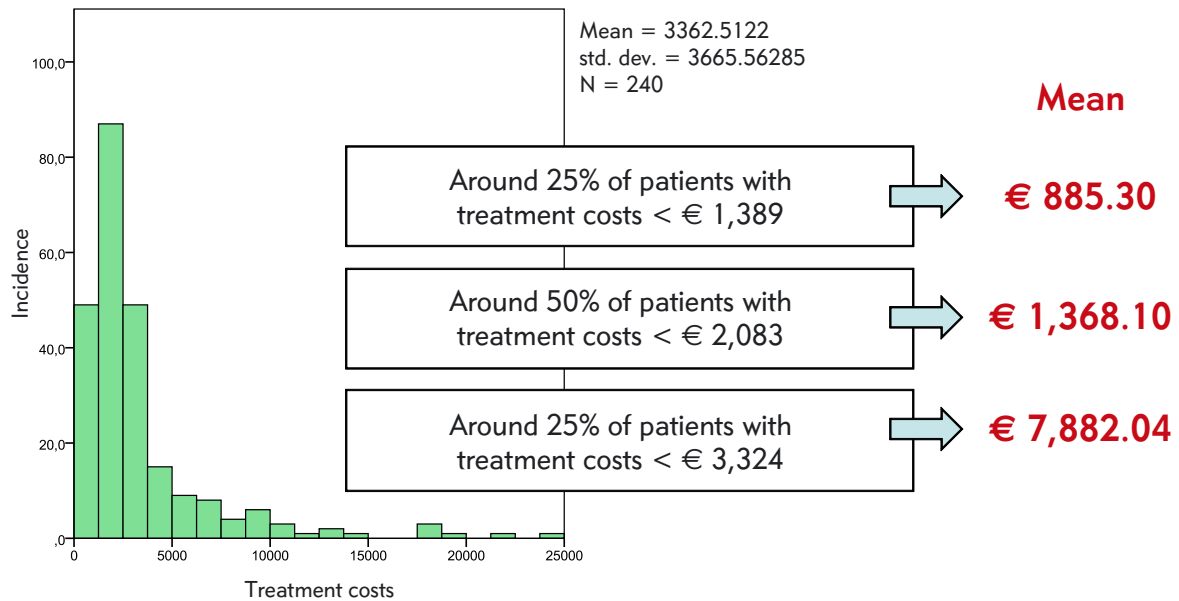
When comparing modern to conventional wound care methods, a substantial reduction can be observed in modern wound management. According to the literature available, the mean duration of treatment for chronic wounds is 301 days or 43 weeks<sup>(8,4)</sup>. In modern wound management, this figure is down to an average of 37.5 days or 5.3 weeks in half of the patients (see Fig. 2). This indicates that the duration of treatment with conventional wound care methods is 8 times as long. The treatment period of 301 days for chronic wounds (see Fig. 4) treated with conventional methods also exceeds the average duration of treatment in the upper quartile (very severe cases of chronic wounds) amounting to 215.8 days by about 50%, based on the average duration of treatment.

In total, the results for modern wound care have shown an average healing time of 92 days or 13.1 weeks to be the arithmetic mean in this study (see Table 3). The mean healing time for wounds treated with conventional methods was 43 weeks, equaling 301 days<sup>(8)</sup>. This means that the duration of treatment with conventional wound care was more than thrice as long as when modern methods were applied.

In the cluster analysis we can see that in about 25% of patients with chronic wounds, the healing time was more than 90 days (4<sup>th</sup> quartile). Even if and when wounds are treated with modern methods of wound care, treatment of one fourth of all patients still remains complex and thus results in cost-intensive treatment periods. In at least half of chronic wound patients however, modern wound treatment is expected to result in cheaper and more efficient treatment courses. A more in-depth analysis of subgroups could produce further results on this issue.

In a further step, first subgroup analyses were conducted with regards to age and wound types. This analysis has shown that different mean treatment periods can be observed depending on the type of wound treated. Especially pressure ulcers and diabetic foot ulcers have shown to take above-average healing times which, in turn, raise the total mean. At the same time, leg ulcer patients normally need a shorter treatment period for their wounds to heal.

Figure 3: Overall Analysis of Treatment Costs



In addition to the type of wound, the age of the patient affected was also discovered to be a relevant indicator for the duration of treatment. This study has shown that patients 70 years or older need a longer mean treatment period until complete healing than patients who are younger than 70 years. In elderly patients, the healing time tends to be on average around 25% longer. So far, there are no reliable reference values for the conventional treatment of chronic wounds. We can observe however that even longterm treatment as may be necessary for pressure and diabetic foot ulcers can be reduced by around half when using modern wound care methods as opposed to conventional treatment options. In an earlier study<sup>(4)</sup> we were able to show on the basis of health insurance data that patients with severe wounds had received several years of treatment in a conventional approach.

Within the framework of data analysis for the continuous evaluation of modern, integrated wound care at the Institute of Health and Nursing Economics, we were further able to determine that patients suffering from pressure or diabetic foot ulcers had a relatively higher rate of abandoning treatment<sup>(6,10)</sup>.

Overall, a nuanced patient analysis in modern treatment produced different results for the variables “age” and “type of wound”. This can be taken into consideration for future integrated medical care contracts and cost-benefit analyses.

### Substantial Differences in Treatment Costs Observed in the Clusters

Analogous to the results and the differences observed in the duration of chronic wound treatment, the treatment

costs describe a similar pattern (see Table 3). While patients in the first Cluster (minor cases) had an average treatment cost of € 885.30, the treatment cost for Cluster 4 patients (very severe cases) was € 7,882.04. Therefore the costs in these two groups differ by a factor of 9 (see Fig. 3). In around half of chronic wound patients, the mean treatment cost is € 1,368.10 (see Fig. 3). It can also be observed that the treatment cost of Cluster 4 patients (very severe cases) is very high at an average of € 7,882.04 and has a significant influence on the overall mean of all chronic wounds.

