Bacterial infections in burn wounds

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Abstract

Thermal injury destroys the skin, the natural barrier to the external environment. As a result the burned area is prone to infection and colonization of microorganisms. The most common pathogenic colonizing bacteria are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *S. aureus* is both a human commensal bacterium, as it is carried by a significant amount of individuals, and a possible infectious pathogen. Other prevalent bacteria in burn wounds are *Acinetobacter baumannii* and *Klebsiella pneumonia*. All these pathogenic species of bacteria can contribute to the inflammatory immune responses induced by thermal injuries. Hyperactivity of human macrophages induces an increased production of cytokines, radical oxygen agents and endotoxins. This leads to a diminished function of the adaptive immune system. Thereby, there is a destruction of cell membranes and denaturation of proteins in endothelial cells. Severe thermal injuries may lead to impaired functioning of the cardiovascular, the excretory, the respiratory and the gastrointestinal system. The most common treatment method used to be treatment with topical antibacterial agents. However, most pathogenic strains have acquired resistance to antibiotics. Several different treatment strategies have been effectively developed. Negative wound pressure therapy has been proved to inhibit bacterial proliferation and to reduce cytokine production. Garlic ointment showed effective inhibition of biofilm formation by all prevalent pathogenic bacterial strains.

Introduction

Burn wounds have been among the most devastating form of injuries during the last decades. Serious thermal injuries cause high mortality and morbidity. Globally, each year 11 million people require medical treatment and 300,000 die due to burns [1]. Remarkable, over 75% of deaths caused by burns are a result of wound infection by bacteria [2-4]. Burn patients are very prone to infections due to the loss of their natural barrier, prolonged hospital stays and therapeutic and diagnostic procedures [4-6]. Furthermore, the incidence of burn wound infection appears to be correlated with both the depth and the size of the wound. Moreover, the longer the wound remains open, the higher the chances on infection [7].

Previous studies have shown that there are several bacterial species which are able to readily infect burn wounds. *S. aureus* and *P. aeruginosa* have been found to be the most common species. Interestingly, the variation in bacterial flora and the colonization rate changes over time after the initial infection [8].

Improved medical treatment and infection control has already reduced the death rate of burn patients significantly. The amount of death-related burns has been halved within the past 40 years [4]. Moreover, bacterial infection can be controlled by the use of antibiotics. A downside of the use of antibiotics is that some pathogenic strains of bacteria may become multidrug-resistant [9].

This review mainly focuses on the bacterial colonization of burn wounds. As the colonization of burn wound starts right after the injury, it is of importance to know which bacterial species colonize burn wounds and how these species affect the human body. Thus, the immune response of the human body to bacterial infection will be illustrated. Furthermore, the possible treatment methods will be examined.

The skin and pathogenesis due to burn injuries

The human skin is an important barrier to the external environment. It is responsible for immunologic protection, thermoregulation and control of fluid levels. Thereby, the natural colonization of microorganisms on the human skin also offers protection against pathogenic microorganisms by bacterial interference [10]. Serious thermal injury may cause disruption in certain physiological functions of the skin. This can result in several harmful conditions such as reduced immunity, infection, scarring and fluid loss as well [11]. Burn injuries cause breaches in the skin by destroying its cellular compounds. The amount of cellular damage varies based on the range of the heat that the skin was exposed to. Thermal burns denature proteins, destroy cell membranes and release oxygen-free radicals. Three different zones can be distinguished within burn wounds. 1. Dead tissue (zone of coagulation), 2. viable tissue, which is at risk for ongoing damage (zone of stasis) and 3. normal skin with minimal injury, which still shows blood flow (zone of hyperaemia) [12].

Furthermore, local and systemic inflammatory responses are also initiated after a burn, which has detrimental effects on both the burn wound and several distant organ systems. The cardiovascular, respiratory, gastrointestinal and renal systems are all affected [13].

Importantly, immediately after a burn the natural microbial flora of the skin surface is removed. This makes the wound prone to infections. It has been found that as the burn wound is bigger and deeper, with the dermis being partially destroyed, the risk of subsequent burn wound colonization and infection is increased [14].

