**Toxicology**

# Microbes indicating the efficiency of cosmetics preservation. Part II. *Staphylococcus aureus*

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## Summary:

*S. aureus* bacteria are used, among others, as an index of official evaluation concerning the efficiency of cosmetics preservation. The thesis discusses its general characteristics, morphology and culture, diagnostics concerning infections caused by staphylococci, the role played by *S. aureus* in the environment, its pathogenicity, sensitivity and resistance to antibiotics, next to current interests of cosmetic microbiology related with this microbe.
The thesis shall aim to strengthen the belief among cosmetic producers and users related with the validity concerning the choice of these indicative microbes utilized to evaluate the efficiency of added preserving agents.

**Key words:** *Staphylococcus aureus,* cosmetics, contaminations, preservation, biofilm, antibiotic.

## Introduction

Part I of the elaboration (*Military Pharmacy and Medicine*, v. 5, No 2, pp. 32-41) concerning indicative microbes detected in cosmetics covered the discussion concerning *Pseudomonas aeruginosa*. Apart from the above mentioned microbe, regulations imposing realization of bacteriological tests on cosmetics also mention *Staphylococcus aureus* [1,2].

*P. aeruginosa* represents a large section of bacteriology, covering gram-negative bacteria, whilst *S. aureus* represents an equally large section of gram-positive cocci. Apart from *Candida albicans* yeast, references relating the two above mentioned bacteria are limited solely by qualitative researches towards bacterial contamination of raw materials and cosmetics, as well as evaluation concerning the efficiency of preservatives added to cosmetic products. Both *P. aeruginosa* and *S. aureus* represent the commonly present bacteria leading to enzymatic decomposition and spoiling of cosmetics. These bacteria are also opportunistic pathogens. Researches conducted by multiple authors also frequently indicate Aspergillus niger representing filamentous fungi.

Dynamic development of cosmetic microbiology, as well as previous tests could probably be expanded and methodically modernized. Nevertheless, practical aspects shall limit the possibilities related with using modern diagnostic achievements in cosmetic industry laboratories.

# General characteristics of *Staphylococcus aureus*

*Staphylococcus aureus* (golden staph) is enumerated among the large group covering bacteria with the shape of coccidia. In culture these coccidia may occur in the form of single seeds (*cocci*) or gain the shape of racemes (*staphylococ*ci), or may occur in the form of beads (*streptococ*ci).

Hence the name staphylococci (from Greek *staphyle — raceme*) comes from accumulations of bacterial cells observed in cultures, as in old cultures it is possible to observe pair layout or sometimes accumulation in chains. Diameter of a single staphylococcal cell is 1 micron. Racemes are formed because staphylococci are divided in two planes and not in one as it is the case in streptococci [3].

Basing on the ability to produce coagulase enzyme, causing blood coagulation, *Staphylococcus* genus was divided into two groups: coagulase-positive (pathogenic) and coagulase-negative. *S. aureus* and *S. intermedius* are coagulase-positive genres. All other staphylococci are coagulase-negative (*coagulaso-negative staphylococci* – CNS). The CNS group covers 30 species. These are commensal skin microbes. Despite the above, some of them may cause the occurrence of infections. These staphylococci, just like *S. aureus* tolerate sodium chloride even up to 7.5% concentration and are often hemolytic. Their identification requires biotype analysis.

Despite the fact that coagulase stands as taxonomic indicator of pathogenicity, yet some natural isolates of *S. aureus* are deprived of the ability to produce this enzyme [3]. What is more, coagulase production activity is observed among other species of coccidia (f. ex. *S. Hyicus* isolated from animals) [4]. Numerous and hard to be identified staphylococcal species are currently combined into groups, f. ex. basing on resistance to Novobiocin, similar pathogenicity, similar metabolic and physiological features [4].

At least 30 species of staphylococci have been reported as gram-positive bacteria with G+C DNA content by means of biochemical analysis, and in particular with the help of DNA-DNA hybridisation. DNA hybridisation of ribosomal RNA (rRNA) and comparative oligonucleotide analysis of 16S rRNA revealed that staphylococci create an adherent group on the level of *Bacillus-Lactobacillus-Streptococcus* species [3].

*S. aureus* species, just like the whole *Staphylococcus* genus gains the gram-positive colour. Microscopic preparations from old cultures may gain irregular colours. *S. aureus* proliferates in oxidative and relatively anaerobic conditions. Despite the fact that it is a relative anaerobe, yet it is the peroxidation of medium (f. ex. by shaking) usually intensified vegetation [4]. As it has been mentioned above, it is a halophil.

In culture based on liquid media it creates a homogenous turbidity, sometimes with flocculent sediment. In case of inoculations from samples containing a mixture of various microbes (especially food or environmental samples) selective foundations are used. Staphylococci are resistant to osmotic stress created by selective foundations: Chapman, with mannitol and NaCl, Blaird-Parker [4].

