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THE ROLE OF *STAPHYLOCOCCUS AUREUS* IN THE CLINICAL DIAGNOSIS OF DIABETIC PATIENTS

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Abstract: Discovering interactions between the etiology of the infection and diabetic patients' immune system activity may be essential for the relevant clinical diagnosis. The dynamics of colonization of the nasal vestibule by *Staphylococcus aureus* and the development of the prevention strategies against infection are different for various populations. Moreover, the colonization of the nasal vestibule might involve both molecular and epidemiological ctorsfa. Researchers have reported that the identification of methicillin-resistant strains *S. aureus* (MRSA) with similar molecular characteristics allows to assess the ability of the microorganism to spread and the risk of infection in diabetic patients. Knowledge of these characteristics allows to take precautions in patients exposed to *S. aureus*. *S. aureus* is an ethiological factors of many severe diseases both in people with weakened immune system and in healthy individuals. Usually, excess weight and obesity contribute to the incidence of diabetes mellitus type 2 (DM2). However, the colonization by *S. aureus* is a probable risk factor for infection. Among *S. aureus* virulence factors, superantigens (SAgs) are essential for pathogenicity. The long-term effect of the superantigen toxic shock syndrome toxin-1 (TSST-1) might be glucose intolerance. This toxin also induces systemic inflammation as a result of the increased exotoxin concentration in blood, and, therefore, may be the causative factor of diabetes. Chronic exposure to staphylococcal superantigens may contribute to the development of diabetes, suggesting a need to conduct targeted therapies against *S. aureus* superantigens.

Rola *Staphylococcus aureus* w diagnostyce klinicznej pacjentów z cukrzycą

Streszczenie: Znajomość zależności między etiologią zakażenia, a aktywnością układu odpornościowego u pacjentów z cukrzycą może mieć kluczowe znaczenie dla diagnostyki klinicznej. Dynamika kolonizacji przedsionka nosa przez *Staphylococcus aureus* oraz zalecenia dotyczące gronkowcowych zakażeń, będą odmienne dla różnych populacji. Charakterystyka kolonizacji przedsionka nosa może obejmować cechy molekularne i czynniki epidemiologiczne. Naukowcy donoszą, że identyfikacja nosicielstwa, szczepami *S. aureus* opornymi na metycylinę (MRSA) o podobnych cechach genotypowych, pozwala ocenić zdolność rozpowszechniania się drobnoustroju oraz ryzyko zakażenia pacjentów z cukrzycą. Znajomość tych właściwości umożliwia wprowadzenie działań zapobiegających zakażeniom wywołanym przez *S. aureus*. Bakteria ta jest czynnikiem etiologicznym wielu ciężkich chorób zarówno u osób z upośledzoną odpornością, jak i u zdrowej populacji. Nadwaga i otyłość stanowią czynnik ryzyka wystąpienia cukrzycy typu 2 (DM2). Natomiast nosicielstwo *S. aureus* może przyczynić się do rozwoju infekcji. Istotne dla patogenności czynniki zjadliwości *S. aureus*, to superantygeny (SAgs). Zaliczamy do nich toksyny zespołu wstrząsu toksycznego (TSST-1), których długotrwałym efektem działania może być nietolerancja glukozy. Wspomniane toksyny indukują również ogólnoustrojowe zapalenie, w wyniku zwiększonego stężenia egzotoksyn we krwi, a zatem mogą być czynnikiem, który indukuje hiperglikemię. Przewlekła ekspozycja na superantygeny gronkowcowe może prowadzić do rozwoju cukrzycy, co sugeruje potrzebę prowadzenia terapii ukierunkowanej na superantygeny *S. aureus*.

Key words: diabetic patients, *Staphylococcus aureus*
Słowa kluczowe: pacjenci chorujący na cukrzycę, *Staphylococcus aureus*

1. Introduction

The test results reveal that patients with diabetes tend to be at higher risk ratio for infections about 1.21 when compared to patients without diabetes [52]. Infections that occur in patients with diabetes have severe course and can be even life-threatening. First of all, clinical symptoms are often mild and masked by the chronic complications of diabetes, which leads to the late medical diagnosis. Secondly, the defect in humoral immunity leads to the inhibition of the normal body's response to the infection [12]. Casqueiro et al.

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confirm that hyperglycemia environment can result in a reduction of the function: lymphocytes T, neutrophils, secretion of inflammatory cytokines and anti-oxidant system [5]. A high percentage of *Staphylococcus aureus* which is the etiological factor for diabetic foot syndrome (DFS) and skin and soft tissue infections (SSTI) confirms that diabetic patients are particularly vulnerable to infections caused by this microorganism [29, 66]. Given the fact that *S. aureus* is an independent predictor of mortality, monitoring of this microorganism must be treated as a priority, as reported by Hernandez et al., in their studies. Therefore, adequate knowledge of these pathogens involved in a potentially invasive disease, is the important tool for effective treatment using antibacterial agents with a properly chosen spectrum of activity [18].

Vu et al., in their study demonstrated adipocytes with insulin resistance resulting from chronic exposure to superantigen TSST-1 [65].

Rodríguez et al. state that the spread of the different strains of *S. aureus* can be controlled through the knowledge of their molecular characteristics and epidemiological mechanisms. In patients with high risk of diabetes type 2 the presence of MRSA colonization of similar molecular characteristics discloses the ability of incidence and risk of *S. aureus* infection [48]. Elimination of *S. aureus* carriage seems to play an important role in the prevention of infections in patients with diabetes who are about to undergo major surgery, which was confirmed by Muñoz et al., revealing an increased frequency of surgical site infections after major heart surgery. Poindexter et al. reports not only confirm increased incidence of staphylococcal colonization among diabetic patients compared to patients without diabetes but also reveal that still the important factors responsible for the higher rates of *S. aureus* carriage in the nasal cavity are not known. Another important issue is the need to control antibiotics' resistance in hospital settings [44].