Bacterial flora

The growth of commensal bacteria is supported by the skin. Bacterial flora protects both directly and indirectly against pathogenic bacteria. The commensal bacteria offer direct protection through production of toxic metabolites and antibiotic substances known as bacteriocins [15]. Furthermore, essential nutrients are provided and adherence of competing

bacteria is inhibited. For example, *Staphylococcus epidermis* binds to keratinocyte receptors and thereby inhibits the adherence of *S. aureus* [16]. *S. epidermis* indirectly protects against pathogenic bacteria by inducing the host to produce antibodies.

Bacterial flora on healthy human skin. The bacterial flora on the human skin can be divided in two categories, namely resident and transient [17]. The resident microorganisms are bacteria which can be continuously found on the human skin. These microorganisms reside mostly under the cells of the outermost horny layer of the epidermis [18]. Nearly all resident bacteria on the human skin are gram-positive. Gram-negative bacteria usually do not favor the dry environment of human skin [15]. The most dominant resident species is *S. epidermis*, a coagulase-negative bacterium. Other similar prevalent species are *S. hominis*, *propionibacteria*, *corynebacteria*, *dermobacteria*, and *micrococci* [14, 19]. The transient flora consists of bacteria which are not always found on the human skin. The medical distinction between resident and transient bacteria can be found in the easy removal through daily hygienic measures such as washing hands [20]. Subsequently, transient bacteria can be acquired by direct contact with contaminated environmental surfaces. A well-known example of a transient bacterium is *S. aureus,* an opportunistic pathogen.

Staphylococcus aureus carriage and infection. *S. aureus* is a gram-positive bacterium which often colonizes burn wounds but it is also prevalent in healthy humans. Thus, *S. aureus* is both a human commensal bacterium and responsible for pathogenic infections [14]. Several studies found that *S. aureus* is carried by a significant amount of humans. About 10-35% of healthy individuals persistently carry this particular bacterium. It is intermittently carried by 20-75% and 5-50% never carries *S. aureus* [21-27]. Furthermore, *S. aureus* can be carried in different areas of the body. It is mostly carried in the nasal area but it is also prevalent on the skin in general, the gastrointestinal tract and the pharynx [24, 28-30]. The fact that *S.* aureus is self-carried makes it an easy candidate for infection. A diminished immune system or a breach in the skin can trigger *S. aureus* to cause minor infections or sometimes even life-threatening diseases. Thus, *S. aureus* could be considered as an opportunistic pathogen. Furthermore, *S. aureus* is known to easily acquire antibiotic resistance. For example, the widely known Methicillin-Resistant *Staphylococcus aureus* (MRSA) have also been found to be prevalent in burn wounds [31, 32].

Bacterial infection in burn wounds. Immediately after a burn wound has been induced the area of the skin is free of bacterial flora. Subsequently, the burn area is accessible to any bacterial species. Microorganisms either from the patient's endogenous skin, gastrointestinal and respiratory flora or from contact with contaminated external environmental surfaces may colonize the burn wound. Taneja et al. studied the bacterial colonization of burn wounds from 71 patients over a period of 14 days [8]. On the first day 33% of the patients were colonized

(Table 1) and *S. aureus* seemed to be the most prevalent bacterium. Furthermore, *S. aureus* accounted for 50% of the bacteria on the 1st day and for 75% on the 7th and 14th day. Other bacteria present on the wounds were gram-negative species like *P. aeruginosa*, *A. baumannii*, *K. pneumonia*, *Enterobacter* and *Escherichia coli* (Table 2). As shown in Table 1, after 7 days nearly all of the patients were colonized and thereby a significant amount of the wounds of the patients were invasively infected. This also resulted in a higher death rate. Overall, out of 214 isolates, *S. aureus* was the most dominant isolate (45.4%) followed by *P. aeruginosa* (13.9%), *Beta-hemolytic streptococci* (9.4%) and *A. baumannii* (9.4%).