Increase is more abundant on enriched foundations, such as TSA or TSA with blood. After 24-48 hours of culture in 35°C, smooth, round and slightly protuberant colonies grow. After holding the culture in room temperature and after exposing it to light the colony reveals a white or orange colour. After 48 hours of proliferation on agar with blood, certain strains from several species, including *S. aureus*, evoke beta haemolysis. As far as pathogenic staphylococci are concerned *Staphylococcus aureus* (next to *S. epidermidis* and *S. saprophyticus*) is the most common and most dangerous etiological factor causing human infections and poisoning, as it is truly comprehencively equipped with all acrimony factors.

Apart from the above, it has numerous enzymes, which can play the role of acrimony factors under certain conditions. These include proteases, lipases, deoxyribonuclease and fatty acid modifying enzyme (FAME). Strains of *S. aureus* isolated from furuncles usually produce lipases,

capable of hydrolysing lipids in the skin, which facilitates its distribution, and what is more, by breaking host's antibacterial lipids, it prolongs bacterial life [3,5].

## Diagnostics

Diagnostics concerning *S. aureus* is performed with suspensions of bacteria collected from colonies that have 24-48 hours. *S. aureus* is usually identified by revealing the capacity to create coagulasis, presence of this factor causes the occurrence of clumging factor (CF), beta haemolysis and anaerobic mannitol decomposition. Diagnostic difficulties may be caused by coagulose-negative strains or differentiation with *S. chleifei* or *S. lugdunensis* strains producing CF [4].

## Isolation and identification of *Staphylococcus* in clinical laboratory

Presence of staphylococci in the sample collected from pathogenic lesions may be suggested as early as on the basis of direct examination of the preparation discoloured according to Gram. Nevertheless, small quantities of bacteria, present particularly in blood samples, make it necessary to proliferate them at first on medium such as agar with blood, TSA or HIA. Samples suspected to be contaminated with other microbes might be cultured on the above-mentioned agar with mannitol inhibiting proliferation of accidental bacterial flora by 7.5% sodium chloride content. Apart from preparing microscoping preparation discoloured according to Gram *S. aureus* may be quickly identified basing on tests for creating catalase and coagulase. What is quite useful in tests on *S. aureus* is also the production of thermostable deoxyribonuclease.

Test result may be confirmed by agglutination of bacterial suspension with latex molecules coated with immunoglobulin G. Ready-made tests with monoclonal serums are available on the market (Staphaurex, Pastaurex). It has been recently described that certain clinical isolates of *S. aureus* do not create coagulase and/or coagulation factor, which may hinder the identification [3].

Commercial tests, basing on the occurrence of fibrinogen binding receptor (CF factor) and

A protein binding immunoglobulin through Fc fragment on the surface of *S. aureus'* cells are commonly used for quick diagnostics [4].

## Natural infestation locations in human organism

Many bacterial species reveal preferences for specific areas in human body. Stephylococci are enumerated among bacteria colonizing skin of every human being, where as commensals they create a natural microflora. *S. aureus* particularly prefers to colonise nasolacrimal duct and bronchia [3]. It was isolated from these places in 20-40% of healthy humans and as much as 60% of hospital personnel [4].

Foetal oral cavity is axenic, however, already during the labour and during 4-12 hours after labour is colonised mainly by bacteria of hospital origin. Oral cavities of infants always reveal the presence of bacteria from *Streptococcus, Staphylococus, Neisseria, Veillonella* and other genus.[6]. Microflora of the human nasal cavity and respiratory tract is mainly filled with *S. aureus* and *S. epidermidis*, and apart from that with diplococci and streptococci. Bronchioles and pulmonary alveoli are axenic. Epidermal layer is not the optimal location for proliferation of microbes, as it is dry, covered with dead corneocytes and it has acidic reaction.

Fatty acids, mainly the unsaturated ones, present in the epidermis reveal a strong activity inhibiting microbial development, especially some strains of *S. aureus*. Apart from dust, the skin of a healthy human being holds numerous bacteria and fungi, most commonly saprophytes. 1 cm$^2$ of epidermal surface has even 10$^6$ bacterial cells, mainly aerobic and anaerobic bacilli (from *Corynebacterium* and *Propionibacterium* genus), aerobic and anaerobic non-haemolysing coccidia, such as *S.epidermidis*, sometimes *S. aureus* and many other types of microbial species (enterococci, enterobacteriaceae, neisseria), in skin folds yeast and filamentous fungi. Because of washing the number of microbes decreases, however, it quickly regains the initial level.

In altered conditions, especially with lowered immunity of the organism, various species of microbes dwelling in the skin can cause infections.

What is problematic is quite a common occurrence of *S. aureus* carriers. It is estimated that about 30% of population are carriers of these bacteria.

In hospital conditions one of the manners to prevent transmission of *S. aureus* is to use protective gloves and masks covering mouth and nose. Implementation of high sanitary regimes in food, gastronomic, cosmetic and pharmaceutical industry proves positive as far as protection against these pathogens is concerned [6].