By comparison to the actual and direct treatment costs for chronic wounds, the annual risk balance payment insurers receive for ulcus cruris patients is currently at € 2,177 (HMG 149 (morbidly classification scheme), skin ulcers excluding pressure ulcers)<sup>(11)</sup>. According to the cost information retrieved from cluster analysis, we can see that more than 50% of all chronic wound patients have lower treatment costs than assumed for the morbidity-related risk payment (Morbi-RSA, here: HMG 149). Treating chronic wounds with modern wound management techniques under an integrated medical care contract is therefore actually profitable for insurers; or even very profitable if only Cluster 1 is chosen as a reference value. For Clusters 3 and 4 however, a more in-depth analysis is required. Possible additional annual payments for patients with chronic wounds within the framework of the Morbi-RSA scheme include:

- For type 1 diabetes patients: € 2,155 (HMG 20)
- For diabetes patients with abnormal peripheral circulation or ketoacidosis: € 2,214 (HMG 16)
- For patients with infected wounds: € 781 (HMG 152)

- For patients suffering from atherosclerosis (mainly HMG105, HMG106 and HMG149): € 4,765 (HMG 104)

A more in-depth analysis of the cost-benefit evaluation is possible when taking into consideration the above-listed additional payments according to the Morbi-RSA scheme. This further analysis would have to be conducted on a case-by-case basis.

**Table 4: Overview of Cost Structure<sup>1</sup>**

Cost Type	Ø Direct daily cost for mobile patients	Direct daily cost for mobile patients (in %)
Physician's fee	€ 7.1	19.45%
Nursing fees	€ 6.3	17.26%
Material costs	€ 17.4	47.67%
Other costs	€ 5.7	15.62%
Total costs	€ 36.5	100.00%

<sup>1</sup> For immobile patients, the daily allowance is around 11% higher.

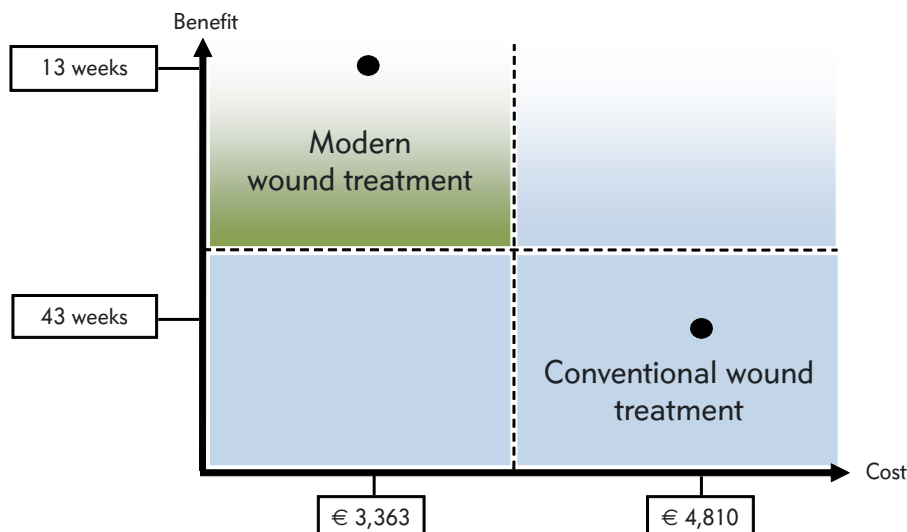
Table 4 contains a detailed list of the distribution of direct costs. Almost 50% of the cost for modern chronic wound treatment is spent on treatment material, in this case wound dressings. Physician's fees account for 19%, nursing fees for 17%. Other costs, including quality assurance measures, further training and qualification measures, account for around 16%.

The direct daily costs per patient treated with modern wound management methods is higher than the direct daily costs for the conventional treatment of chronic wounds as indicated so far. This was described in earlier studies<sup>(2,4)</sup>. The costs amount to less than € 20 per day. However, the calculation for the costs of conventional treatment only takes nursing and material costs into consideration. This fact puts the earlier comparison into perspective. Nevertheless, we can state that the direct daily treatment costs for modern wound management are a little higher than for conventional treatment. The eventual cost reduction observed when comparing the two treatment options results from a much shorter duration of treatment in modern wound management. This is a result earlier studies have shown as well.

The calculation of direct treatment costs (Table 4) in this study is extremely valid as the data used was taken directly from the integrated medical care contract and the quality of this information was assured in advance. Nevertheless, even this calculation had to exclude the indirect costs incurred. The mere fact that a patient can be released from the hospital earlier because he or she has been cured further lowers the costs of modern wound management as compared to conventional treatment. Any hidden or indirect costs resulting from the patient's health status could also be included in the model analysis which would probably widen the gap between the costs for modern and conventional treatment even further.

When comparing the cost structure in these four different groups of patients, the influence of major cases on the overall treatment situation becomes apparent (see Figure 3 and Table 3).

**Figure 4: Comparing the Treatment Options in a Graph**



### High Opportunity Costs

Figure 4 shows that the mean treatment costs for modern wound care are around € 1,500 lower than the costs of conventional treatment.

When considering the total population (see incidence rate) the costs for not using the cheaper wound treatment alternative (opportunity costs) are considerable. Assuming the difference in costs between the two treatment options amounting to € 1,500 can be achieved and saved for all 650,000 patients calculated earlier, the opportunity costs would go into the billions. And we should bear in mind that this health economic calculation only includes the direct costs. Any hidden or indirect costs would further increase these opportunity costs or the additional money spent in alternative to modern wound treatment.