Other studies showed wound colonization with similar species of bacteria. Nonetheless, the proportions of the different species seemed to differ. Keen et al. showed that the most prevalent colonizing bacteria were *A. baumannii* with 22% out of 780 isolates. Other following dominant bacteria were *P. aeruginosa* (20%), *K. pneumonia* (20%) and only then *S. aureus* (13%) [9]. Furthermore, another study showed that *P. aeruginosa* appeared to be the most prevalent bacterium with 50% prevalence followed by *E. cloacae* (27.8%) and *S. aureus* (9.3%) (118 isolates) [3]. The high prevalence of *P. aeruginosa* could be explained due to their high

Table 1. Result of gross wound appearance, invasive wound infection, colonization of wounds & mortality.

Result	**Post admission time of sampling**				
	1st day	**3rd day**	**7th day**	**10th day**	**14th day**
Number of patients (n) sampled	71	64	36	36	24
Number of patients colonized	24 (33%)	44 (68.7%)	34 (94%)	34 (94%)	24 (100%)
Grossly clean wound	71 (100%)	61 (95.3%)	17 (47.2%)	18 (50%)	06 (25%)
Invasive wound infection	0 (nil)	04 (9%)	17 (50%)	8 (23.5%)	02 (8.3%)
Polymicrobial wound colonization	6 (25%)	15 (34%)	08 (23.5%)	13 (38.2%)	14 (58.3%)
Patients died (n=16)*	0 (nil)	01 (6.25%)	06 (37.5%)	02 (12.5%)	04 (25%)

*Three died after 14th day.

Table 2. Results of bacterial cultures from wounds on different days.

Organism	**1st day**	**3rd day**	**7th day**	**10th day**	**14th day**
No. of patients colonized	24	44	34	34	24
S. aureus (n=101)	12 (50%)	29 (65.9%)	25 (73.5%)	17 (50%)	18 (75%)
P. aeruginosa (n=22)	4 (16.7%)	2 (4.5%)	8 (23.5 %)	4 (11.8%)	4 (16.7%)
A. baumannii (n=21)	5 (20.8%)	8 (59.2%)	4 (11.8%)	2 (5.9%)	2 (8.3%)
β Hemolytic Streptococci (n=21)	1 (4.2%)	4 (9.1%)	4 (11.8%)	4 (11.8%)	8 (33.3%)
K. pneumonia (n=15)	2 (8.3%)	6 (13.6%)	3 (8.8%)	2 (5.9%)	2 (8.3%)
Enterobacter (n=9)	2 (8.3%)	4 (9.1%)	3 (8.8%)	-	-
Proteus mirabilis (n=8)	-	2 (4.5%)	4 (11.8%)	1 (2.9%)	1 (4.2%)
E.coli (n=7)	1 (4.2%)	2 (4.5%)	1 (2.9%)	2 (5.9%)	1 (4.2%)
Enterococci (n=7)	2 (8.3%) 3	3 (6.8%)	1 (2.9%)	1 (2.9%)	-
Cirtobacter (n=2)	-	-	-	1 (2.9%)	1 (4.2%)
α-haemolytic Streptococci (n=1)	-	-	-	1 (2.9%)	-

n = number of isolates.

resistance to antibiotics. Moreover, gram-negative bacteria grow well in the moist environment of burn wounds. As a wound becomes colonized, the bacteria form protective structures known as biofilms. Bacteria secrete polysaccharides which form a protective matrix, known as a glycocalyx [33, 34].

Overall, in most studies, the gram-positive *S. aureus* was found to be the most common bacteria in the first days of burn wound colonization [35]. Over time, gram-negative bacteria as *P. aeruginosa, A. baumannii* and *K. pneumonia* became more prevalent. Noteworthy is that many isolates of these bacteria *S. aureus, P. aeruginosa, A. baumannii* and *K. pneumonia* showed resistance to antibiotics, strengthened by biofilm formation. Table 3 shows the percentage of isolates with acquired resistances to antibiotics [2]. Remarkable is that all of the isolates show signs of multi-drug resistance, both *S. aureus* and the gram-negative isolates as well. Furthermore, the study performed by Taneja et al. revealed similar high antibiotic resistance of the above mentioned prevalent bacteria. Additional investigated antibiotics in this study were methicilin and gentamicin [8].