The aim of the study is to determine the impact of *S. aureus* infection on the clinical course of diabetes mellitus.

2. Risk factors for infection in patients with diabetes

Diabetes mellitus is the metabolic disease caused by a defect in insulin action or secretion, which leads to hyperglycemia [55]. As a result of poor glycemic control or ketoacidosis, secondary defects in phagocytic cells can occur in diabetic patients. The process of chemotaxis and phagocytosis, when normally bacteria are killed by neutrophils, can be weakened in patients with diabetes [49]. Infectious diseases are common in diabetic patients. The main pathogenic mechanisms are: the increased virulence of certain pathogens in hyperglycemic environment, reduction of chemotaxis activity and production of the interleukins in response to the infection, as well as a decrease in the activity of phagocytes and the immobilization of neutrophils [3, 5, 17].

2.1. Immunodeficiency

Infection in patients with diabetes causes many pathophysiological changes. The complement system contains plasma proteins and cell surface proteins which promote opsonization and bacterial phagocytosis by leukocytes. The complement system is activated and may increase the lysis of microorganism and mediates a B-cell antibody production [9]. Decreased level of C4 was observed in patients with DM and that may cause malformed neutrophils formation and decreased response to cytokines. In the process of phagocytosis, lipopolysaccharide (LPS) induces mononuclear cells and monocytes, but in response to stimulation, they release lower amounts of interleukin-1 (IL-1), and interleukin-6 (IL-6). Lymphocytes and macrophages decrease IL-10 production as a consequence of increased glycation. Glycation reduces also the myeloid cells' expression of the cell surface of the major histocompatibility complex class I (MHC), and this reduces cell-mediated immunity. Lymphocytes T and NK cells reduce the release of interferon gamma (IFN-γ). Additionally, T cells and macrophages inhibit tumor necrosis factor (TNF-α) [46, 30]. Impaired immunological defense in hyperglycemic environment applies to phagocytic activity, polymorphonuclear leukocytes and chemotaxis. Hyperglycemic environment increases the intracellular concentration of glucose in tissues that do not consume insulin, and this decreases the concentration of NADPH. Exhaustion of NADPH, as a cofactor, disables the action of the molecules that play an important role in the antioxidative mechanisms of a cell. As a result, this leads to the increase in tissue sensitivity to oxidative stress. Some studies have shown that HbA1c < 8.0% has an influence on the proliferative activity of lymphocytes Th (helper cells – CD4+) and their response to antigens. In diabetic patients glycation of the immunoglobulin is proportional to the increasing concentration of HbA1c, which may impair the biological function of antibodies [5, 22].

2.2. Obesity

Numerous studies have shown the strong relationship between obesity and diabetes [9, 39, 55]. In obese patients adipocytes function is impaired and this leads to metabolic disorders and chronic inflammation. Obesity and diabetes mellitus type 2 lead to microbiome

changes, staphylococcal carriage, and noticeable infection. An important role in the development of DM2 is played by e.g. superantigen TSST-1, as a result of the presence of the long-term glucose intolerance. In vitro and in vivo studies have shown that the chronic presence of exotoxin TSST-1 induces not only insulin resistance but also lipolysis and inflammation in adipocytes [65]. However, other studies have shown a variety of risk factors and therefore, not all the people with obesity develop DM2 [70]. The comparison study of B lymphocytes and inflammatory cytokines in obese diabetic patients without symptoms of diabetes has shown similar properties and impaired functioning. Obese people in response to the inflammation secrete pro-inflammatory cytokines typical for the chronic infection. Zhai et al. compared obese patients with and without diabetes and have shown increased production of polyclonal antibodies by adaptive B-cells in patients with diabetes. Additionally, in these patients, there is an impaired response to vaccination, and there is often a generalized inflammation [70]. Dai et al. conducted studies in diabetic patients with elevated BMI and with normal BMI, and evaluated the expression and the function of the proinflammatory cytokines released by T-cells and monocytes [7]. It was found that obese patients with diabetes have increased production of proinflammatory cytokines, and their adipose tissue began to accumulate more M1-type macrophages and T-lymphocytes stimulating IL-17 production. The comparison of IL-17 production in obese patients with and without diabetes has shown that only in obese diabetic patients the release of IL-17 was upregulated. Production of IFN-y by T cells was increased in both study groups. Activation of monocytes in obese with DM2 is possible by the action of both IFN-y and IL-17, whereas in diabetic patients with normal BMI only IFN-y functions properly. Dai et al. revealed that the immune system and BMI play an important role in pathogenesis of DM2 [7]. DeFuria et al. have highlighted that in the development of inflammation in obesity and/or DM2, B-lymphocytes play an important role [9]. In the case of malfunction, these cells formed a mechanism dependent on contact B-cells promote proinflammatory T-cell functions, resulting in stimulated secretion of proinflammatory cytokines. Researchers suggest that the target of inflammation treatment in obese and / or diabetic patients are B-lymphocytes [9]. Frasca et al., evaluated the effects of an influenza vaccine. The study group consisted of obese patients in different age. The evaluation of the immune response after vaccination has demonstrated that leptin causes disorders of B-cell levels in obese patients. This was confirmed by an increase in IL-10 secretion and immune activation markers (TLR4, TNF-α, micro-RNA) [13].

2.3. Staphylococcal carriage

S. aureus methicillin resistant strains (MRSA) are pathogenic factors of many diseases. Carriage of *S. aureus* acts as the pathological and epidemiological factor in the development of infection. The front of the nostrils is an ecological niche for the development of *S. aureus* carriage. According to the *S. aureus* carriage healthy people can be divided into three groups – about 20% of people are solid carriers, 60% are sporadic carriers and about 20% who were almost never carriers of *S. aureus.* Carriage (particularly MRSA) is dangerous in patients with skin wound, who are undergoing surgery, hemodialysis or peritoneal dialysis (CAPD), infected with HIV, AIDS patients and patients with intravascular devices. The high risk group would include diabetic patients due to increased frequency of chronic wounds, the presence of intravascular devices and common infectious diseases.