Comparing the two treatment options, we can see that there is an enormous potential for improving both costs and benefits when applying modern, integrated wound treatment.

Against the background of extremely high opportunity costs going into the billions considering the total population of chronic wound patients, this study has shown the need for comprehensive integrated modern wound treatment including an analysis and an evaluation of different courses of treatment. After all, longer treatment periods with modern wound care methods also require further observing and analysis.

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# Classification of Wounds at Risk (W.A.R. Score) and their Antimicrobial Treatment with Polihexanide

– A Practice-oriented Expert Recommendation

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## Summary

Currently, there are no generally accepted definitions for wounds at risk of infection. In clinical practice, too many chronic wounds are regarded as being at risk of infection, resulting in an excessive use of topical antimicrobials in terms of frequency and/or duration of use.

Based on expert discussion and current knowledge, a clinical assessment score was developed and is now introduced. The objective of the introduced Wounds At Risk (W.A.R.) score is to allow for decision-making on the indication for the use of antiseptics on the basis of polihexanide. The proposed clinical classification of wounds at risk shall make the decision for wound antiseptics more simple and allow for an appropriate general treatment regimen with the focus on prevention of wound infection.

The W.A.R. score is based on a clinically oriented risk assessment using concrete patient circumstances. The indication for use of antiseptics is the result of the addition of differently weighted risk causes, for which points are assigned. Antimicrobial treatment is justified in the case of three or more points.

**Key words:** Wounds at Risk, wound at risk of infection, W.A.R. Score, polihexanide

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## 1. INTRODUCTION AND PROBLEM

So far, there is no generally accepted definition for the synonymous terms “wound at risk” and “wound at risk of infection”.

As a result of this lack of clear definitions, many wounds are therefore indiscriminately classified as being at risk of infection. The fact that antiseptic agents are applied too often or longer than necessary is thus often a consequence of a safety culture which is not based on evidence or empirical findings. In addition, it is important to recognize groups of patients at risk or critical wound conditions in order to avoid severe infections through consistent wound management.

## 2. OBJECTIVES

This expert recommendation is to make a contribution to defining the term “wound at risk” and to provide guidance in deciding when local, antimicrobial measures are useful treatment options for avoiding wound infections. Due to the fact that there are almost no evidence-based guidelines on this issue, this recommendation mirrors the consensus of an interdisciplinary and interprofessional group of experts based on an analysis of the current state of medical research and the group members’ own practical experience<sup>(3)</sup>.

Polihexanide was chosen as an antiseptic reference substance due to its favorable benefit-risk profile in wound treatment and the relatively comprehensive data available (15,22,23).

### 3. RISK ASSESSMENT FACTORS

To assess the probability of wound infection, not only the current pathogen load and the type(s) of pathogen are important to know but also their virulence and their interactions with the patient's immune system play a crucial role.

The following general infectiology equation will make the connection clearer:

$$\text{Risk of infection} = \frac{\text{number of pathogens} \times \text{pathogenicity of pathogens}}{\text{patient's immune response}}$$

That means: the higher the denominator, i.e. the better the patient's immunocompetence, the lower the risk of infection. If, on the other hand, the numerator decreases, especially as a consequence of a reduction of the number of pathogens, the risk of infection can also be reduced.

#### 3.1. Pathogen Load

The microbiological detection of microorganisms in a secondarily healing wound does not automatically mean the wound is infected. Depending on the amount of pathogens, their proliferative properties and the host's immunological response, we can distinguish between the following microbial wound situations:

- *Contamination*: Microorganisms are present and attached to the tissue (microbial attachment), but they are not (yet) multiplying.
- *Colonization*: Microorganisms are present and multiplying. The host does not (yet) show any significant immunological reaction.
- *Critical colonization*: Significant increase of pathogens without the host showing any classical signs of infection; however, wound healing is delayed due to e.g. toxin formation.
- *Local infection*: Clinically visible immunological host response showing the typical signs of infection: redness (erythema > 2 cm measuring from the wound edge with a tendency to grow quickly indicates a spreading infection and a risk of the infection generalizing)<sup>(25)</sup>, swelling, local hyperthermia of skin/tissue, pain, functional impairments and for instance an increase in the amount and the viscosity of wound exudate, perceptible smell, stagnating wound healing process<sup>(14,25)</sup>.
- *Generalized infection*: In addition to the local reactions described, there are also signs of a systemic host reaction such as leukocytosis, an increase in C-reactive protein and fever<sup>(13)</sup>.

A merely quantitative approach to a wound's microbial load (e.g. reference value > 10<sup>5</sup> pathogens per gram of tissue) cannot be introduced as a standard procedure in clinical practice and is also not sufficient for assessing the risk of infection. If a patient has a relevant disposition and/or the microorganisms are particularly pathogenic, a wound contaminated with virulent pathogens can for instance already pose a threat to the patient in itself even if the pathogen load is low. A typical example of that is a colonization with methicillin-resistant *Staphylococcus aureus* (MRSA).

The term "critical colonization" is still being discussed controversially as this state cannot be clearly defined neither from a microbiological nor from a clinical point of view; nevertheless, the term is used in practice<sup>(14)</sup>. Pathogen virulence and the host's immune response are two important factors when it comes to whether a colonized wound will become critically colonized or infected or not. Accordingly, it is important to pay close attention to the above-mentioned clinical signs and symptoms of wound infection during the wound process. If necessary, local and/or systemic antimicrobial measures are to be taken.