When sneezing or coughing microbes from nasal cavities and upper respiratory tracts may be distributed through droplet route and hence create bioaerosoles being an epidemiological threat. That is why in certain places, especially during the treatment process it is essential to wear protective masks [6].

## Pathogenicity

Apart from weakening the organism and lowering its immunity, the following ailments leading to infection with *S. aureus* were described:

1) Leukocyte chemotaxis defect, Wiskott-Aldrich immunodeficiency syndrome;
2) Down syndrome, Jacobs syndrome;
3) Diabetes, rheumatic disease;
4) Defect of intracellular killing, granulomatous disease;
5) Opsonization defect;
6) Hypogammaglobulinemia, hypergammaglobulinemia IgE;
7) Breaking the skin continuity (burns);
8) Presence of a foreign matter;
9) Valves, vascular catheters;
10) Influenza infections;
11) Cardiac diseases;
12) Neoplastic diseases. Prophylactic administration of antibiotics [7].

## Virulence factors, pathogenesis, forms of infection

Majority of infections caused by *S. aureus* have multiform pathogenesis [3]. That is why it is often difficult to establish which of the numerous virulence factors causes the given form of infection. [8]. Virulence factors include:

### 1. Capsular polysaccharide (Microcapsule).

As far as clinical isolates of *S. aureus* are concerned, their majority has a superficial polysaccharide, but they lose it quickly in laboratory cultures. It is called the microcapsule, as it can be seen only under electron microscope after being coated with antibodies. It does not resemble other bacterial capsules that can already be visible under optical microscope. The role of the capsule is not yet entirely clear. It may hinder phagocytosis, although in vitro tests reveal this loss only when there is no complement. On the contrary, comparison between the activity of pathogenic strain with capsule and a mutant with damaged capsule in *endocarditis* suggests that polysaccharide hinders colonization of damaged heart valves [3].

### 2. Protein A

Protein A is a superficial protein of *S. aureus*, which is covalently bound with peptoglycan layer. It occurs in more than 90% of strains. It binds the Fc region of IgG molecules, enabling their activity towards staphylococci [8], and simultaneously blocking the opsonization caused by Fc. This usually leads to inhibition, opsonization and phagocytosis [3].

Immunoglobulins bound by the A protein activate the complement, which consequently leads to occurrence of severe inflammatory reaction [8].

Protein A intensifies the virulence. Mutants of *S. aureus* that do not have the protein A are more efficiently phagocytized in vitro [3].

### 3. Leukocidin

*S. aureus* contains a toxin which poses a specific influence on multinuclear leukocytes, and hence its name. Phagocytosis conducted by leukocytes stands as an important prevention against each infection, including the staphylococcal one, so leukocidin acting on leukocytes is an effective virulence factor. This toxins shall be discussed in greater detail in the following part of the thesis. *S. aureus* is characterised by activity of many other cellular proteins and polysaccharides, correlated with virulence. These include: superficial proteins supporting colonization of host's tissues, factors that most probably inhibit phagocytosis and bind immunoglobulins.

## Toxins that pose a threat to tissues of the host and cause pathogenic symptoms

Most strains of *S. aureus* reveal adherent factors on surface of protein cells, and these factors enable bacterial access to host's proteins and hence facilitate binding between blood clots and damaged tissues. These tissues are the subject of intense researches. What is worth mentioning is the affinity to fibronectin. The place binding *S. aureus* is the fragment with 27 Kd mass, which captures trypsin from the final segment of the Fn molecule (soluble fibronectin). It is assumed that Fn opsonization can intensify in vivo phagocytosis and *S. aureus* damage, and yet simultaneously facilitate bacterial settling in damaged tissues or cells covered with Fn. Staphylococcal teichoic acid is responsible for binding fibronectin. Fibronectin binding proteins have also been detected in CNS strains. Frequency of fibrinolectin binding by staphylococcal cells varies depending on the species [5].

Interaction between *S. aureus* and collagen may play a certain role in facilitating the adhesion between bacteria and damaged tissues. Receptor facilitating collagen adhesion is observed in species evoking *osteomyelitis* and septic *arthritis* [3].

## Toxins posing threat to cell membranes

*S. aureus* may have several various types of protein toxins, which pose threat to cell membranes under in vitro conditions. These toxins include:

### 1. Alpha toxin

The most well-known and most dangerous for membranes. It occurs as monomer bound with cell membrane of sensitive cells. Alpha-toxin is systematically released during infections and results in a septic shock. Subunits of the toxin may create hexametric rings, with central orifice through which bacterial cell content is released. Sensitive cells in human organism have a specific receptor, which enables to be bound with small concentrations of the toxin. In case of large concentrations, toxins react in a non-specific manner with membranous lipids and this leads to occurrence of bigger pores, through which divalent cations and small molecules can pass.

Human blood platelets and monocytes are particularly sensitive to toxin, as the surface of these blood cells contains places, which allow toxin binding. Series of secondary reactions lead to creation of eicosanoids and cytokines, and these molecules cause the occurrence of inflammatory mediators. In case of severe infections evoked by S. aureus these reactions can lead to septic shock. Mutant strains of S. aureus, deprived of alpha toxin, prove to be less virulent in various animal infection models.