Table 3. Relative frequency of resistance to antibiotics in bacteria prevalent in burn wounds.

Antibiotic	*P. aeruginosa* (%)	*A. baumannii* (%)	*K. pneumonia* (%)	*S. aureus* (%)
Ceftazidime	96.3	92.3	91.7	72.2
Amikacin	57.1	92	87.5	66.7
Imipenem	17.9	7.7	4.3	66.7
Meropenem	18.5	3.8	33.3	31.6
Piperacillin	30.8	7.7	50	31.6
Ciprofloxacin	61.5	92.3	70.8	31.6
Cefepime	39.3	7.7	25	31.6

Intermediate resistant samples have not been counted as resistant. If a patient has had at least one culture resistant to an antibiotic for a specific organism, we considered that organism resistant to that antibiotic.

Immune response

The immune system immediately becomes active after a thermal injury has been induced. Both the innate and adaptive immune system play a role in burn wounds. Severe burn injuries induce an immunosuppressed state leading to possible infectious complications, sepsis and multiple organ failures [4, 13]. These are the major causes of morbidity and mortality. An overview of the immune response as a result of thermal injury is shown in Fig. 1.

Thermal injury activates a wide variety of human macrophages and endothelial cells which results in inflammatory responses [36, 37]. Hyperactivity of macrophages mainly leads to the production of the cytokines interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) [4]. Thereby there is an upregulation of the production of IL-6 and prostaglandin E_2 (PGE_2). These inflammatory cytokines cause a deficiency of the immune system. Increased levels of IL-1β, TNF-α and IL-6 lead to a state of fever and the production of acute-phase proteins [38]. Besides, these cytokines contribute to cascades of secondary cytokine production. The cytokine levels