The treatment of infections caused by MRSA makes many problems arise and staphylococcal infections prevention is growing in importance. Studies show that as the result of the elimination of carriage in high risk groups of patients, the reduction in the number of infections is reported. Carriage elimination is critical in preventing infections in high risk groups of patients, therefore further research is needed to determine the exact high risk group [23].

The mechanisms leading to the carriage of *S. aureus* in the nasal cavity are multifactorial. *S. aureus* nasal cavity carriage can be the result of predisposed conditions and inhibition. There are four reasons for nasal colonization by *S. aureus*. Firstly, *S. aureus* must come into contact with the nasal cavity. Secondly, *S. aureus* must adhere to specific receptors in the nasal cavity. Thirdly, *S. aureus* must overcome the organism's defense systems and ultimately *S. aureus* must be able to multiply in the nasal cavity. Wertheim et al. conducted study on the hospitalized patients with bacteraemia caused by *S. aureus* who were carriers of this bacteria and compared results with patients with bacteraemia without carriage of *S. aureus*. The researchers found that 21% patients had their nasal cavities colonized with *S. aureus* [67].

The constant presence of *S. aureus* in the nasal cavity is one of the etiological factors of recurrent infection. Ecological niche for *S. aureus* is the front part of the nostrils, where the re-colonization occurs during a few weeks to several months. The incidence of carriage depends on many factors, including diabetes which contributes to the occurrence of carriage, sometimes for many years. *S. aureus* affinity to the epithelium of the nasal cavity is dependent on the teichoic, lipoteichoic acids and fibronectin, which adhere to the epithelial cells [61]. Colonization of the front part of the nostrils by *S. aureus* promotes colonization of other

parts of the body, mucous membranes or abraded skin. Microorganism's invasiveness is associated with the presence of cell surface adhesins, secretion of exotoxins inhibiting phagocytosis. The presence of these factors facilitates feeding the bacteria and impairs immune system responses. The most common staphylococcal superantigens that can be identified in the nasal cavity are: staphylococcal enterotoxin SE and toxic shock syndrome toxin 1 (TSST-1). They stimulate T-cells and macrophages to release inflammatory cytokines. Superantigens cause massive release of cytokines that directly link to the variable domains of T-cell receptor major histocompatibility complex class II molecules on antigen presenting cells. Polyclonal T-cells cause the massive release of cytokines which can lead to the toxic shock syndrome [14, 37]. Burian et al. led the research explaining why enterotoxin gene cluster EGC SAgs do not work properly in patients who are carriers of *S. aureus* [4]. The members of EGC like: SEG, SELN, SELO, SEI, SELM, and/or SELU, activate T-cells and antibody response. Also T-cells are activated by non-EGC SAgs like: SEC, SEA or TSST-1, and their antibodies are common in healthy population and patients with bacteraemia. SAgs genes are transcribed, in the nasal cavity, though antibodies against non-EGC and anti-EGC. Carriers demonstrate the presence of antibodies against SEA and SEC, which are mitogenic effect on the present strain of bacteria, but they have low level antibodies EGC SAgs. It can be concluded, that carriers of *S. aureus*, don't induce antibody response against EGC SAgs [4, 19, 62]. In addition, bacteraemia enhances the potency of the anti-EGC SAgs, which are only rarely formed in patients with bacteraemia [53, 64]. Other researchers have reported that low levels of non-EGC antibodies, detectable *in vivo*, do not indicate bacteraemia [53, 56, 64], table I.

Table I
The risk coefficient of *S. aureus* carriage

Adults	*S. aureus* carriage rate
Adults receiving insulin	(34.0–53.4%)
Adults healthy	(10.7–34.2%)
Diabetics receiving oral anti-diabetic agents	(11.0–35.0%)

Reference: [56]

Clinical studies

Carriers of *S. aureus* have diverse characteristics, depending on ethnicity, race, gender and age. Colonization of *S. aureus* may predispose to staphylococcal carriage. Lipsky et al. investigated several factors that may be associated with the staphylococcal carriage comparing diabetic out-patients with non-diabetic control group. The number of *S. aureus* carriers of the nasal cavity and on the skin noticed in 59 diabetic patients was significantly higher (30.5%) compared to 44 in the control group (11.4%) ($p = 0.02$), but the ratio did not significantly differ between insulin-dependent diabetic patients (31.0%) and non-insulin-dependent diabetic patients (30.0%). No correlation ($p > 0.05$) with antibiotic treatment, age, race or clinical condition was observed in diabetic patients who were carriers of staphylococcus. In contrast, an inverse correlation to the glucose concentration (based on fasting plasma glucose and glycosylated hemoglobin) was found in hospitalized patients ($p < 0.03$). Additionally, during the 1-year follow-up staphylococcal colonization appeared more often in diabetic patients than in the control group. The results confirm the increased incidence of staphylococcus colonization in patients with diabetes compared to non-diabetic patients, and disclose that insulin and other demographic factors do not affect the higher rates of *S. aureus* carriage in the diabetic patients' nasal cavities [28] (table II).