### 2. Beta-toxin

Beta-toxin is a sphingomyelinase, which threats membranes rich in sphingomyelin. This is confirmed by decreased virulence among mutant strains with beta-toxin deficiency. Most strains of *S. aureus* isolated from people do not produce beta-toxin, unlike majority of strains isolated from inflamed cows' udders. Most probably the beta-toxin plays an important role in pathogenesis underlying this disease among animals. Lysis of sheep erythrocytes is the classical test related with beta-toxin.

### 3. Delta-toxin

Delta toxin is a very small peptide produced by majority of *S. aureus* strains. It is also produced by *S. epidermidis* and *S. lugdunensis*. Its role in pathogenicity remains unknown.

### 4. Epsilon-toxin and leukocidin

Epsilon-toxin and leukocidin are binary proteins, occurring separately, but acting together on cell membranes. Epsilon-toxin contains 3 protein components, namely A, B and C. B and C components create leukotoxin revealing weak hemolytic activity, whereas its components A and B are hemolytic and poorly leukotoxic. In case of subcutaneous infection in rabbits leukocidin evokes dermonecrosis. What is more, when concentration threats cell membranes it results in releasing inflammatory mediators from human neutrophils.

## Superantigens, enterotoxins and toxin of toxic shock syndrome

Superantigens stimulate T cells in a non-specific manner, with omission of normal antigen identification and stimulate release of citotoxins evoking toxic shock syndrome (TSS) symptoms. *S. aureus* can possess two different types of

enterotoxins with activity of superantigen and with 6 various serotypes (A, B, C, D, E and G), as well as toxic shock syndrome toxin (TSST-1). Enterotoxins are responsible for staphylococcal food poisonings, they evoke diarrhoeas and nauseas, and they can lead to TSS [3]. Moreover, two epidermolytic toxins (ET) were characterised, coded by chromosome and plasmid genes. In reality these are two form of toxins: ET A and ET B, which differ as far as antigens are concerned, but they both have properties of proteases. Maybe the highly specific epidermal protein stands as the aim of these toxins [3]. They damage desmosomes within the granular layer of the epidermis, by detaching the dead superficial layers. In neonates they cause epidermal exfoliation with presence of vesicles [8].

## Other extracellular proteins

Coagulase is one of many *S. aureus'* enzymes enumerated as components of extracellular proteins. It coagulates human and rabbit blood plasma [5]. Coagulase binds with host's prothrombin and creates a complex known as staphylotrombin. Its proteolytic activity is revealed in a complex occurring as a result of fibrinogen conversion into fibrin.

Test confirming the presence of coagulase is the traditional determinant utilised to identify *S. aureus* in clinical laboratories. It is assumed that *S. aureus* protects itself against host's defence by evoking the occurrence of thrombus.

Clumbing Factor — CF, commonly known as "bound coagulase". It coagulates fibrinogen without participation of coagulase activator. It participates in screening staphylococci against leukocytes and antibacterial agents in serum. Glass test (rabbit serum and suspension of the examined strain of *S. aureus* create flocks appearing within 30 seconds) is used as a routine test in microbiological diagnostics.

Genetic tests proved that coagulase and CF are different protein units. Mutant of *S. aureus* deprived of coagulase remain the CF activity, whereas mutants with CF contain coagulase.

Many strains of *S. aureus* produce staphylokinase revealing proteolytic activity. Production of this particular type of lytic enzyme by bacteria, supports their distribution. Its operating mechanism is identical as in case of streptokinase, which is used in treating *coronary thrombosis* [3].

Staphylokinase does not reveal any direct enzymatic activity. It is indirectly responsible for fibrinolytic activity of *S. aureus*, as it only causes transformation of pasminogen into plasmin.

Endonuclease produced by *S. aureus* remains stable even after cooking for 15 minutes. Endonuclease test is also used on a routine basis.

To conclude discussion concerning infectious factors, it is worth emphasising that there exists a relation between strains isolated from particular disorders and expression of infectious factors indicating their role in pathogenesis. Symptoms of human diseases may be recreated on animals with some factors in forms of pure proteins [3].

## Forms of infections

*S. aureus* evokes the following forms of infections:

### I. Superficial dermal infections

Superficial dermal infections constitute the biggest issue in cosmetic microbiology. This concerns infections caused by various microbes, and *S. aureus* is the main among the above. Although dermatology and medical cosmetics deal with skin infections, yet the basic knowledge on reasons underlying the causes of infections and pathogenic changes should be passed on specialists trained in cosmetology and cosmetics.

Forms of skin infections:
  1) Inflammation of hair follicles (the most limited infection).
  2) Hordeolums (small furuncles present on verges of eyelids).
  3) Furuncles (more expanded infections);
  4) Furunculosis (abscesses).
  5) Multiple furuncles: combined abscesses covering hair follicles and surrounding tissues, they usually occur on the neck and the back [8].
  6) Impetigo and other superficial infections [3].