#### 3.2. Microbiological Evidence and Pathogenicity

Regardless of the pathogen isolation method (in a qualitative approach through a smear test, in a semi-quantitative approach through an extended smear test or in a quantitative approach e.g. through douching or tissue biopsy), today's microbiological standard wound diagnostics are still characterized by a number of shortcomings. Generally, the following factors limit the efficiency of the microbiological standard diagnostics in wound treatment<sup>(26)</sup>:

- the pathogen isolation rate from the wound
- the effectiveness of pathogen isolation (e.g. smear test vs. biopsy)
- the limited information on the relevance of the pathogens found, if any, and
- the time needed for microbiological findings
- the chronicity of the wound
- the location where pathogens are found (i.e. how deep into the wound)

The reproduction of results with regards to the recovery rate of microorganisms is often unsatisfactory. Most of the time, material is taken out of the wound at a given spot and does not adequately reflect the conditions in deeper layers of the wound bed. In addition, the pathogens responsible for a given wound healing disorder or an infection can often not be identified if protected by biofilm<sup>(26)</sup>.

Even if one or more species of microorganisms can be detected, the validity of the standard findings is still limited with regards to its clinical relevance. Normally, the standard findings only provide information on the

genus and possibly the species of the microorganisms found including their susceptibility to selected antibiotics. It does not give any information on the existence or lack of specific pathogenicity factors. The pathogenicity of a strain depends on the virulence factors produced by that specific strain. Thus, certain virulence factors relevant for wound infection such as the production of the enzymes coagulase, metalloproteinases or staphyloxanthin (a carotenoid pigment with cytotoxic and, at the same time, antioxidant properties), are more likely to appear in *Staphylococcus aureus* isolates than for instance in *Staphylococcus epidermidis* isolates; but even within one species these virulence factors are strain-dependent. “Classical” microbiological findings do not distinguish between the pathogenic and less pathogenic strains of a species. The only conclusions as to the virulence can be drawn from the susceptibility to antibiotics which is only indirectly relevant as possible antibiotic resistances only become relevant if systemic antibiotic treatment is required. In case of secondary sepsis following wound infection however, knowledge about antibiotic susceptibility can be crucial for the patient prognosis<sup>(1,6)</sup>.

A further limitation results from the natural period of two to five days that is required for bacterial cultures to grow after samples have been taken (time of clinically recognized risk) and for achieving microbiological results. During this period, the patient’s medical condition can already deteriorate and even result in a clinical infection<sup>(1,6,13)</sup>.

Easy-to-use quick tests are generally available. These tests are highly specific for some pathogens and have not yet been sufficiently analyzed for their practicability. Thus, there is currently a need for action already with regards to recognizing a clinical problem. The results of microbiological diagnostics can then later be used to confirm or contradict this decision. In case of the patient passing on to a systemic infection, the microbiological findings do however facilitate the choice of necessary systemic treatment options (e.g. choosing the necessary antibiotic) and, with regards to epidemiological aspects, do provide important insights on the range of pathogens to be expected locally<sup>(1,13,30,47)</sup>.

One particularly important aspect of the microbiological findings focusing on the pathogen and its antibiotic resistance properties is information on evidence of multiresistant bacteria. This especially includes MRSA strains (including CA-MRSA stains involving a high risk of necrotizing infection), extended-spectrum beta-lactamase (ESBL)-producing, gram-negative bacteria or glycopeptide-/vancomycin-resistant enterococci (GRE, VRE). If these pathogens are found, it is always appropriate to take

an antiseptic measure as antibiotic-resistant bacteria (even if they might lack strain-specific virulence factors for causing a local wound infection) can have a negative effect on the prognosis of the patient affected in case of a systemic infection or even on that of neighboring patients in case of an unintended cross-contamination<sup>(1,6,7,25,46,47,48)</sup>.

### 3.3 The Patient’s Immunological Status

Clinical experience has shown that an otherwise largely healthy person can be suffering from an ulcer cruris (caused by a chronic condition) without having an infection for years<sup>(41)</sup>. Chronic wounds are therefore not automatically wounds at risk. On the other hand, the factors weakening a patient’s immune response are often the same factors also responsible for delays in wound healing which might therefore contribute to the wound becoming chronic; these factors are for instance: poorly controlled diabetes mellitus, immunosuppressives, malnutrition or arterial vascular disease. The actual risk of infection of patients with either chronic or acute wounds largely depends on their general immunological situation.

## 4. WOUNDS AT RISK (WOUNDS AT RISK OF INFECTION)

From a clinical perspective, there are two categories of factors posing a particular risk of infection (see Figure 1):

- Endogenous factors: These factors have an influence on the development of a wound infection and are basically a consequence of a weakening of the patient’s immune system.
- Factors posing an external (exogenous) burden: These factors are influenced by the amount and pathogenicity of the pathogens and by their susceptibility to antiseptic measures.

Below, some of these risk situations will be briefly discussed.

### 4.1. Wounds with an Endogenous Risk of Infection

The term “immunosuppression” is a general description of any processes suppressing immunological reactions. Immunosuppressed patients tend to suffer from complicated and often also chronic wounds more frequently than other patients. These wounds can be further subcategorized into neoplasia (e.g. squamous cell carcinoma), infections (e.g. lues maligna), vasculitis (e.g. secondary vasculitis in systemic lupus erythematosus – SLE) and specific wounds (e.g. ulcerating graft-versus-host disease – GvHD). Especially bacterial superinfections with e.g. erysipelas formation, a phlegmon or even sepsis can be a severe complication which is more frequent in immunosuppressed patients than in immunocompetent ones.