Methicillin-resistant *S. aureus* (MRSA) colonizes the front nose in diabetic patients which may cause an increase in morbidity and mortality. Alizargar et al. collected anterior nares swabs from 494 patients with diabetes. Out of the 494 patients, 210 (42.5%) had a positive result of nasal colonization by *S. aureus*, out of which 122 (57%) were MRSA (24.7% of all patients

Table II
The frequency of isolation *Staphylococcus aureus* in patients with diabetes

Clinically diagnosis	The most commonly isolated microorganism	Frequency isolation	References
Acute purulent meningitis (APM)	*Staphylococcus aureus*	31%	67
Sepsis	*Staphylococcus aureus*	21.3%	67
Bacteraemia	*Escherichia coli* *Staphylococcus aureus*	23.9%	54
Diabetic foot syndrome (DFS)	*Staphylococcus aureus*	15.6%	33
Skin and soft tissue infections (SSTI)	MRSA MSSA	7.4% 13.2%	36
Carriers of the nasal cavity and on the skin	*Staphylococcus aureus*	30.5%	32

with DM). The study showed a high incidence of MRSA infections in diabetic patients, which leads to the conclusion that epidemiological studies and assessment of MRSA colonization risk factors may be helpful in the effective monitoring of patients with diabetes [1]. *S. aureus* as a source of endogenous microflora is a common factor for surgical site infection (SSI) after major heart surgery (MHS). All patients undergoing MHS must have their microbial flora in the nasal cavity tested. Every year brings new observational studies evaluating the effect of staphylococcal carriage on the development of SSI after MHS. Among others, Muñoz P. et al. conducted annual observational studies in which patients undergoing MHS were examined for nasal *S. aureus* transport prior to surgery. Of the 357 patients studied, 96 (27%) were carriers of *S. aureus* in the nasal cavity, of which 9 (9.4%) had strains of MRSA. The results have shown that the independent predictor of the development of SSI was the presence of *S. aureus* in the nasal cavity, which, for patients with diabetes, was RR: 5.9, p = 0.003. The results confirm that the carriage of *S. aureus* in the nasal cavity increases the frequency of hospital SSI after major heart surgery MHS [35].

McKane et al. have analyzed intensive care unit (ICU) patients who were positive with methicillin-resistant *S. aureus* (MRSA) (N = 76 913 patients). MRSA colonization on admission was associated with earlier exposure to pathogens while providing healthcare services. The researchers mark out: the time prior to the hospitalization (OR = 2.4 95% – CI = 1.3 – 4.7; p < 0.01), a stay in a nursing home (OR = 3.8 95% – CI = 2.3 – 6.3; p < 0.01) and contact with the health care system (OR = – Cl 8.0 95% = 4.2–15.1; p < 0.01). Among the comorbidities associated with MRSA colonization, diabetes has also been mentioned (p < 0.01). The risk factor associated with the adoption of ICU, did not demonstrate a statistically significant connection with MRSA colonization (OR = 1.1 95% – CI = 0.6–1.8; p = 0.87). The study shows that the use of screening is helpful in defining risk groups which are exposed to MRSA colonization [32] (table III).

Table III
Meta-analysis of risk factors associated with MRSA colonization and admission to the ICU and the hospital

Comorbid conditions	Odds Ratio hospital	Odds Ratio ICU	p-value hospital	p-value ICU
Diabetes mellitus	2.30	3.78	< 0.01	< 0.01

ICU – intensive care unit; Reference: [32]

3. Staphylococcal infections in patients with diabetes

3.1. Staphylococcal superantigens

Virulence factors produced by *S. aureus* include enzymes, toxins and cell surface proteins. Several enzymes produced by *S. aureus* cause tissue destruction and the spread of toxins. Toxins cause cytolysis and tissue damage, e.g. the presence of enterotoxins and exotoxins may cause septic shock syndrome. Superantigens are made of structurally and functionally related molecules which include TSST-1 and enterotoxin [12].

Currently there are 24 serologically different superantigens: TSST-1, Staphylococcal enterotoxins (SES) (serotypes A, Bn, Cn, D, E and G) and SE-like (SE-I) superantigen (serovars H, I, and JX). TSST-1 and SE-X have different amino acid sequence. These superantigens have one seat with a low-affinity major histocompatibility complex class II (MHC-II) which binds to the folds O/B. The location interacts with the α-chains of MHC II molecules. Binding to MHC II superantigen TSST-1 interacts also with an antigenic peptide which is a peptide-binding groove of the molecule. The β chain of T-cell receptors (Vβ-TCRs) binding site of TSST-1 is located in a groove between the O/B fold and β-grasp domains [58, 65].

3.2. Skin and soft tissue infections

Skin and soft tissue infections (SSTI) can cause substantial morbidity in diabetes [29]. Skin lesions occur in 20–30% of patients with diabetes and metabolic disorders and patients with abnormal sweating, itching, hyperalgesia [55].

Cultures of *S. aureus* clones USA 100 can be often found on the skin and in the front of the nostrils. This may indicate the origin of endogenous USA 100. Patients with diabetes are heavily colonized with *S. aureus* clones USA 100 , in large amount over 10^{13}/person [66]. Exacerbation of symptoms on the skin may occur as a result of staphylococcal infections. For the same reason, there is a disorder in immune response that occurs as a result of the presence of allergens and viruses. Abnormal body response is caused by superantigens and alpha-toxins that can cause skin inflammation [58].

Clinical studies

Lipsky et al. collected data from clinical studies of 3030 patients with diabetes hospitalized with positive bacterial cultures and diagnosed with SSTI. The data were collected from 97 hospitals in between 2003–2007. The results of swabs taken from wounds revealed mixed cultures in 53.0% of cases of infection at different localization and in 58.7% of cases of foot infections. Patients with foot infections com-

pared with patients with infections of other localization were positive for MRSA (9.6% vs 7.4%, $p = 0.06$) and methicillin-sensitive *S. aureus* (MSSA) (16.4% vs 13.2%, $p < 0.05$), respectively. During the study period, the percentage of MRSA positive swab results in patients with infections at different localizations increased from 14.0% to 24.6%, $p = 0.006$, and from 11.6% to 21.9%, $p < 0.0001$ for patients with foot infections, respectively [29].