7) *Staphylococcal Scalded Skin Syndrome (SSSS)*. Disease is observed among children. Staphylococcal toxin, exfoliatin, leads to occurrence of large blisters in the epidermis, which burst and uncover live epidermal layers. Follicular impetigo with limited amount of blisters and staphylococcal scarlet fever with exfoliating rash occur in benign form.

## II. Deep infections

*S. aureus* causes dreadful deep infections in people who are weakened and chronically ill, with traumatic injuries, burnt or with immunosuppression. These include [3]:

### 1. Bone marrow and bone infections
 *S. aureus* is the most common etiological factor underlying acute bone marrow and bone infection, especially among children. Patients get infected through blood route, by dissemination from a distant focus.

### 2. Pneumonia
Associated with occurrence of abscesses in pulmonary tissue. This particularly concerns patients with weakened immunity, most commonly as complications after flu or after aspirating a foreign body.

### 3. Infection of the heart muscle
Infection of the heart muscle is enumerated among severe illnesses evoked by *S. aureus*. Some authors believe that *S. aureus* may initiate *endocarditis* by adjoining the undamaged cells in the *endothelium*. This was confirmed in tests focusing on cultures of epidermal cells infected with *S. aureus*, and researches proved the occurrence of bacterial adjoining to surface of endothelial cells and bacterial internalization in phagocyte resembling process. Other researchers assume that in case of bacterial adjoining, *endocarditis* may develop even with incredibly small cellular damage.

## III. Diseases caused by staphylococcal toxins

*S. aureus* has the capacity to produce two different types of enterotoxins, holding 6 different serotypes and toxic shock syndrome toxin (TSST-1), and it causes staphylococcal food poisoning and toxic shock syndrome (TSS).

**Staphylococcal food poisoning**
Poisonings are evoked by staphylococcal enterotoxins proliferated in food. Enterotoxins are present in several antigen types (A, B, C, D, E and G), whereas the most commonly observed poisonings are the ones of type A. Symptoms of poisoning include sudden vomiting and diarrhoea occurring after 1-5 hours after consuming food contaminated with staphylococcus. With proper rehydration of the patient's organism poisoning abates spontaneously after 24-48 hours. In USA the record number of staphylococcal poisonings was noted in 1987 and covered 1513 000 cases next to 1210 deaths. In 1997, the annual costs of foodborne illnesses caused by *S. aureus* in USA reached 1.2 milliard dollars. [9].

**Toxic Shock Syndrome (TSS)**
It has been described in 1978 as a disease caused by various, toxic producing strains of *S. aureus*. Currently the toxin responsible for occurrence of TSS was marked as TSS-1 (TSST-1). This is a multiple organ disease with fever, vomiting, diarrhoea, erythematous rash, exfoliating skin on palms and feet, muscle pains and lowered arterial pressure. The disease may lead to multiple organ dysfunction syndrome, shock and death [8]. The disease was reported among women during menstruation. It was mainly related with the use of absorbing tampons, which led to proliferation of large amounts of bacteria in vagina.

TSST-1 may be produced by various strains participating in different types of infections. It causes fatal shock among laboratory animals. It has been proven that TSST-1 can produce coagulase-negative staphylococci and lead to TSS.

## IV. Hospital infections

Staphylococci are indicated at the top of the list containing microbes causing hospital infections. This results from their wide distribution, easy colonisation of skin and mucous membranes, as well as the fact that they easily gain antibiotic resistance genes. Due to the above hospital is a place where selection of resistant strains may occur fairly easy [4].

These infections can be related with structure of medical devices, starting from straight catheters to complicated connections and exchangeable cardiac valves, may be evoked by *S. aureus*

and similar *S. epidermidis*. During a short period after implementation the device becomes coated with complex of host's protein mixture. It was proved that as far as mixture of these proteins is concerned, fibrinogen is a dominating component responsible for adhesion of *S. aureus*.

However, fibrinogen is divided on medical devices remaining in human organism for a longer time (f. ex. venous catheters) and it does not facilitate bacterial adhesion anymore. Instead of the above, fibronectin becomes the dominating ligant that facilitates bacterial adhesion [3]. Treatment of such cases based solely on antibiotics is related with certain difficulties and it is sometimes essential to remove the medical device. As some strains infecting patients in hospitals are resistant to majority of antibiotics, what remains to be used in therapy is vancomycin as staphylococci did not develop resistance against this drug [3].

## Epidemiology and lighting infections caused by *S. aureus*

*S. aureus* easily colonises skin and surface of mucous membranes, especially the damaged ones. Regardless of their age, race and gender, people are carriers of *S. aureus* located mainly in nasal atrium and around anus [8], and this may cause various forms of infections gaining the character of an epidemics.

*S. aureus* is disseminated in hospitals, not only in the environment, but also among patients and personnel. What concerns health service workers, the carrier state level reaches 60%. And it constitutes the main factor of hospital infections.