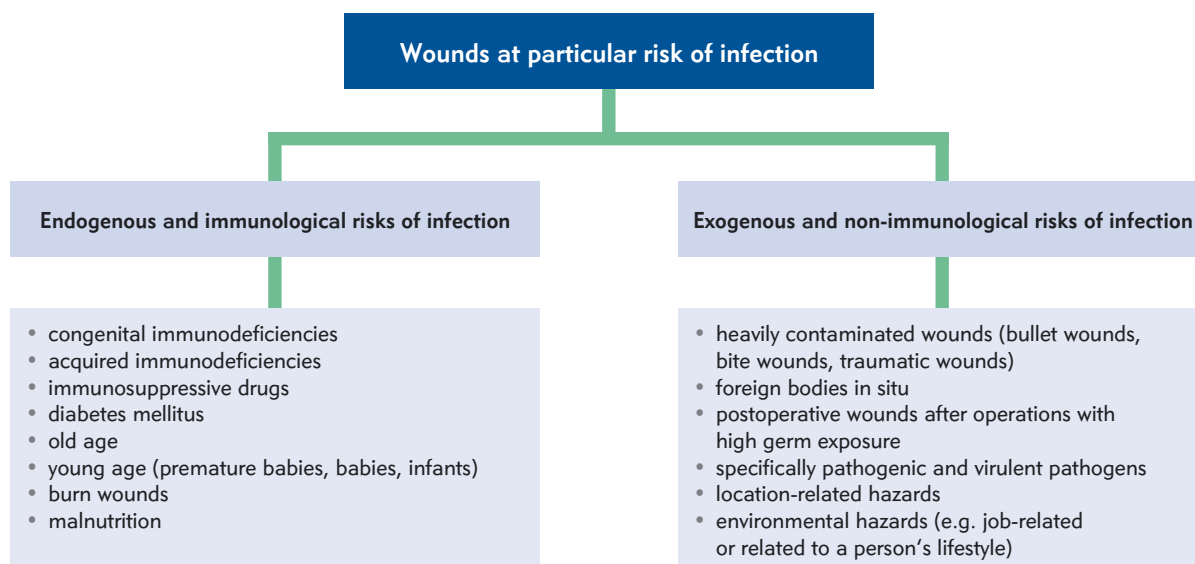


Figure 1: Wounds at particular risk of infection

In immunodeficient patients, a general distinction needs to be made between congenital and acquired immunodeficiencies.

#### 4.1.1. Congenital Immunodeficiencies

The largest share of congenital immunodeficiencies (around 50%) is made up by disorders of antibody production. Typical examples of such conditions are: agammaglobulinemia, common variable immunodeficiencies (CVID) and the hyper IgM syndrome. Other congenital immunodeficiencies are due to a B cell disorder such as X-linked agammaglobulinemia, a T cell disorder such as the Nezelof syndrome or on a combined B and T cell disorder such as severe combined immunodeficiencies (SCID), the Wiskott-Aldrich syndrome (WAS), the DiGeorge syndrome or the Louis-Bar syndrome.

#### 4.1.2. Acquired Immunodeficiencies

Acquired immunodeficiencies are much more frequent and can for instance be caused by the following factors:

- Infectious diseases, e.g. AIDS (acquired immune deficiency syndrome), cytomegaly, measles
- Neoplasias, e.g. leukemia, lymphomas
- Drugs, e.g. glucocorticoids
- Autoimmune diseases, e.g. systemic lupus erythematosus, rheumatoid arthritis
- Impaired cellular immune response and cellular senescence
- Age
- Malnutrition
- Splenectomy
- Burns
- Diabetes mellitus

(4,12,39,40)

#### 4.1.2.1. Immunologically Relevant Drugs

Drugs can be subcategorized by active ingredients:

##### *Glucocorticoids*

- e.g. prednisone, prednisolone, dexamethasone. These agents directly inhibit T cells and indirectly also B cells.

##### *Cytostatics*

- Alkylating agents, e.g. cyclophosphamide, melphalan. These agents inhibit cell division through alkylation of DNA.
- Antimetabolites, e.g. methotrexate (MTX), azathioprine. These agents inhibit both DNA and RNA synthesis as purine, pyrimidine or folic acid analogs.
- Intercalating agents, e.g. doxorubicin, mitoxantrone. These agents interact non-covalently with DNA and thus inhibit replication.

##### *Antibodies, e.g.*

- anti-CD3 – catumaxomab
- anti-CD20 – rituximab
- anti-CD25 – basiliximab

##### *Immunosuppressives*

Changes in immunophilins, e.g. through ciclosporin, tacrolimus, sirolimus. Calcineurin inhibition for instance leads to inhibition of T cells.

Finally, there is a number of other immunosuppressive drugs: mycophenolate mofetil for example leads to an inhibition of both B and T cells by inhibiting guanosine nucleotide synthesis <sup>(39)</sup>.

#### 4.1.3. Risk of Infection in Patients Suffering from Diabetic Foot Syndrome/Diabetic Podopathy

Peripheral neuropathy with a loss of sensory, motor and autonomic functions is the primary cause of ulcerations affecting the feet of diabetic patients upon trauma or pressure.

Local factors potentially encouraging wound infection in diabetes patients include defects of the skin's acid mantle caused by seborrhea and sudomotor dysfunction, skin cracks, rhagades or mycosis-related skin defects facilitating pathogen invasion or a very large amount of pathogens combined with a lack of sensory warning signals. Impaired arterial circulation of the legs can be an additional factor. As of today, we can assume that microangiopathy does not result in the occlusion of arterioles and capillaries. Nevertheless, a thickening of the basement membrane can be observed in the small arterioles leading to reduced oxygen permeation into the tissue. However, the autonomically induced opening of all arterio-venous shunts and loss of postural constriction results in permanent hyperperfusion. Glycated hemoglobin transports lower amounts of oxygen. Reduced erythrocyte deformability leads to a further reduction of oxygen supply to the tissue. The resulting abnormal protein biosynthesis leads to a lowered local resistance to infection<sup>(10,18,42)</sup>.

The generally reduced immune response in diabetic patients is attributed to decreased leukocyte and monocyte activity and the resulting reduction of chemotaxis and phagocytosis. Systemic and local immune deficiencies are also the reason why a large part of diabetic patients with infected wounds do not experience classical inflammatory reactions, fever or leukocytosis<sup>(8,18,36,37,42)</sup>.