3.3. Diabetic foot syndrome

Diabetic foot syndrome (DFS) is one of the most common causes of lower extremity amputation in developed countries [31]. DFS is defined as ulcer or destruction of deep tissues of the foot in patients with diabetes, with the presence of neurological disorders and peripheral vascular disease of the lower limbs at different levels. Pathological changes can be found in all of its structural elements: vessels (atherosclerosis and hardening of the walls), nerves (neuropathy), skin (inflammation, loss of the elasticity, trophic changes, calluses, ulcers), muscles (atrophy, contractures) and bones (local osteoporosis, osteomyelitis, avascular necrosis) [55]. Diabetic foot ulcers (DFU) is a complication that occurs in diabetes. DFU poses a risk of further infection, gangrene and in the end, limb amputation. The most frequently mentioned risk factors for DFU include ischemia, neuropathy and ischemia, abnormal neuroischemia. Studies have shown that only 10% of patients develop DFU that is not complicated by ischemia or neuropathy, and 90% of them develop abnormal neuroischemia, neuropathy and ischemia [69]. SAgs cause activation of TCR-Vβ region present on T-cells. Activated T-cells stimulate the production of proinflammatory cytokines and maintain the chronic inflammation. The consequence of the presence of SAgs is the complication in the wound healing. Vu et al. examined the patients with DFU from whom *S. aureus* strains USA100 were isolated. This group of clones revealed increased production of sags that may interfere with the growth of bacterial cells' culture, and as a consequence impair the wound healing. Vu et al. showed that 88% of the strains isolated from patients with DFU had a SEI-X gene, which contains the gene for TSST-1. Typically, *S. aureus* strains have the gene for either SEl-X or TSST-1. Sel-x was located in the core chromosome of all *S. aureus* strains except for USA200 strains, which encode TSST-1 within pathogenicity islands. Researchers suggested that the therapy neutralizing or reducing SAg production by *S. aureus* may be beneficial in the management of patients with DFU. The significant virulence factor in the skin infections is *S. aureus*' α-toxin. The toxin synergizes with SAgs in some disease conditions. Researchers evaluated α-toxin production among the DFU isolates and reported that USA300 isolates had the highest of this toxin production [66].

Clinical studies

According to the definition, DFS pertains to infection, ulceration, or destruction of the foot's deep tissues, resulting from the injury of peripheral nerves or blood vessels. Depending on etiology, DFS is divided into neuropathic, ischemic, or mixed type. Małecki et al. conducted a study to determine the most common pathogens responsible for infections associated with DFS. Medical records of patients treated in clinical hospital between 2008 and 2010 were collected. A total of 102 patients have been identified, from whom samples were taken and tested for antibiotics sensitivity. 199 bacterial strains were isolated, in total. Majority of them were gram-positive bacteria, especially *S. aureus*, coagulase-negative *Staphylococcus* and *Enterococcus faecalis*. High percentage of infections was caused by *S. aureus* (15.58%). However, out of all the etiological factors *Staphylococcus* spp. was 26.63%, which in particular consists of coagulase-positive *S. aureus* isolated in most cases of DFU [31]. The study suggests that *Staphylococcus* spp. predominates among the etiological factors of DFS infection and the appropriate patient education that encourage regular foot care in order to prevent DFS and its complications should be conducted.

Chronic infections caused by *S. aureus* generate resistance to antibiotics. The presence of methicillin-resistant *S. aureus* (MRSA) may cause the formation of small colony variants (SCVs). Cervantes-Garcia et al. have reported isolation of MRSA strains in patients with DFU, where 36% of cases contained genes *mecA* [6]. Cervantes-Garcia et al. described the first cases of patients with type 2 diabetes who had diabetic foot infections caused by MRSA-SCV, and the DFU not treated with gentamicin. *S. aureus*-SCV is localized intracellularly, which may interfere with the use of antibiotics. The authors report that SCV isolates were present in patients with long-term treatment with antibiotics or DFU infection lasting for a long time. *S. aureus*-SCVs strains are responsible for the misidentification of infections in patients by routine. Automated systems are used to detect *S. aureus* isolates. Mistakes can be the consequence of short incubation periods or low discrimination levels in data bases. The key to a proper *S. aureus*-SCVs recognition and successful recovery is the use of an extensive set of cultures and identification techniques [6]. This study makes these isolates an important outcome because SCV variants had neither been previously reported nor identified with DFU infections. The isolation of MRSA-SCVs in diabetic foot ulcers and patients diagnosed with type 2 diabetes mellitus indicates that

the treatment is more complex. The use of adequate antibiotics is especially important in order to avoid antibiotic resistance [6].

3.4. Sepsis

S. aureus infections are the second leading cause of sepsis in the USA [21]**.** Infections in diabetic patients can be severe and life-threatening because the clinical signs are often masked by the chronic complications of diabetes, which leads to the delayed medical diagnosis. What contributes more to this condition, is the inability to control the infection caused by the mutual dependence and defect in immune cell and in the humoral response [41]. Sepsis is a systemic inflammatory response syndrome (SIRS) resulting from the infection. SIRS is characterized by a sudden onset of at least two of these symptoms, body temperature above 38°C or below 36°C, heart rate greater than 90/minute, respiratory rate greater than 20/minute or $PaCO_2$ below 32 mmHg, the number of leukocytes more than 12 000/μl or less than 4 000/μl, or more than 10% of the immature form of neutrophils [25]. Infectious diseases caused by *S. aureus* cause some of the most severe hospital-associated and community-acquired illnesses. Staphylococcal superantigens (SAgs) secrete staphylococcal toxins that are major *S. aureus* virulence factors. SAgs mediate many clinical signs such as fever, hypotension, multi-organ dysfunction and rash. SA-gs are also capable of enhancing the toxic effects of endogenous endotoxin. SAgs are associated with many life threatening *S. aureus* illnesses such as TSS, septicemia. SAgs cross-link the Vb chain of the T-cell receptor (TCR) to the MHC II molecule on antigen-presenting cells independent of cognate antigen specifity, inducing a powerful activation of T-cells and macrophages with massive production of cytokines. Activation of 0.0001–0.001% of the body's T-cell population is the normal response to the antigen, but SAgs can activate 20–30% of T-cells and in some cases up to 70% of the total T-cell population. The number of activated T-cells and macrophages secreting large quantities of cytokines, is responsible for the clinical symptoms associated with SAgs and staphylococcal toxic shock syndrome (TSS), such as capillary leak, hypotension, rash, and fever. Researchers' observations provide evidence, that SAgs mediated in inhibiting antibody response [21, 25]. T-cell exhaustion is the most common explanation used to account for T-cell answer during TSS. T-cell exhaustion in humans with TSS has not been observed. B-cell function, including activation state (lack of T-cell help), chemotaxis and migration could be affected during TSS which would therefore inhibit the development of SAgs and *S. aureus*-specific antibodies, and memory response [24]. In patients with staphylococcal pneumonia and infective endocarditis sepsis state is a consequence of staphylococcal infection. The significant number of patients develop sepsis after surgery and systemic effects in these patients may result from the superantigen action. Infectious strains are likely to produce the toxins and certain superantigens, notably SEA, TSST-1, and SEC, which are overrepresented in sepsis cases. Superantigens may be produced in focal sites of infection protected from hemoglobin peptides and then secreted into the bloodstream [58].