Epidemiological justification of infections caused by *S. aureus* traditionally leads through marking with bacteriophages, yet it is associated with many limitations. Molecular indicating methods are still within the experimental phase. Nonetheless, even the recent attempts to implement these methods resulted in advancement related with understanding pathogenesis of staphylococcal diseases. Genes coding potential virulence agents were cloned. This facilitates molecular researches focusing on their model activity, both in vivo and on animals. Epidemiological investigation concerning sources of these infections, it is essential to establish mutual relations between strains

isolated from patients. Indicating systems must be reproducible, differentiating, easy to interpret and user friendly.

Pulsating electrophoresis still remains to be the most reliable method, as it is based on genomic DNA being cut by restrictive enzyme, which creates large genomic fragments (between 50 and 700 kb) [3].

Epidemiological risk in hospitals comes both from patients as well as carriers from the personnel of the facility. There are various routes of transmitting strains, yet the hands of the personnel play an important role, being the route of direct contact [8]. In order to eliminate MRSA carrier-state, patients may undergo disinfecting procedures or may be treated with antibiotics, while carriers of epidemic strains, especially MRSA, should be isolated.

## Protecting the organism against infections

Phagocytosis is the main mechanism used by our organism to fight staphylococcal infections. Antibodies produced during developing infection neutralize toxins and facilitate opsonization. On the side of staphylococcus, capsule and A protein may resist phagocytosis. Staphylococcal film covering implanted medical devices is also resistant to phagocytosis. Apart from the above mentioned capsule and protein, *S. aureus* also has the ability to avoid the protection of infected organism due to other, quite numerous agents that have the capacity to interfere with defensive mechanisms. However, there are no detailed data on the role of virulence played by these agents [3].

### Specific prophylaxis

Despite previous achievements in immune researches on *S. aureus*, there still is no available vaccine that could fight staphylococcal infections. What is currently being taken into consideration is the method based on preventing disease, especially in hospitalised patients, by using immune serums obtained from voluntary donors or by administering human antibodies monoclonal to superficial capsule polysaccharide. These monoclonal antibodies may prevent bacterial adherence, as well as evoke phagocytosis. This is to intensify immune properties of their serums,

anticipated for administering them to patients before surgical procedures. Prototype of such vaccine based on polysaccharide of *S. aureus'* capsule was implemented into the stage of tests on volunteers [3].

Another staphylococcal vaccine based on fibronectin binding protein results in immunity against inflammation of cows' udders. Maybe such vaccine could also be used in humans [3].

# Treatment

## Antibiotic therapy

From the beginning of using penicillin *S. aureus* revealed considerable activity in gaining resistance to the above. Due to these reasons, from the early stage of the antibiotic era *S. aureus* required introduction of new drugs. Consequently, the phenomenon of resistance to other medications appeared shortly after their implementation. Most strains of *S. aureus* are characterized by resistance to penicillin/ampicillin related with production of beta induced lactamase of plasmid origin [5].

Rapid gaining resistance occurred due to genetic mechanisms covering accepting extrachromosomal plasmids or additional genetic chromosomal information by means of transposons [10] and by mutations in chromosomal genes [3].

Characteristic bacterial pumps play a significant role in antibiotic resistance. These are proteins, which recognize various types of antibiotics and remove them from bacterial cell cytoplasma. This process plays the main role in resistance to tetracycline, fluorochinolin, macrolide and more and more frequently — beta-lactam.
Many of them occur in gram-positive bacteria.

Nowadays, extrahospital infections related with *S. aureus* area usually treated with beta-lactams. Whereas intrahospital infections are usually caused by strains resistant to antibiotics, hence these can be treated solely with vancomycin [3].

Resistance to methicillin stands as the indicator of multiresistance (*Methicilin Resistant S. aureus* — MRSA). Despite the fact that MRSA relates to methicillin resistance, yet majority of these strains are multi-resistant. Literature also describes strains resistant to all clinically used medications, except for glycopeptides (vancomycin and teicoplanin). These strains evoke intrahospital infections that can have epidemic forms [3].

In case of certain enterococci, laboratory tests discovered plasmid related with resistance to vancomycin and the resistance determinant was moved from enterococci to *S. aureus*. Therefore, there exists a threat that this phenomenon may occur in natural conditions.

The list presented below enumerates sensitivity to antibiotics basing on data from the mid nineties of the last century [7].

1) Benzylpenicillin (about 70-95% resistant strains);
2) Ampicillin (resistance as above);
3) Penicillin/inhibitor – active towards penicillinase(+);
4) Methicillin – resistant strains may appear in hospital environment, cross resistance with beta-lactams and partially also with chinolones and lyncosamines;
5) Isoxazol penicillins (cloxacillin, dicloxacillin, floxacillin);
6) Cephalosporins I, II and III generation (cefotaxime, ceftriaxone);
7) Fusidic acid;
8) Lyncosamides (lyncomycin, clindamycin);
9) Macrolides (there are resistant strains)
10) Tetracyclins (minocycline) (there are resistant strains);
11) Aminoglycosides (netylmycin);
12) Polipeptides (vancomycin, teicoplanin);
13) New chinolones (ciprofloxacin, ofloxacin, pefloxacin);
14) Cotrimoxasole (there are resistant strains)
15) Imipenem, meropenem) [7].