#### 4.1.4. Risk of Infection in Burns and Scalds

Burns and scalds are thermal injuries of the skin, skin appendages and sometimes the underlying tissue; burns are injuries resulting from fire or contact with hot objects while scalds are caused by hot fluids or steam.

Infections are severe complications of these injuries and sepsis resulting from large burns accounts for more than 50% of fatalities. Around one week after trauma, every burn wound can be assumed to be colonized by microbes or already infected. The conditions in burn wounds are ideal for microorganisms. The risk of infection results from the long-term destruction of the skin barrier, the generally reduced immune response and impaired microcirculation<sup>(4,11,24,29,48)</sup>.

## 4.2. Wounds with an Exogenous Risk of Infection

### 4.2.1 Risk of Infection in Postoperative or Iatrogenic Wounds

Unplanned secondary wound healing in postoperative wounds originally intended to close in primary wound healing often leads to an increased risk of microbial wound healing disorders<sup>(38,47,49)</sup>.

### 4.2.2. Bullet Wounds, Bite Wounds and Contaminated Wounds

#### 4.2.2.1. Heavily Contaminated Wounds

Abrasions, lacerations, contused wounds and impalement traumas acquired outdoors can be heavily contaminated by exogenous organisms. In addition to organisms of the skin flora including MRSA, especially Clostridium and Bacillus spp., potentially even rabies viruses are introduced into the wound.

#### 4.2.2.2. Bite Wounds

Bite wounds are tissue injuries inflicted by humans or animals. Approximately 60-80% of bite wounds are caused by dogs, around 20-30% by cats. In rural areas, human bites are quite rare; in urban areas however, they account for up to 20% of all bite wounds. The greatest problem with regards to bite wounds is that infectious diseases such as tetanus, hepatitis or rabies can be passed on that way. Damages to tendons, muscles or nerves can also be a consequence. While transmission of zoonoses like rabies, cat bite disease, cat pox, rat-bite fever, tularemia, brucellosis or leptospirosis through animal bites and transmission of tetanus through animal bites or accidental trauma have become quite rare, the pathogens contained in the oral flora can lead to severe wound infections or even result in disseminated infection and sepsis. However, the number of bacterial strains isolated from a bite wound is often relatively low which can be due to the selective diagnostic methods used. In cat and dog bites for instance *Pasteurella canis*, *Pasteurella multocida* or *Mannheimia haemolytica* are frequently the underlying causes of wound infection while *Staphylococcus aureus*, *Streptococcus pyogenes*, *Capnocytophaga canimorsus*, *Neisseria* and *Moraxella* spp. account for fewer cases. In 39% of animal bites and in 50% of human bite wounds, anaerobic organisms were detected. The most frequent ones among these anaerobes are *Bacteroides*, *Fusobacterium*, *Peptococcus*, *Veillonella*, *Porphyromonas* and *Prevotella* spp.. Human bite wounds can result in wound infections but they can also lead to systemic infections with *Eikenella corrodens*,  $\beta$ -lactamase-resistant anaerobes, ESBL-producing organisms and MRSA. Finally, transmission of hepatitis B or syphilis

is also possible. The fact that cat bites have a higher risk of infection than dog bites due to the puncture-like wounds and a deeper inoculation of pathogens also needs to be taken into consideration. The risk of infection in human bite wounds was  $\geq 20\%$  depending on localization, in dog bites it was between 3 and 17%<sup>(31)</sup>.

As a detection of pathogens cannot be conducted during the early stages of prevention, the risk of wound infection needs to be minimized by a combination of surgical means and antiseptics and, if necessary, antibiotic treatment.

While primary closure is the uncontested method of choice in bite wounds in face and head, more and more experts tend to think that also in other regions of the body delayed wound closure should only be taken into consideration for wounds that are already infected and for those at special risk of infection; after all, the combination of surgical treatment and antiseptics is a major factor determining the success of treatment. In addition to the benefit of achieving faster wound healing if aiming for primary closure, the cosmetic results of this method are also better. Within a period of up to 12 hours, primary stitching can be used to close the wound following surgical and antiseptic treatment and, if necessary, "single shot" antibiotic prophylaxis. Primary closure can even be applied to infected wounds after inserting a drain<sup>(31)</sup>.

#### 4.2.2.3. Bullet Wounds

A bullet wound is a particular type of acute, traumatic wound. These wounds are caused by the blunt force of bullets or bullet fragments and are a combination of laceration and contused wound. The projectile directly tears apart the tissue when entering, traversing and exiting the body. Depending on the form of the projectile, the tissue displaced on the sides of it may experience very high radial acceleration, major heat generation and tissue destruction (cavitation effect). Injuries caused by mole guns are particularly dangerous because in addition to the projectile contaminated soil is introduced deep into the wound.

Treatment options for bullet wounds include exploration and debridement of the bullet channel and, if necessary, vascular reconstruction, bone stabilization with an external fixator and exploration of the abdominal and chest cavities<sup>(16,46,47)</sup>.

#### 4.2.3. External Hazards

As the skin plays an important role in defense against infections, the wound poses a potential threat which may be more or less serious depending on external factors such as a person's profession, personal hygiene or the location of the wound.

#### 4.2.3.1. Job-Related Hazards

Many patients suffering from chronic wounds are absolutely able to work. In a 2007 study on the Disease Management Program the average age of chronic wound patients was found to be 68 years ( $\pm 12$ ). Especially in rural areas, many people work in agriculture well beyond the actual retirement age. This may result in hygienically difficult conditions caused by the pathogen load in the environment, people's shoes and clothing. Work at construction sites, car garages, ports, recycling facilities and landfills or in a woodworking company may be just as problematic. When taking up a patient's personal data it is thus very important to collect information about the work environment as well. For instance, if a company requires its employees to wear steel-toed safety shoes for work, pressure relief will be impaired and the warm and humid atmosphere in the shoes will promote the proliferation of pathogens. The same holds true for gumboots worn for stable work.