Clinical studies

Petrovici et al. conducted studies assessing etiology, clinical features and results of diabetic patients with invasive disease. A retrospective study was conducted in the period from 2008 to 2010, in the Clinical Infectious Diseases Hospital of Iasi, Romania. The study included 75 diabetic patients with sepsis with proven microbial etiology (positive cultures from normally sterile sites) and patients with sepsis with clinical symptoms of the suspected etiology (positive cultures from the pus). Among the 75 diabetic patients with severe cases of sepsis (44%) there were more cases of patients with insulin-dependent diabetes than with non-insulin-dependent diabetes (40% vs. 4%, $p < 0.005$). The most common isolated agents were *S. aureus* and *Escherichia coli*, which were found in 16 (21.3%) cases out of both species, the next was coagulase negative staphylococci (66%). Multiple septic disseminations were in 17 (22.6%) cases and meningeal involvement was documented in 10 (15.6%) cases. MRSA was reported in 53.3% cases of the invasive strains of *S. aureus* [41]. These results confirm that patients with diabetes are particularly exposed to staphylococcal superantigens (TSST-1, SEC and SEA), which in favorable conditions cause sepsis [41]. Targeted treatment should be focused on the staphylococcal virulence factors.

S. aureus bacteraemia (SAB) can lead to endovascular and metastatic infections, and complications can occur at almost all sites of the body. Increased morbidity and mortality in people with SAB is determined by the presence of adhesins and toxins despite the appropriate antimicrobial treatment. These factors behave like superantigens (SAgs) and lead to a massive release of proinflammatory cytokines. The result of these changes is inflammatory response, which leads to endothelial leakage, hemodynamic shock, multiorgan failure, and in the end to death [36]. Vandenesch et al. investigated the relationship between PVL and CA-MRSA infections. CA-MRSA isolates that were collected from all over the world very often contained PVL [62]. Risk factor for MRSA infection is usually the nasal cavity colonization. It was found that 82% of patients with bacteraemia caused by MRSA had previously strains of *Staphylococcus* existing in the nasal

cavity [15]. Risk factors for infection HA-MRSA relate to prior hospitalization, antibiotics, surgery and the presence of prosthetic or other implant devices. Other comorbidities (diabetes, cancer), colonization of the nasal cavity and prosthetic devices are the most common causes of CA-SAB [53].

Patients with diabetes are at the increased risk of colonization of *S. aureus*, which is connected with the frequent infections of the skin, soft tissue, and sepsis. Stoeckle et al. conducted retrospective studies on 71 patients with diabetes and 252 without diabetes, who have been infected through the bloodstream. The majority of microorganisms isolated from the blood cultures of the above mentioned groups of patients with diabetes were: *E. coli* (28.2% versus 37.3%, p = 0.156) and *S. aureus* (23.9% vs. 15.9%, p = 0.117). Bloodstream infections (BSI) in diabetic patients (25.8 episodes/1000 admissions), and in patients without diabetes (5.8 episodes/1000 admissions) was reported. The incidence of BSI was 4.4 times higher in diabetic patients than in non-diabetic patients (< 0.0001). Moreover, bacteraemia occurred 2.3 times more frequently in patients with diabetes than in patients without diabetes. Research conducted by Stoeckle et al. led to the conclusion that patients with diabetes are at the increased risk of BSI and complications of infection [60].

Hernandez et al. collected data on episodes of community onset bacteraemia of unknown origin (CO-BSI) at the university hospital between 2005 and 2011. Incidents of CO-BSI were reported in 745 patients. The most frequent comorbidity noticed was diabetes mellitus in 151 (20.3%) patients [18] (table IV).

Table IV
Isolated microorganisms and antibiotic susceptibility in community-onset bacteraemia of unknown origin

Microorganisms	Total n = 745 (%)	CA-BSI n = 340 (%)	HCA-BSI n = 405 (%)	p-value
Methicillin-sensitive *S. aureus*	64 (8.6)	35 (10.3)	29 (7.2)	< 0.128
Methicillin-resistance *S. aureus*	14 (1.9)	5 (1.5)	9 (2.2)	< 0.452

CA-BSI – community acquired bloodstream infections
HCA-BSI – health care associated bloodstream infections
Reference: [18]

S. aureus was one of the risk factors for CO-BSI. In multivariate analysis with regard to *S. aureus*, the odds ratio for diabetes was OR 1.72 (1.01–2.91). *S. aureus* (including methicillin-resistant strains) was equally isolated both in patients with health-care-associated (HCA-BSI) and community-acquired (CA-BSI) bloodstream infections. It should be noted that 17.9% of *S. aureus* strains were resistant to methicillin. The results obtained by Hernandez et al. led to the conclusion that knowledge of the pathogenic bacteria responsible for CA-BSI is important in the selection of appropriate therapy, especially for *S. aureus*, which is an independent predictor of mortality [18], table V.