Apart from the ability to gain antibiotic resistance, *S. aureus* also gains resistance to antiseptics and disinfectants such as quaternary ammonium compounds, which may facilitate its survival in hospital environment [3]. There is no rationale that a new, effective antibiotic shall appear, and there are no modifications of previously used medications towards this direction (1). Just like previously, as well as in the future scientists shall continue to undertake attempts to find new generations of

antimicrobial medications, including the ones fighting *S. aureus*. What also evokes interest are the attempts to find agents inactivating enzymes responsible for basic bacterial functions, f. ex. division of cells [3].

## Bacteriophage treatment

Phenomenon of expanding resistance to antibiotics caused interest in the ability to therapeutic use of bacteriophages. Bacteriophages were administered orally, locally and parenterally. Therapy covered wound infections, abdominal inflammation, pneumonia, osteomyelitis, sepsis, skin inflammation, abscesses caused by various drug resistant pathogens, f. ex. *S. aureus* [8]. As bacteriophages are captured within the organism by reticuloendothelial system, researches focused on their excessive administration were undertaken in order to verify whether their certain mutants could pass through and initiate efficient pathogen fighting [11]. An inevitable advantage related with bacteriophage treatment is the specifity of bacteriophages towards bacteria, and hence they can be used to fight a specific pathogen, without killing host's physiological flora.

Bacteriophages replicate within bacterial cell and lead to its lysis and release into environment of filial phages that can attack following cells, and so a small dose of phage can prove sufficient to fight the infection. Bacteriophage therapy is practically safe and does not evoke any side effects.

Due to the above mentioned properties, in case of infection caused by bacteria resistant to all available antibiotics, bacteriophages may become the last resort [11].

Negative aspects of bacteriophage therapy include:
1) Selection of bacterial strains resistant to bacteriophages, and resistant to antibiotics.
2) Phages, just like other viruses, require appropriate cellular receptors that are essential for absorption. Because of the above, phage therapy may be undertaken after precise pathogen identification.
3) Determined phage therapeutic preparation may prove inefficient in case of strains isolated from other geographic areas.

4) Phages may have antigen properties, which limits their repeated usage in the same patient.
5) Bacteriophage therapy cannot be used to treat infections caused by intracellular pathogens (*Salmonella*, *Yersinia*, bacilli).
6) The feature of entering in the so-called lysogenic cycle, where DNA connects into bacterial genetic material may stand as an obstruction related with pharmaceutical use of moderate bacteriophages. In this situation phages do not destroy bacterial cells, which become insensitive to repeated infection with the same phage.
7) Certain bacteriophages are capable of lysogenic conversion, namely of changing bacterial phenotypic features (they gain genes coding virulence factors).
8) Phages, such as *S. aureus* participate in horizontal transfer of enterotoxin A, staphylokinase and beta lysine.

To conclude with, it is Essentials to state that phage therapy requires knowledge on bacterial genes transferred by viruses, as well as certainty that the genetic material does not contain virulence genes.

## Search for new therapeutic options

Previously used antibiotics treat during later stages of disease's development by killing the pathogen that has already proliferated within the organism. In such case microbe could even colonise the organism and evoke pathogenic lesions. Currently it is possible to treat the patient during the phase of pathogen adhesion to host's cells.

Certain bacterial structures participate in the adhesion, and on the other hand also certain carbohydrates from the surface of eukaryotic cells. Researches on the use of these compounds to inhibit the adhesion before bacteria settle on the surface of host's cells were undertaken [11]. Another method of new therapy is to use aminoacyl-tRNA synthetase enzymes. It was detected that certain compounds act as inhibitors on bacterial synthetases, and simultaneously act slowly on host's cellular synthetase. There are known natural synthetase inhibitors. One of them, pseudomonic acid acts, among others, on synthetases of *S. aeruginosa*. This inhibitor acts 80 000 times faster

on synthetases of bacterial cells than on mammal cells [11].

## Current focus on *S. aureus* in cosmetic microbiology

Majority of publications concerns the manners of preservation by means of various compositions based on preparations with properties revealing antimicrobial activity. In studies on searching for optimal cosmetics preservatives, *S. aureus* remains an unchangeable index of their efficiency. Current interest focuses on non-chemical preservatives, mainly plant extracts. Extracts from *Nitraria refusa* were examined in search for such preservatives. This is a traditional agent used commonly as an anti-inflammatory factor and in case of cicatrisation. Two fractions (polar and non-polar) of *Nitraria* leaves were tested towards antimicrobial properties in relation to human pathogens.