Truck drivers are an occupational group which is particularly vulnerable for pressure ulcers. After long periods of sitting in a plastic seat without any possibility to change position, pressure marks can develop in the sacral area. Many people affected wait a long time before seeing a doctor because they underestimate the problem, they're "ashamed" or even afraid of losing their job. These skin lesions are at risk of infection due to their location and an insufficient air supply. The same group is also frequently affected by pilonidal sinus.

Also, jobs in the healthcare sector should not be underestimated. Different facultative pathogens or pathogens can be found in hospitals, doctor's offices and nursery homes. In case of an injury, these can pose a particular risk (e.g. infection with MRSA)<sup>(34)</sup>.

#### 4.2.3.2. The Significance of Personal Hygiene

The risk of wound infection increases if the dressings are handled with a lack of hand hygiene. Gardening is a risk factor just like home improvement or cleaning. Water pipes, shower heads and tap aerators can contain *Pseudomonas* and other water pathogens which can be transmitted during wound irrigation. A lack of hygiene can also lead to cross-contamination during foot baths<sup>(2)</sup>.

#### 4.2.3.3. The Location of the Wound as a Risk Factor

Some body regions have a higher microbial load than others<sup>(2,11)</sup>. This includes the perineal region, feet, face wounds or stomach wounds with an existing anus praeter. Due to the fact that dressings are much more difficult to attach to hairy skin, there is a risk of wound pathogens passing under the sides of the dressing especially close to hair follicles<sup>(32)</sup>. This problem can also be observed for wounds close to external fixators, tubes, tracheal cannulas and catheters<sup>(28)</sup>.

Table 1: Risk Classification of Wounds at Risk of Infection (Wounds at Risk)

Risk Category	Risk Definition (based on risk factors and different indications)	Score (W.A.R.)
Category 1	<ul style="list-style-type: none"> <li>a) acquired immunosuppressive disease (e.g. diabetes mellitus)</li> <li>b) acquired immunodeficiency caused by medical treatment with e.g. cyclosporin, methotrexate, glucocorticoids, antibodies</li> <li>c) solid tumors</li> <li>d) hematologic systemic disease</li> <li>e) postoperative wound healing disorder leading to (unintended) secondary wound healing</li> <li>f) wounds with a particularly high pathogen load resulting from their location (e.g. perineum, genitals)</li> <li>g) problematic hygienic conditions resulting from a person's social or professional environment (e.g. farmers, truck drivers)</li> <li>h) patient age &gt; 80 years</li> <li>i) young patient age (premature babies, babies, infants)</li> <li>j) age of wound &gt; 1 year</li> <li>k) wound size &gt; 10 cm<sup>2</sup></li> <li>l) chronic wounds regardless of their cause if &gt; 1.5 cm deep</li> <li>m) long-term inpatient treatment &gt; 3 weeks</li> </ul>	<p><b>One</b> risk point for every one of these risk factors that applies to the patient's situation. (check as many as apply)</p> <p>The points are added up.</p>
Category 2	<ul style="list-style-type: none"> <li>a) Severe acquired immunodeficiencies (e.g. HIV infection)</li> <li>b) heavily contaminated acute wounds</li> <li>c) bite wounds, stab wounds and bullet wounds if between 1.5 and 3.5 cm deep</li> </ul>	<p><b>Two</b> risk points for every one of these risk factors that applies to the patient's situation. (check as many as apply)</p> <p>The points are added up.</p>
Category 3	<ul style="list-style-type: none"> <li>a) burn wounds affecting &gt; 15% of the body surface</li> <li>b) wounds directly connected to organs or functional structures (including joints) or containing exogenous material</li> <li>c) profound congenital immunodeficiencies such as agammaglobulinemia; severe combined immunodeficiencies (SCID), etc.</li> <li>d) bite wounds, stab wounds and bullet wounds if &gt; 3.5 cm deep</li> </ul>	<p><b>Three</b> risk points for every one of these risk factors that applies to the patient's situation. (check as many as apply)</p> <p>The points are added up.</p>

Afterwards, all points are to be added. A **score  $\geq 3$  points** indicates that, from a clinical perspective, the wound is at risk of infection; it can therefore be considered a clinical indication for the use of local antiseptics.

Note: Regardless of this recommendation, there may be other treatment indications per se requiring local antiseptic treatment, e.g.

- eradication of pathogens as required by the RKI's regulations if multiresistant pathogens are found
- critically colonized wounds

Furthermore, each patient's individual medication needs to be taken into consideration. Systemic antibiotic prophylaxis is recommended for patients at risk of endocarditis, e.g. for patients with pre-existing defects of heart valves or a mechanical valve replacement.

Unfavorable external conditions can pose a risk of infection to any wound. Therefore, a careful analysis of a patient's living conditions with regards to hygiene and the patient's activities is necessary to assess his or her individual risk<sup>(41,45)</sup>.

## 5. RISK SCORE (W.A.R. SCORE)

A checklist serving as a score for wounds at risk is established to pursue the objective of facilitating a clinically usable, substantial form of risk assessment with regards to a patient's specific situation (Table 1). The indication for use of antiseptics is the result of the addition of differently weighted risk causes, for which points are assigned. Antimicrobial treatment is justified in the case of three or more points.

This score is to serve as a proposal for discussion and was developed on the basis of comprehensive clinical experience due to the fact that there is a lack of reliable data. Without any doubt, this score will have to be tested in practice and will be adapted continuously.