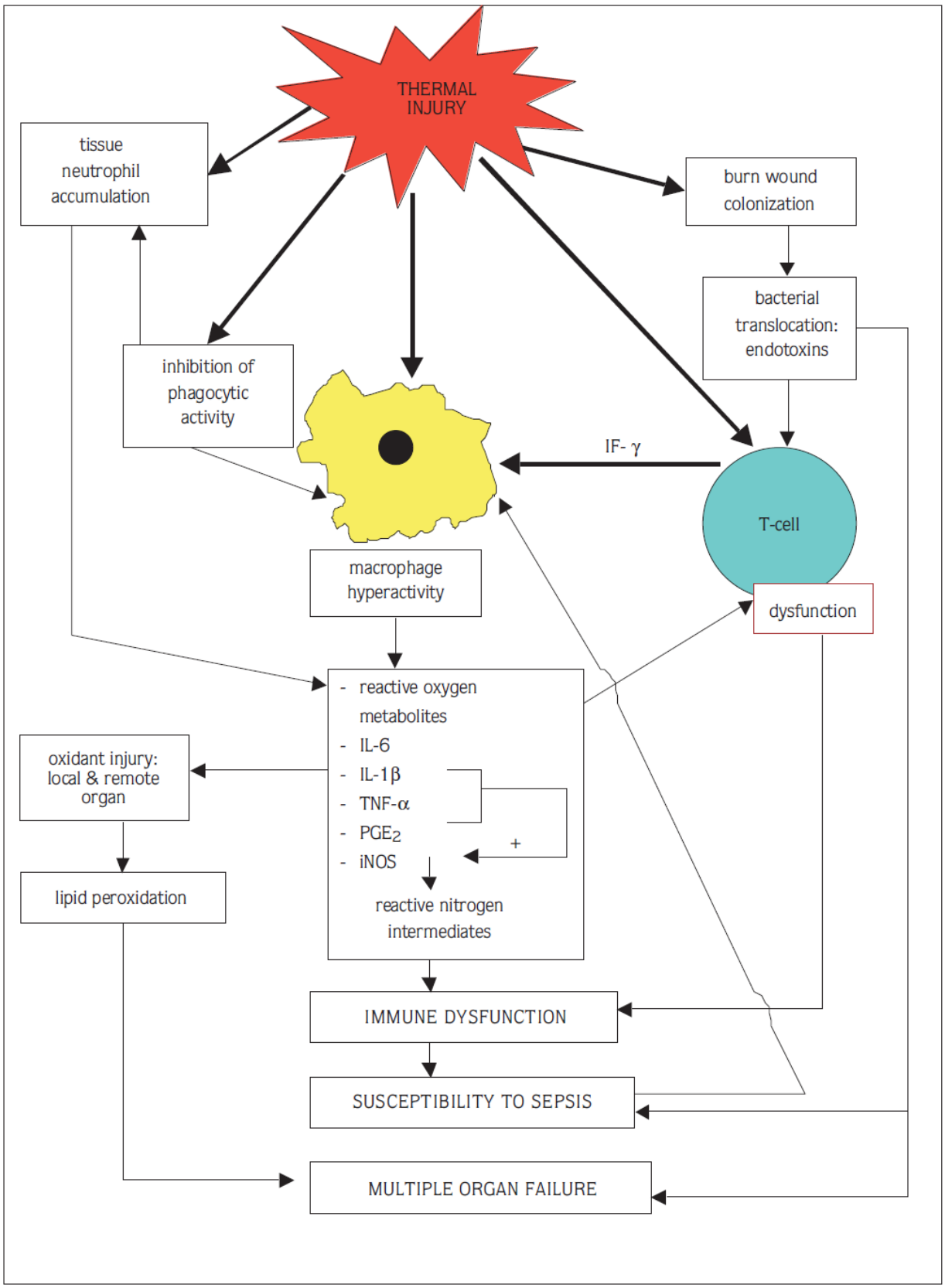

Figure 1. Overview of the immune response as a result of thermal injury.

peak at 7 days after the injury has been induced. PGE_2 promotes the proliferation of T helper cells into Th-2 cells which produce the anti-inflammatory cytokines IL-4 and IL-10 [39]. Increased levels of IL-4 and IL-10 inhibit Th-1 cell activation and antigen presentation of macrophages. Overall, the changes in cytokine levels diminish T cell proliferation and cause an imbalance in T cells, which leads to a dysfunction in the adaptive immune system. Several studies have suggested that dysregulation in T-cell functioning, glucocorticoids and T-helper cytokines contribute to the post burn immune dysfunction. Another following result of the increased serum levels of inflammatory cytokines is a possible susceptibility to sepsis [40, 41].

Thermal injury also initiates production of burn toxins and oxygen radicals. Reactive oxygen metabolites can initiate destruction of cell membranes and denature proteins by lipid peroxidation [42]. This process is mainly seen in the first few hours and first days post burn injury. Furthermore, there appears to be a correlation between the amounts of oxygen radicals and the severity of distant organ failure [43].

Additionaly, after a thermal injury has been induced the wound is prone to infection. Gram-negative bacteria like *P. aeruginosa* contain endotoxins, also referred to as lipopolysaccharides, on the outer membrane of the cell wall. Endotoxins are able to activate macrophages and neutrophils which lead to high productions of oxidants and proteases [44]. After the burn, endotoxins are found in the blood circulation. Remarkable is that these are not only derived from bacteria colonizing the burn wound. Burn injury leads to a reduced blood volume which has destructive effects on the mucosal of the gut barrier. Subsequently, translocation of gut bacteria results in an increased circulation of endotoxins [45].

Table 4. Systemic responses to burn injury.

Cardiovascular system	**Excretory system**	**Respiratory system**	**Gastrointestinal system**
Acute (hypovolemia) phase	**Acute (hypovolemia) phase**	- Hypoxemia - Pulmonary hypertension - ↑Airway resistance - ↓Pulmonary compliance	- Adynamic ileus - Gastric dilatation - Delay in gastric emptying - Gastrointestinal hemorrhage - ↑Gastric secretions - ↑Ulcer incidence - ↓Intestinal & colonic motility - ↓Mesenteric blood flow - ↓Nutrient absorption - Bacterial translocation - Hepatic injury
- ↓Blood flow - ↓Cardiac output - ↑Capillary permeability - ↑Peripheral vascular resistance	- ↓Renal blood flow - ↓Glomerular filtration rate		
Hypermetabolic phase	**Hypermetabolic phase**		
- ↑Blood flow - Edema formation - Cardiac arrhythmias - Myocardial infarction - Myocardial dysfunction/cardiac instability	- ↑Renal blood flow - ↑Glomerular filtration rate - Impaired tubular functions - Acute renal failure		