Table V
Univariate analysis of predictors of *Staphylococcus aureus* bacteraemia in patients with community-onset bacteraemia of unknown origin

Risk faktor: *S. aureus*	No n = 667 (%)	Yes n = 78 (%)	p-value
HCA-BSI	367 (55.0)	38 (48.7)	0.290
Prior hospital admission	187 (28.0)	16 (20.5)	0.158
Comorbility Diabetes mellitus	127 (19.0)	24 (30.8)	0.015

Reference: [18]

Laupland et al. conducted the study on the population in Canada, which refers to about one million adults with severe bloodstream infection in the period 2000–2002. 342 residents met the criteria for severe bloodstream infections (BSI), while the total annual incidence was 15.7 per 100.000 people. Significant risk factors for bloodstream infections were related to several demographic conditions and chronic diseases like diabetes mellitus (relative risk RR 5.9, 4.4, –7.8). The most common etiological factors were *S. aureus*, *E. coli*, and *Streptococcus pneumoniae*, respectively: 3.0, 3.0 and 1.9 per 100.000. Diabetes was the risk factor for BSI in 63 patients, whose estimated risk of exposure to infection of the bloodstream was 80.73. The incidence of serious bloodstream infections in patients with diabetes, compared to patients without diabetes was 78 per 100.000. The etiological factor for severe infections of the bloodstream was *S. aureus* in 46 patients and hospital infection occurred in 19 patients. Laupland et al. showed that epidemiological knowledge of the BSI, especially in high- risk patients, which include patients with diabetes, is important to ensure proper healthcare and take the precautions [26]. McKane et al. hypothesized that diabetes can correlate with an increased risk of CA-BSI. 2551 patients in critical condition were admitted to the ICU, where the incidence of CA-BSI in patients with diabetes accounted for 13.6%. 63% out of the the 2551 ICU patients had diabetes. The most common microorganisms isolated from the test group were: *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Candida albicans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Enterococcus faecium*. The researchers found that diabetes is a predictor of CA-BSI. The risk of the blood infection was significantly higher in patients with diabetes (adjusted odds ratio 1.42) compared to

patients without diabetes. In addition, adjusted risk of sepsis was significantly higher in patients with diabetes (1.26) compared to patients without diabetes. McKane et al. reported that diagnosed diabetes and glycated hemoglobin (HbA1c) levels greater than 6.5% are independent predictors of CA-BSI in ICU patients. All that information confirm that diagnosed diabetes and chronic hyperglycemia increase the risk of infection [32], table VI.

Table VI
The frequency of bloodstream infection in patient with diabetes mellitus

BSI	Concomitant diabetes mellitus	References
Community onset bacteraemia of unknown origin (CO-BSI)	20.3%	56
Healthcare-associated bloodstream infection (HCA-BSI)	18.2%	61
Community-acquired bloodstream infections (CA-BSI)	13.6%	35

A retrospective cohort study using administrative data was carried out in 1999 in Canada. A group of diabetic patients was compared to a group of non-diabetic patients (n = 513.749 in each group). Risk ratios were calculated for infectious diseases and death caused by the outbreak of infectious diseases, for people with and without diabetes. The study was repeated to confirm the stability of the predictions for the similarly defined second group, using data from 1996. The risk factor for infectious diseases and death due to the infectious disease for patients with diabetes compared to patients without diabetes was 1.21 (99% CI 1.20–1.22). In contrast, the risk ratio was 2.17 for infectious diseases, and 1.92 for the death due to the infection. Many individual infections occurred more frequently in patients with diabetes, particularly severe bacterial infections. The results obtained from the studies lead to the conclusion that diabetes is a risk factor for increased morbidity and mortality due to the infectious diseases, and micro- or macrovascular complications. In addition, the infection as a complication of diabetes should be also considered [52].

3.5. Infective endocarditis

The etiological agent of the infective endocarditis (IE) is *S. aureus* and accounts for about 40 000 cases/year in the USA [21]. Infective endocarditis is the heart endothelial cells' infection caused by different bacterial species. This life-threatening infection is usually caused by *S. aureus*.

The changes that can be observed is the presence of the large growing vegetation on the heart endothelium. These include host components (tissue factor, fibronectin and fibrinogen), host cells, and bacteria [33]. The formation of the growing vegetations is dependent on the cell surface, which adhere to *S. aureus* virulence factors. Pragman et al. showed that TSS toxin-1 (TSST-1) plays an important role in initiating the infective endocarditis that is caused by the strains which produce superantigens. [15].

Xiong et al., published that in patients with infective endocarditis persistent bacteremia strains type USA200 (producing exotoxins: TSST-1, SEC and α-toxin) were isolated, which carry the tstH gene that encodes TSST-1 [68]. Correlation between the presence of tstH and TSST-1 protein production is 1:1 and about 90% of infective endocarditis cases are associated with USA 200 strains and TSST-1 production [20]. Mattis et al. showed that superantigen staphylococcal enterotoxin (SE) C is also highly important in the infective endocarditis. Huseby et al. showed that secreted cytolysins' β-toxin contribute to the infective endocarditis progression [20]. Researchers' reports confirm increased incidence of staphylococcal colonization in diabetic patients compared to patients without diabetes. However, they still do not know the critical factors responsible for the higher rates of carriage of *S. aureus* in the nasal cavity and the need to control the increasing antibiotics' resistance in hospital settings is constantly growing. In other studies MRSA MW2 (an SEC-producing strain) encoding multiple Sags were used, including SEC and SEl-X. MW2 and its derivatives that were lacking sec or selx were tested on the rabbit model of IE and sepsis. This study demonstrated that SEC is the dominant virulence SAg in strain MW2, and *S. aureus* bacteraemia when alone, does not account for the lethal outcome in infection but the virulence SAg exhibits high mortality [50].