## 6. THE SIGNIFICANCE OF POLIHEXANIDE FOR WOUNDS AT RISK

Against the background of the well-known modes of action of polihexanide and the resulting positive risk benefit assessment, it seems sensible to use polihexanide-containing products on wounds with a clear indication of being a wound at risk in the risk situations described above<sup>(13,19,20,22)</sup>.

Bite wounds however are an exception. In these wounds, surface-active antiseptics such as octenidine, polihexanide or chlorhexidine can theoretically facilitate eradication due to their surface tension; in contrast to alcohols and povidone iodine which are systemically absorbed however, they will not be effective in the deeper layers of the wound. This hypothesis is supported by the fact that –

Table 2: The Properties of Polihexanide (acc. to (13))

Effectiveness of Polihexanide	Tolerance of Polihexanide
broad antimicrobial effect	good clinical tolerance
very low blood and protein errors (limited mucous effectiveness due to mucins)	selective, specific mechanism of action
remanence, post-antiseptic effect	biocompatibility index > 1 (ratio of cytotoxicity (IC50) and microbicidal effectiveness (RF > lg 3 = pathogen reduction by more than 3 logarithmic steps), tested in FBS (fetal bovine serum))
concentration-dependent promotion of wound healing <sup>(44)</sup>	no known toxic risks
anti-inflammatory properties	no known resorptive risks
no known development of resistance	long-term effect
reduction of biofilm and fibrin development <sup>(21)</sup>	low risk of contact sensitizations

in contrast to povidone iodine and alcohols – these agents are not absorbed when applied to the wound. Upon the pressurized application of octenidine to a stab wound in a child's hand, severe long-term edematous effects with tissue damage were observed; pressurized application into the tissue is therefore contraindicated. Analogous effects could probably be observed for polihexanide and chlorhexidine. Irrigation of deep wounds (e.g. bite wounds) with surface-active, highly protein-binding antiseptics is only indicated if the irrigation solution can drain away (drainage)<sup>(17,27,30,31,35,43,50)</sup>.

The basic principles of therapeutic application of polihexanide in infected wounds are defined in an expert recommendation<sup>(13)</sup>.

Table 2 provides an overview of the effectiveness and tolerance of this substance.

A clear distinction needs to be made between the situation described earlier and a (potentially repeated) application to wounds at risk which requires substantial reasons. The relevant risk factors are listed in Table 1.

In general, treatment with antiseptic solutions or an auxiliary use of antimicrobial wound dressings is only useful if integrated into a monitored clinical treatment approach. The frequency of dressing changes will vary depending on the general state of the wound. Infected wounds often require daily dressing changes. In wounds at risk of infection (W.A.R.!) it depends on the state of the wound and the frequency required may vary between two and seven days. After a maximum of 21 days, any application needs to be reconsidered. If the wound is still at risk (W.A.R.), the further therapeutic approach needs to be defined on a case-by-case basis<sup>(13)</sup>.

If an infection is suspected to be deep and passed on to other parts of the body, systemic antimicrobial therapy is required<sup>(13,25,47)</sup>.

## 7. APPLICATION, AVAILABLE PRODUCTS AND MODE OF APPLICATION

The range of standard concentrations for polihexanide solutions for antiseptic wound treatment varies from 0.01% and 0.02% to 0.04%. The solution is for local application only, to be used e.g. as an irrigation solution (lavage), in irrigation-suction drainages or in moist wound dressings. As polihexanide has a delayed onset of effect and the pathogens' reaction to the agent may vary over time, a **minimum period of 10 to 15 minutes** upon broad application to the wound bed needs to be allowed in order to be effective. It is therefore an *intermittent, repeated application*.

If polihexanide is used as an antimicrobial component in wound dressings and gels, the continuous eradication of wound pathogens and the thus increasing safety adds to the general benefits of moist wound care.

The polihexanide-containing products used in wound care come in different dosage forms (antiseptics, wound irrigation solutions, gels, wound dressings) and have different properties due to their respective support material. They have specific indications (drugs = antiseptics) or intended purposes (medical products) and are to be evaluated in view of different criteria with regards to their clinical effectiveness.

It is therefore important to use these products exclusively in accordance with their given indications or intended purposes<sup>(13)</sup>.

Contaminated wound, not at risk of infection (e.g. occasional wound, trivial wound)	Cleansing	0
Colonized wound, not at risk of infection (e.g. unproblematic, chronic wound)	if necessary debridement	
Colonized wound <b>at risk of infection</b> (see Table 1)	<b>Antiseptic/antimicrobial treatment</b>	I
Critically colonized wound	Cleansing if necessary debridement	
Locally infected wound	<b>Antiseptic/antimicrobial treatment</b> Cleansing Surgical debridement	II
Systemic infection, infected wound	<b>Systemic antimicrobial treatment</b> <b>Antiseptic treatment</b> Cleansing Surgical debridement	III

Figure 2  
Therapeutic intensity in antimicrobial wound treatment

### Contraindications

According to present knowledge, there are relative contraindications for using polihexanide:

- for antiseptic joint irrigation (cartilage toxicity)
- on any part of the CNS including meninges or intralumbar application
- in the middle and inner ear and in the inner eye
- during the first 4 months of pregnancy (afterwards, only use after a thorough benefit-risk assessment)
- if allergic to polihexanide

(5,9,13,15,22,30,33)

### 8. CONCLUSION AND SUMMARY

The W.A.R. Score described in Table 1 is a useful tool for optimizing risk assessment for wounds at risk of infection. It facilitates the classification of these wounds in a general treatment regimen.

Based on the classification according to different, microbiological states of the wound and the respective clinical situation, a simple and practical multi-tier pattern for the treatment of this wound can be derived (see Figure 2).

That way, a replicable range of methods based on the respective requirements can be provided for clinical practice and every wound can be adequately treated upon assessing the risk at hand.

Conflict of interest:

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