The destruction of cells, proteins and lipids induced by endotoxins, oxygen radicals and other inflammatory mediators lead to tissue damage and multiple organ failure. Several systems in the human body are affected by burn injuries. The cardiovascular, the excretory, the respiratory and the gastrointestinal system all show signs of impaired functioning (Table 4) [13]. Severe failure of these organ systems lead to a high probability of mortality.

Treatment methods of burn injuries

Due to the high numbers of morbidity and mortality an effective treatment is demanded. A various amount of treatment methods have been developed during the last decades. The most commonly used primary treatment for severe infections of burn wounds has been the use of antibiotics, or topical antibacterial agents. This treatment has widely been used for the prophylaxis and treatment of infections for the last few decades [46]. Topical antimicrobial agents are effective in significantly reducing the bacterial flora on the wound as well as preventing the systemic spread of infecting pathogens [4, 47]. A widely used topical antimicrobial agent, for inter alia against *S. aureus*, is the combination of silver and cerium. Silver, Ag-SD, acts on the bacterial cell wall and binds strongly to bacterial DNA. Thereby, cerium forms a barrier over the wound which offers protection against bacterial colonization and infection [14, 48, 49]. However, too much usage of these antimicrobial agents led to an increased resistance of bacteria like *S. aureus* and *P. aeruginosa* to silver, cerium and also multiple other used antibiotics. Thus, these problems require different strategies in the treatment of burn injuries and infection or novel antibiotics have to keep being developed.

Nonetheless, alternative treatment methods have been developed in the past years. An example is the Negative Pressure Wound Therapy (NPWT), an increasingly used method since the 2000s. Recent studies proved the effectiveness of this particular treatment, as it clears the bacteria from the wound [50]. Other studies found that NPWT significantly reduces the presence of *P. aeruginosa* within a contaminated open fracture wound [51]. Furthermore, a recent study by Liu et al. investigated whether NPWT prevents sepsis as a result of burn wound infection by *P. aeruginosa*. The results indicated that NPWT inhibited bacterial proliferation. Thereby, the *in vivo* inflammatory response was reduced which led to a protection of the internal organs [52].

Other studies directed their treatment methods at inhibiting biofilm formation. It has been demonstrated that garlic (*Allium sativum*) or garlic extract (allicin) effectively inhibits the growth of bacterial pathogens [53, 54]. Nidadavolu et al. found that garlic ointment inhibited biofilm development and eliminated 24h established biofilms as well. Garlic ointment inhibited the biofilm development by *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *A.baumannii* and *Klebsiella pneumonia*. Moreover, 24h established biofilms by *S. aureus*, *S. epidermidis* and *A. baumannii* were disrupted [55].

Conclusion

This review has highlighted the effects of bacterial colonization on burn wounds. The most prevalent bacteria colonizing burn wounds are the gram-positive *S. aureus* and the gram-negative *P. aeruginosa*. Furthermore, the gram-negative bacteria *A. baumannii* and *K. pneumonia* were also commonly present. About half of the human population either persistently or intermittently carries *S. aureus* which leads to easy infection of patients in hospitals. All these species of pathogenic bacteria induce inflammatory responses in burn wounds. Several cytokines and reactive oxygen agents are produced and may cause dysfunction of the immune system. Severe burn injuries may lead to multiple organ failure. Thus, treatment of burn wounds and prevention of infection is necessary. Most species of pathogenic bacteria have acquired resistance to antibiotics as a result of prolonged usage. Novel types of antibiotics have to keep being developed in order to effectively treat infection of burn wounds. Several other types of treatment methods have also been developed. NPWT and garlic ointment have proved to effectively inhibit bacterial proliferation. Nowadays, the morbidity and mortality due to burn injuries has already been significantly reduced over the last few decades. However, the mortality rate, mostly as a result of infection, is globally still high. Thus, faster or more effective measurements are necessary.

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