Chloroform fraction was more effective in case of *E. coli* and *S. aureus* strains. Main components of this fraction include fatty acids: palmitin acid (28.04%) and unsaturated acids (48.78%): linolen acid (29.69%) and alinolen acid (19.09%). These results indicate that selective extraction is essential in order to obtain fraction with high biological activity, as these fractions can be used as preserving components in food, cosmetic and pharmaceutical industry [12].

It seems that studies on properties of extracts from *Aquilaria crassna* (*Thymelaeaceae*) or *Krisana agarwood* plants may also be interesting. Extracts from this plant are used in Asia for a long time to produce incenses, cosmetics and pharmaceutics. In Thailand, leaves of young Aquilaria crassna are used to make tea. It is assumed that Krisana leaves have therapeutic properties, such as antidiarrhoeal, antidiabetic and antibacterial activity. It has been confirmed that water extract from leaves of *Aquilaria crassna* reveal antimicrobial properties towards *S. aureus* and *S. epidermidis*, and especially in treating diarrhoeas caused by *S. aureus* and skin infections related with *S. epidermidis* [13].

The same situation can be observed among plants from *Anthemideae* family, where certain species reveal considerable activity towards *S. aureus*, just like towards certain bacilli and enterococci [14].

Nevertheless, plant extracts are of dark brown and greenish colour, which spoils the look of products. In order to avoid this problem a discolouration of various concentrations were used (5, 10, 15, 20, 25 and 30%) of extracts from cashew leaves by immersing bags filled with 10, 15, 20 and 25g of activated carbon for 0 to 6 hours [15].

Antimicrobial properties of ether oils used in traditional medicine as active components of medications and cosmetics evoke continuous interest.

As far as *S. aureus* is concerned, washing liquids containing solely oils met pharmacopoeial criteria, whereas in case of gentle balm even in 0.5% concentration they did not reveal any preserving activity. Significant increase in antibacterial activity was obtained after adding solvent to 0.5% tea tree oil, yet it was not only a considerable increase in antifungal properties.

These studies are also interesting for dentists [16]. Studies covered sensitivity of *S. aureus* against 12 various ether oils, which, apart from two, inhibited proliferation of these bacteria. These results suggest that ether oils may be used as active components of oral preparations. However, further in vitro studies should be conducted in order to confirm possible antibiotic properties and low toxicity of these preparations in oral environment [16].

Studies also focused on antimicrobial activity among commercial essential oils: lavender, tea tree and lemon in washing liquid or in gentle balm. Inhibitive efficiency towards *S. aureus* was obtained with 1% addition of oils in washing fluid and with 0.5% addition in case of the gentle balm. Preservation with the use of mixture containing several preservatives has certain advantages. It has been shown that addition of most commonly used allergenic preservatives may be significantly lowered when these are combined with phenoxyethanol [17]. The same different associations between preservatives revealed more efficient activity inhibiting *S. aureus*.

Cosmetic cream was efficiently preserved with concentrations not exceeding minimal approved phenoxyethanol concentrations with allergenic diasolifynol urea preservative (diazolidinyl urea) [18]. Literature also contains

theses describing the suitability of xylitol in cosmetic preservation. Xylitol is a natural sugar obtained from plants, fruits and vegetables. Its antimicrobial properties were described in literature. Studies evaluated preservative efficiency of the following composition: water, xylitol, blycol (copolymer and polyether functional siloxane) with addition of 1% retynil palmitate (RP). Analyses were conducted with the use of *E. coli, S. aureus, P. aeruginosa, C. albicans* and *A. niger* microbes. After 7 days of exposure, microbes revealed various increasing actions. All microbes were eliminated, except for *A. niger* [19].

In other studies MIC C-8xylitol monoesther in relation to tested *S. aureus* strain varied between 1% and 1.25%. Similar values were obtained in case of *E. coli* and *C. albicans*. In test conditions, xylitol reveals antimicrobial properties that are sufficient enough and hence can be considered as alternative preservative agent in cosmetics [20].

Aiming to eliminate sources of microbiological contamination among raw materials used to produce cosmetics, scientists undertook researches focused on powdered calcium carbonate and implemented elements of genetic tests into traditional diagnostic methods. This is to ensure optimal detection of microbes when establishing the risk of microbiological hazards [21].

As far as the presented list containing the current literature is concerned, it is dominated by topics related with searching for optimal compositions covering preserving agents, which are evaluated basing on efficiency of their activity on *S. aureus*.

## Summary

Article covers the current overview of knowledge on staphylococcus aureus. Particular attention has been paid to properties of this bacteria, namely: common occurrence in external environment and in human organism, many infective factors, diversity of evoked infections and poisoning, ability to gain drug resistance, lack of effective peculiar prophylaxis, opportunistic features of staphylococci when organism is weakened, as well as perspectives relating fight against infections. The final part of the elaboration presents an overview of literature focusing on the topic of *S. aureus* in cosmetics and cosmetology. Knowledge presented in this elaboration also constitutes a set of arguments supporting the efficacy of placing this pathogen among bacterial indicators of cosmetic contamination and efficiency of preservative agents.

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