An international study on *S. aureus* strains isolated from patients with infective endocarditis revealed higher frequency of SAg genes encoding TSST-1, SEC, SEG, and SEI among IE isolates compared to isolates collected from soft tissue infections [38]. Sepsis and IE are serious infections of the blood and heart valves. *S. aureus* strains that are exceptional at causing IE in rabbits produce TSST-1, SEB, and/or SEC [59]. Furthermore, we have previously shown that SAg-deficient strains, when expressing TSST-1 ectopically, increased ability to generate septic vegetations compared to TSST-1-negative isogenic strains [45]. These results highlight the critical role of SAgs in IE and explain the high prevalence of these genes in IE patients' strains.

Lee et al. showed the association between SAg-in duced vascular leakage and TSS. IE with *S. aureus* bacteraemia develops on infection sites in susceptible

individuals which produce SAgs and can lead to hypotension and cause a systemic buildup of SAg. In studies Lee et al. rabbits were treated with TSST-1. This animal model showed that reducing vegetation size and bacterial burden in infected rabbits will be possible by avoiding SAg-induced changes that increase difficulty in IE management. Obtained data suggest that SAgs'effects are vascular leakage, hypotension and immune system dysregulation, which could interfere with the cardiovascular system in the end [27]. All the defence factors protecting from the IE development depend on therapeutic agents that neutralize SAgs.

3.6. Acute purulent meningitis

Acute purulent meningitis (APM) is rapidly progressive bacterial infection of the meninges and subarachnoid space. APM's beginning is sudden and it's course dynamic and dominated by meningeal signs and increased intracranial pressure. Severe status within a few hours is deteriorating and can reach up to life-threatening. APM is characterized by a large number of inflammatory CSF cells, majority of which are neutrophils. Untreated APM spreads to the nervous tissue of the brain, leading to the meninges and brain inflammation. The patient's condition is usually serious. The major APM symptoms include: headache, fever above 39°C, nausea, vomiting, photophobia and meningeal signs [42, 47].

Clinical studies

Petrovici et al. analysed such factors like: etiology, clinical symptoms and the results in patients with diabetes and bacterial meningitis, the determination of the nervous system during invasive [42]. In a three-year-retrospective study, 445 adults at the age of over 18 years with a diagnosis of APM (positive cultures from normally sterile sites) or suspected APM (positive cultures from pus) were examined. The studied group was divided into 2 subgroups: diabetic patients (95 out of 445) and non-diabetic patients (350 out of 445). APM was diagnosed in 16 out of 95 patients with diabetes (16.8%) and in 43 out of 350 (12.3%) patients without diabetes ($p = 0.322$). Positive cultures were isolated simultaneously from CSF and other fluids (blood and pus). The most frequent clinical symptoms observed in patients with diabetes and without diabetes were consciousness disorders (68.8% vs. 23.3%) and fever (37.5% vs 88.4%). *S. aureus* was the most common etiological agent in meningitis both in patients with diabetes and without diabetes (31.3 vs. 23.3%). This study shows a relatively high frequency of meningitis episodes in diabetic patients in sepsis. Bacterial meningitis was often microbiologically validated in the CSF in the group of diabetic patients. Petrovici et al. report in this study that the risk of the delayed bacterial meningitis diagnosis was unfortunately high due to the chronic complications of diabetes [42].

4. Vaccination

Salgado-Pabón et al. were searching for an effective *S. aureus* vaccine. A vaccination with purified SAgs or SAg toxoids alone or in combination with cytotoxins has proven to be a successful strategy to protect rabbits against lethal doses of *S. aureus* clonal types (USA100, USA200, USA300, USA400). This vaccination provides sterilizing immunity. Protection of rabbits after contact with CA-MRSA TSST1+ USA200, SEC+ USA400 or SEB+ USA400 strains, provides vaccination with TSST1, SEC or SEB, respectively [51].

TSS1-neutralizing antibodies production is stimulated by three different immunizing TSST1 toxoids. TSS1- antibodies protect rabbits from a TSST1 lethal challenge in an LPS enhancement model. In the rabbit pneumonia model exposed to *S. aureus* MNPE (TSST1+, SEC+ and α-toxinHI) trivalent vaccine which is composed of TSST1G31S (or TSST1S32P), SEC and α-toxinH35L provides complete protection against this lethal challenge. It protects rabbits from infective endocarditis and lethal sepsis providing pentavalent vaccine which contains TSST1G31S (or TSST1S32P), SEC, α-toxinH35L, β-toxin and γ-toxin when treated with a lethal dose of *S. aureus* MNPE [51].

Salgado-Pabón et al. report that multivalent vaccines directed against virulence factors produced by staphylococci are to protect from dangerous diseases caused by *S. aureus*. Studies show that neutralization of superantigens and cytolysins causes inactivation of *S. aureus*. The same will be for coagulase-negative staphylococci, when we remove an alternative way of complement and neutrophils opsonization. Vaccination strategies must be based on understanding *S. aureus* infection pathogenesis and using animal models with immune system and cardiovascular physiology that resemble the human ones [51].

5. Conclusions

1. The elevated glucose levels in diabetic patients with increased dynamics of the infection result in the difficulty to control the infection properly.
2. The dynamics of colonization of the nasal vestibule by *S. aureus* connected with high risk of infection diabetic patient. Among *S. aureus* virulence factors, superantigens (SAgs) are essential for pathogenicity.
3. The role that superantigens play in causing infectious diseases in immunodeficient patients must

be highlighted. An appropriate vaccine with multiple toxoids may provide protection against *S. aureus* infection in patients with diabetes.

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